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Comparison of *bax*, *ibpAB* and *cspH* Genes in *Escherichia coli* Isolates From Urine and Fecal Samples

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ABSTRACT

Background and Aim: Antimicrobial resistance (AMR) in *Escherichia (E.) coli* presents challenges in its clinical management. This study aimed to quantify the prevalence and relative expression levels of *bax, ibpA, ibpB, and cspH* genes in *E. coli* isolates obtained from urine and fecal samples of patients diagnosed with urinary tract infections (UTIs).

Materials and Methods: In this cross-sectional investigation, 50 *E. coli* isolates (25 urinary and 25 fecal) were collected from patients diagnosed with UTI in Sanandaj, Iran. Antimicrobial susceptibility was determined using Kirby-Bauer disk diffusion method. Biofilm-forming capacity was quantified using microtiter plate assay. Quantitative Real-Time PCR (qPCR) was performed to determine gene expression levels of target genes.

Results: Antimicrobial susceptibility testing of urinary isolates revealed maximal sensitivity to nitrofurantoin, ofloxacin, and norfloxacin, while the highest resistance rates were observed for amoxicillin, nalidixic acid, and trimethoprim-sulfamethoxazole. In fecal isolates, the greatest sensitivity was recorded for nitrofurantoin, ofloxacin, and norfloxacin. The genomic prevalence of the *bax*, *ibpA, ibpB*, and *cspH* genes in urinary isolates was 100%. In fecal isolates, *ibpA* was ubiquitous (100%), while *bax, ibpB, and cspH* were detected in 96% of the strains. Analysis of relative expression levels showed that the expression of all 4 genes was significantly higher in urinary isolates compared to fecal isolates. No significant difference was observed in biofilm formation capacity between two groups ($P>0.05$).

Conclusion: This investigation identifies a significant differential expression of *bax, ibpA, ibpB, and cspH* genes between fecal and urinary *E. coli* isolates, whereas biofilm-forming capacity remained consistent across sources.

Keywords: Antibiotic Resistance, *bax*, *cspH*, *Escherichia coli*, *IbpA*, *IbpB*, PCR, Urinary Tract Infection

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1. Introduction

Escherichia (E.) coli is identified as the primary etiological agent responsible for approximately 58% to 80% of all diagnosed urinary tract infections (UTIs). Although the vast majority of *E. coli* strains exist as commensal, non-pathogenic inhabitants of the human intestinal tract, specific virulent serotypes, such as O157:H7, are recognized as clinical triggers for foodborne toxicoinfections, acute diarrhoea, and severe urinary tract pathologies **(1, 2)**. Symptoms typical of UTIs

include pollakiuria, persistent dysuria, and the presence of hematuria or pyuria within the urine. Furthermore, as a classic Gram-negative bacillus, *E. coli* possesses the inherent capability to transition into the systemic circulation following localized infections, potentially precipitating life-threatening conditions such as bacteremia and septicemia **(3, 4)**. Uropathogenic *E. coli* (UPEC) represents a specialized subgroup of strains fundamentally involved in the pathogenesis of various UTIs, particularly in cases of cystitis **(5, 6)**. Successful colonization and persistence of *E. coli* within the urinary tract depend on its ability to withstand fluctuating environmental pressures, such as osmotic stress, pH variations, and host immune responses. Small heat shock proteins (sHSPs), specifically the *ibpAB* operon, are critical for maintaining proteostasis by preventing protein aggregation under the high-stress conditions of the gut, such as bile salt exposure and oxidative pressure. Consequently, the induction of specialized stress-response mechanisms—specifically those involving molecular chaperones and HSPs—is hypothesized to be a critical determinant of both commensal survival in the gut and uropathogenic virulence in the bladder **(5, 7)**. The molecular interface between commensalism and pathogenicity is governed by the bacterium ability to orchestrate rapid transcriptomic shifts in response to extracytoplasmic stress. Central to this adaptive success is the mobilization of specialized genetic loci that maintain cellular homeostasis under physiological extremes, such as those encountered during the transition from the nutrient-rich intestinal lumen to the chemically restrictive and osmotically challenging environment of the urinary tract. Among these critical fitness determinants, the *bax* gene has been characterized for its role in mediating cellular tolerance to high urea concentrations and hypersalinity; while its specific enzymatic pathways are still being elucidated, it is increasingly recognized as a vital marker for cellular resilience under environmental extremity **(8, 9)**. The open reading frame (ORF) of the *bax* gene spans 825 nucleotides and encodes a protein composed of approximately 274 amino acid residues (BioCyc database). Based on extensive computational analysis, the promoter region of the *bax* gene is predicted to be under the rigorous regulatory control of the σ^{70} (sigma 70) factor **(8-10)**. *IbpA* and *IbpB* represent sHSPs of 16 kDa belonging to the A-family of HSPs. Their expression can surge several hundred-fold in response to heat shock. Under conditions of acute proteotoxic stress, these proteins prevent irreversible protein aggregation. Specifically, *IbpA* and *IbpB* modulate the biophysical properties of protein aggregates to facilitate more efficient degradation and refolding by the Hsp70–Hsp100 bichaperone system. A deficiency in *ibpA* and *ibpB* in *E. coli* can lead to attenuated bacterial growth and diminished cellular stability, or an excessive accumulation of HSPs at temperatures ranging from 45°C to 50°C. Upon heat shock, these proteins undergo polymerization to form functional heterooligomers. The *IbpA* and *IbpB* genes reside within an operon regulated by a sigma32-dependent promoter upstream of the *IbpA* ORF. The ORFs are separated by 112 base pairs, and both genes possess specific independent ribosome-binding sites **(7, 11-14)**.

Cold shock proteins (CSPs) constitute a highly conserved family of small nucleic acid-binding proteins (~7.4 kDa) characterized by the structural presence of cold shock domains (CSDs). In *E. coli*, nine *CSP* paralogs have been identified (*cspA* through *cspI*), which interact with single-stranded RNA and DNA. Under cryostress, mRNA molecules often form secondary structures that stabilize the transcript but inhibit translation. By increasing the intracellular concentration of *CSPs* during cold stress, the bacterium prevents the formation of these inhibitory structures, thereby facilitating the expression of cold-tolerance genes **(15-18)**. Because the formation of stable secondary structures through base pairing typically inhibits both transcription and translation, *CSPs* act to enhance gene expression by actively preventing the formation or removal of these structures. This physiological function of *CSPs* is frequently referred to as RNA chaperone activity. The cspH is a small, 70-amino acid protein within this family, sharing 77% sequence identity with cspF. While microarray analysis has demonstrated that *cspF* undergoes a regulated transcriptional response in the presence of urea, the regulatory frameworks governing both *cspF* and *cspH* remain poorly understood **(18-21)**.

Biofilm formation is a paramount indicator in the development of UTIs caused by uropathogenic *E. coli*. In these strains, biofilm architecture is a fundamental stage of the infection cycle; the capacity for surface attachment and subsequent biofilm maturation are key virulence traits associated with colonization and invasion of the host tissues **(7, 22)**. The *ibpAB* operon is a critical stress-response element that is significantly induced during the growth of *E. coli* biofilms; consequently, a deficiency in these proteins impairs biofilm development **(7, 8)**. Genes encoding heat and cold shock proteins exhibit high expression levels during the biofilm lifecycle; specifically, during biofilm maturation, the expression levels of the *cspH*, *ibpA*, and *ibpB* genes have been observed to increase by approximately 57-, 8.7-, and 34-fold, respectively **(23, 24)**. Systematically examining the frequency and functional roles of these specific genes in the context of biofilm formation and cellular tolerance to adverse environmental conditions, such as urea, allows us to

better elucidate the underlying pathogenicity of these bacteria. This study investigated the prevalence and transcriptomic expression of the *bax, IbpA, IbpB,* and *cspH* genes in *E. coli* strains isolated from matched urine and stool samples of UTI patients referred to Sanandaj health centers during the 2023-2024 period.

2. Materials and Methods

2.1 Study Design and Isolate Collection

In this cross-sectional investigation, 50 *E. coli* isolates (25 urinary and 25 fecal) were collected from patients diagnosed with UTIs in Sanandaj, Iran between 2023 and 2024 (fecal samples were taken from people with *E. coli* UTI). The sample size was based on previous studies and the lowest frequency of the studied genes, which was 80%, and considering 30% changes after the intervention with alpha of 0.05 and beta of 0.90, equal to 23 people for each group of urine and fecal samples, which ultimately resulted in 25 samples for each group in this study. Inclusion criteria for the uropathogenic cohort required a clinically and laboratory-confirmed UTI (defined as $>10^5$ CFU/mL in midstream urine), while exclusion criteria were strictly defined to eliminate confounding variables; these included patients currently undergoing antibiotic therapy or those with a history of chronic renal failure. Furthermore, patients who had received antimicrobial therapy within the preceding 14 days, those with indwelling urinary catheters, or individuals with known anatomical abnormalities of the urinary tract were excluded, as these factors could significantly alter the natural stress-response signatures of the isolates. Upon isolation, samples were inoculated into Brain Heart Infusion (BHI) broth and subsequently preserved in 18% glycerol at -80°C for future molecular analysis.

2.2 Microbiological and Biochemical Identification

The definitive identification of *E. coli* was achieved through a systematic battery of microbiological tests. These included Gram staining and cultivation on selective and differential media, such as Blood agar and Eosin Methylene Blue (EMB) agar. Furthermore, the biochemical identity of the isolates was confirmed through oxidase tests and other standard differential biochemical assays **(25)**.

2.3 Antimicrobial Susceptibility Testing

Bacterial susceptibility and resistance profiles were determined using the Kirby-Bauer disk diffusion method. The selection of antibiotics and the interpretation of results were based on the 2023 and 2024 Clinical and Laboratory Standards Institute (CLSI) guidelines **(26)**. *Escherichia coli ATCC 25922* was utilized as the quality control reference strain. The antibiotic panel consisted of amoxicillin (AMX) (25 µg), cefixime (CFM) (5 µg), norfloxacin (NOR) (10 µg), ofloxacin (OFX) (5 µg), nalidixic acid (NA) (30 µg), nitrofurantoin (FM) (300 µg), trimethoprim-sulfamethoxazole (SXT) (1.25/23.75 µg), and ciprofloxacin (CP) (5 µg). Following inoculation on Mueller-Hinton agar, disks (manufactured by Padtan Teb Co., Iran) were applied to the medium and incubated at 37°C for 24 hr. The resulting zones of inhibition were measured and categorized as sensitive, intermediate, or resistant according to the CLSI (2024) interpretive standards **(26)**.

2.4 Quantification of Biofilm Formation

Biofilm-forming capacity was quantified using a microtiter plate assay in BHI medium supplemented with 0.2% glucose. Briefly, 200 µL of bacterial suspension (comprising 10 µL of a 1.5×10^3 CFU/mL inoculum and 190 µL of BHI broth) was added in triplicate to the wells of a 96-well flat-bottom polystyrene plate. Sterile BHI broth was employed as a negative control. Following an 18-hr incubation at 37°C, the planktonic suspension was discarded, and the wells were washed three times with phosphate-buffered saline (PBS, pH 7.2). Biofilms were fixed with 220 µL of methanol and air-dried at room temperature. The wells were then stained with 0.1% crystal violet (220 µL) for 15 min, followed by three washes with PBS to remove excess dye. After drying, 220 µL of 30% acetic acid was added to each well to solubilize the bound crystal violet. The absorbance of the resulting solution was measured using an ELISA reader (BioTek Synergy, USA) at a wavelength of 595 nm **(20)**.

2.5 DNA Extraction and Multiplex PCR Analysis

Genomic DNA was extracted from the isolates using a commercial DNA extraction kit (Sinaclon Co., Iran) based on instructions. Oligonucleotide primers targeting the genes of interest were designed utilizing Primer3 Software. Targeted amplification was performed in a 20 µL reaction volume using PCR Master Mix (Sinaclon Co., Iran). The resulting amplicons were analyzed via electrophoresis on a 1.5% agarose gel and visualized using UV transillumination after staining with GelRed (or alternative nucleic acid stain) to confirm the presence of DNA bands. The PCR thermal cyclying included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation (95°C for 30 sec), annealing (58°C for 30 sec), and extension (72°C for 45 sec), with a final extension at 72°C for 5 min. A multiplex PCR strategy was optimized for the simultaneous detection of the *bax, ibpA, ibpB*, and *cspH* genes (Table 1).

2.6 RNA extraction and Transcriptional Analysis via Real-Time PCR

The total bacterial RNA extraction was carried out with Total RNA Extraction Kit from Sinaclon Co., following the manufacturer's instructions. RNA sample concentration and ratio were determined by measuring absorbance at 260 nm and 280 nm on a BioTek Synergy HTX Reader spectrophotometer (USA). For evaluating RNA purity and integrity, samples were run on a fresh 1% agarose gel stained with a safe dye, then electrophoresed in 1× TAE buffer at 100 V for 30 min.

The cDNA was synthesized using Sinaclon Co. reverse transcription kit. Quantitative Real-Time PCR (qPCR) was conducted using the Power SYBR Green PCR Master Mix (SinaClon, Iran) in a total reaction volume of 20 µL. For normalization, the 16S rRNA was used as a reference gene (relative expression). Finally, the normalized results were reported as the difference between groups using formula $2^{-\Delta\Delta Ct}$.

2.7 Statistical Analysis

Statistical analyses were performed using SPSS Software version 20. Mann-Whitney test was used to evaluate the relationship between the control and experimental groups, considering the nonparametric nature of the data; P-values less than 0.05 indicated statistical differences.

Table 1. Primers used in the study.

Gene		Primers (5'- 3')	Product length (bp)	References
bax	F	CTTGCTTGAACGCGTAGACA	163	Designed by Primer3
	R	CCCTTTCACTTTACCTGGCG		
ibpA	F	GAGTAATGGCGGCTACCCTC	205	Designed by Primer3
	R	GCGTTCAAAGTTGCGTTCAG		
ibpB	F	GATGCGTCAATGGATCGGTT	241	Designed by Primer3
	R	CATAAGCCCTTGATGCAGCC		
cspH	F	TTCATTATCCCCTCCGACGG	151	Designed by Primer3
	R	AGAGATAAACATTTGCCGCTGT		
16s rRNA	F	TCCAGGTGTAGCGGTGAAAT	236	Designed by Primer3
	R	TGAGTTTTAACCTTGCGGCC		

3. Results

3.1 Antimicrobial Susceptibility Test Results

Phenotypic characterization of antimicrobial susceptibility revealed that uropathogenic isolates exhibited the highest resistance frequencies against ampicillin (80%), nalidixic acid (72%), and trimethoprim-sulfamethoxazole (60%). In contrast, these urinary isolates demonstrated maximal susceptibility to nitrofurantoin (84%), norfloxacin (68%), and ofloxacin (68%).

Within the fecal isolate population, resistance was most pronounced against nalidixic acid (80%), ampicillin (76%), and trimethoprim-sulfamethoxazole (68%), whereas a universal susceptibility rate of 100% was observed for nitrofurantoin (Figure 1). The results also showed that while specific resistance frequencies (such as those for nalidixic acid and ampicillin) appeared higher in the fecal cohort, the overall antimicrobial resistance profiles between the urinary and fecal isolates did not reach statistical significance (P>0.05), suggesting a high degree of similarity in the resistome across these two anatomical niches within the same host.

3.2 Biofilm Formation Results

The capacity for biofilm formation was quantitatively assessed via microtiter plate assay across 25 urinary and 25 fecal specimens. Among the urinary isolates, the vast majority (96%) were characterized by a weak biofilm-forming phenotype, while a small subset (4%) exhibited moderate biofilm production. Similarly, within the fecal cohort, 92% of the isolates demonstrated weak biofilm formation, with 8% showing moderate capacity. Transcriptomic analysis confirmed significant upregulation across all target genes, including *cspH*. Comparative statistical analysis indicated that biofilm-forming potential did not differ significantly between the urinary and fecal *E. coli* isolates (P>0.05).

3.3 Molecular Detection Results

The genomic prevalence of *bax, ibpA, ibpB,* and *cspH* genes was found to be 100% across all evaluated urinary isolates. In the fecal population, *ibpA* gene was ubiquitous (100% frequency), whereas *bax, ibpB, and cspH* genes were each identified in 96% of the isolates (Table 2). The diagnostic specificity of the amplicons was confirmed through agarose gel electrophoresis, which revealed discrete bands corresponding to the anticipated molecular weights for each target locus.

Analysis of relative expression levels showed that the expression of all 4 genes was significantly higher in urinary isolates compared to fecal isolates, but it is noteworthy that there was only a significant correlation bet ween the relative expression levels of cspH (P=0.02) and ibpA (P=0.00) genes in urinary and fecal samples (Figure 2).

Table 2. Frequency of *bax*, *ibpA*, *ibpB* and *cspH* genes in urinary and fecal *E.coli* isolates.

Gene / Frequency	ibpA	ibpB	bax	Reverse Primer (5'–3')
Urine	100%	100%	100%	100%
Stool	100%	96%	96%	96%

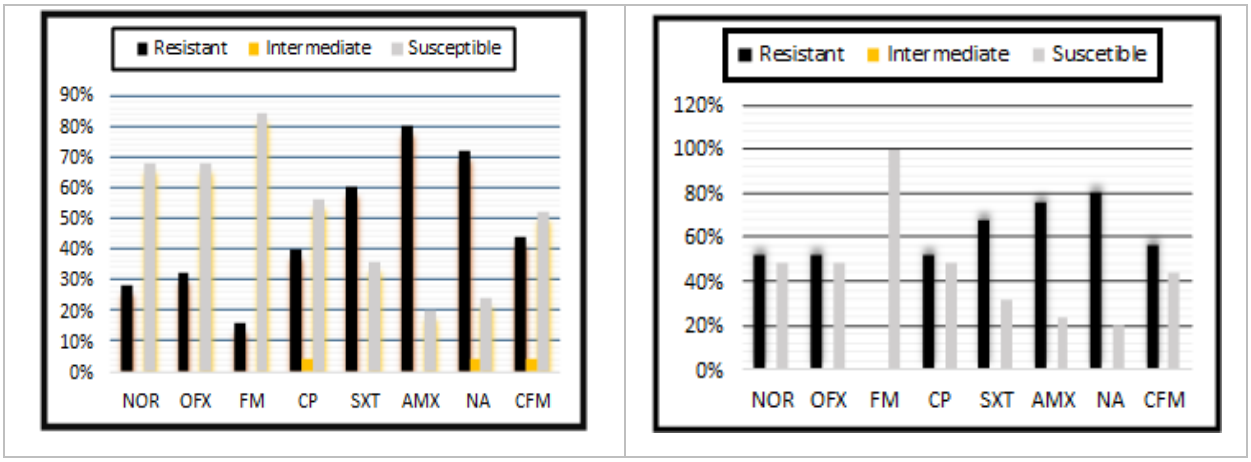
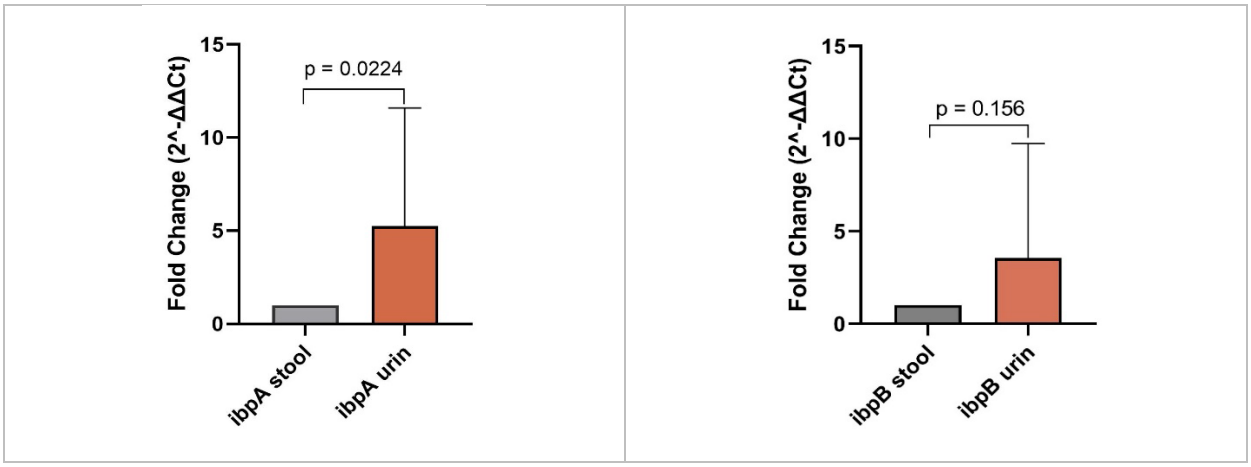


Figure 1. Antibiogram results. The charts show antibiogram results on urine samples (left) and stool samples (right) **(Prepared by Authors, 2026)**.



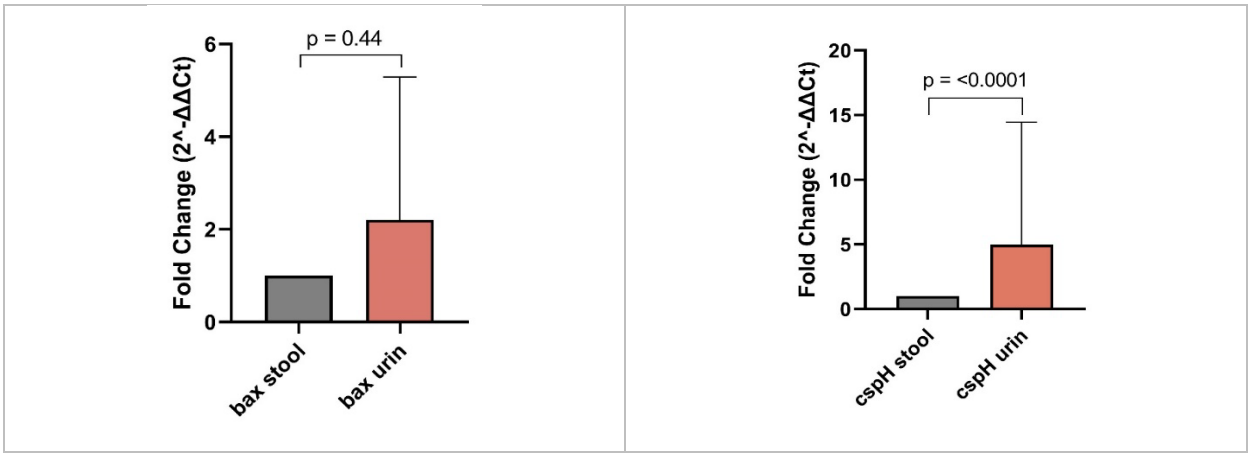


Figure 2. Relative expression levels of target genes assessed by Real-Time PCR. The charts show relative expression levels of *bax*, *ibpA*, *ibpB* and *cspH* genes in urinary and fecal isolates **(Prepared by Authors, 2026)**.

4. Discussion

The present study evaluated the antimicrobial resistance profiles, biofilm-forming capacity, gene prevalence, and gene expression of *bax, ibpA, ibpB*, and *cspH* in 50 *E. coli* isolates, comprised of 25 urinary and 25 fecal strains obtained from the same patients with urinary tract infections.

To our knowledge, this is one of the few studies to compare these genes in matched urinary and fecal isolates from the same host, which reduces the effect of patient-to-patient differences on the results and provides a more reliable comparison of niche-specific molecular adaptations **(27, 28)**. Antimicrobial susceptibility testing highlighted that although fecal isolates showed slightly higher resistance rates for some antibiotics, the overall difference between the two groups was not statistically significant (*P*>0.05). These resistance patterns are consistent with findings reported by other researchers. Nikzad et al **(3)** studied uropathogenic *E. coli* isolated from pregnant women in Karaj, Iran, and reported high resistance rates to ampicillin and other commonly used antibiotics, with nitrofurantoin remaining one of the most effective agents. The strong activity of nitrofurantoin observed in our study is in agreement with Biswas et al **(1)**, who reported that nitrofurantoin continues to be an effective first-line treatment for uncomplicated urinary tract infections in many regions. The similarity in resistance profiles between urinary and fecal isolates supports the view expressed by Vautrin et al **(6)**, who suggested that intestinal *E. coli* strains may serve as a reservoir for uropathogenic strains and share similar resistance characteristics.

Biofilm analysis showed that majority of isolates in both groups exhibited weak biofilm-forming capacity. No statistically significant difference was found in biofilm-forming capacity between the two groups (*P*>0.05). This finding is supported by results from other studies. Mahale et al **(22)** compared biofilm production between uropathogenic and commensal *E. coli* and found no significant difference in biofilm-forming capacity between the two groups, which is consistent with our results. Naziri et al **(29)** also studied biofilm formation in uropathogenic *E. coli* and pointed out that biofilm production is a complex process that depends on many different genetic factors beyond stress-response genes alone, including structural adhesins such as type 1 fimbriae and curli fibers regulated by csgD. Yan et al **(30)** confirmed that *csgD* plays a central role in biofilm architecture in *E. coli*, and that the regulation of this process is largely independent of chaperone-encoding genes such as *ibpA* and *ibpB* **(30-32)**. The lack of significant difference in biofilm formation between urinary and fecal isolates in our study, despite marked differences in gene expression, may indicate that isolates direct their metabolic resources toward individual cell survival rather than biofilm matrix production, as also suggested by Qasemi et al **(33)**, who reported that genetic determinants of biofilm formation are distinct from those mediating general stress tolerance in *E. coli*.

PCR analysis detected all four genes (*bax, ibpA, ibpB*, and *cspH*) in 100% of urinary isolates. In fecal isolates, *ibpA* was found in 100% of strains, while *bax*, *ibpB*, and *cspH* were each detected in 96% of strains. This high prevalence in both groups suggests that these genes are part of the core genome of *E. coli* and provide basic survival advantages in different environments. Tantoso et al **(34)** analyzed the core genome of *E. coli* and reported that stress-associated genes are broadly conserved among *E. coli* isolates regardless of their origin, which is consistent with high prevalence we observed in both urinary and fecal samples **(34, 35)**. The slightly lower frequency of *bax, ibpB,* and *cspH* in

4% of fecal isolates may reflect natural genetic variation within the gut commensal population.

The most important finding of this study is related to gene expression. Quantitative real-time PCR showed that all four genes were expressed at significantly higher levels in urinary isolates compared to their matched fecal counterparts (P<0.05 for all genes). This consistent pattern across *bax, ibpA, ibpB*, and *cspH* indicates a global upregulation of stress-response machinery in uropathogenic strains **(36)**. However, examining the correlation between expression levels in paired urinary and fecal samples from the same patients, a significant positive correlation was observed only for *cspH* (P=0.02) and *ibpA* (P=0.00). This suggests that while *bax* and *ibpB* are also significantly upregulated in urine, their expression levels are not directly coupled between the two anatomical sites at the individual patient level **(37)**.

The elevated expression of ibpA and ibpB in urinary isolates is biologically plausible given the unique physicochemical stressors present in the bladder. These genes encode ssHSPs that function as molecular chaperones, preventing irreversible protein aggregation under proteotoxic conditions **(14, 38, 39)**. Miwa et al **(7)** showed that *IbpA* acts as an aggregation sensor in *E. coli* and can regulate its own expression at the post-transcriptional level, indicating that this protein is highly responsive to environmental stress. Piróg et al **(40)** demonstrated that *IbpA* and *IbpB* form a functional heterodimer that provides protection against protein aggregation under stress conditions. The urinary environment exposes *E. coli* to high urea concentrations, extreme osmolarity fluctuations, and nutrient limitation, all of which can induce protein misfolding and denaturation. Sun et al **(23)** reported that *ibpA* expression in *E. coli* is sensitive to sub-inhibitory antibiotic concentrations, further supporting the role of this gene in responding to environmental stress **(23, 27)**. The heightened expression of these chaperones in uropathogenic isolates likely reflects an adaptive response to the proteotoxic stress encountered during urinary tract colonization. Similarly, the increased expression of *cspH* in urinary isolates, which also showed significant correlation between paired samples, warrants consideration. CSPs act as RNA chaperones that facilitate mRNA stability and translation under fluctuating environmental conditions by preventing the formation of inhibitory secondary structures **(20)**. Yair et al **(15)** showed that cold shock proteins; CspC and CspE target specific cellular RNAs and contribute to the survival of septicemic *E. coli* in hostile environments. While originally characterized in the context of cold stress, members of the Csp family have been increasingly recognized for their roles in adaptation to various stressors, including osmotic shock and oxidative stress **(13)**.

The significant upregulation and correlation of *cspH* in our urinary isolates suggests that this RNA chaperone facilitates rapid post-transcriptional adaptation to the stringent conditions of the urinary tract. The elevated expression of *bax* in urinary isolates, while not showing significant correlation between paired samples, follows the same directional trend observed for the other genes **(27, 41)**. Kim et al **(8)** identified *bax* as a gene associated with tolerance to high urea concentrations and osmotic stress in *E. coli*, noting that its expression may serve as an indicator of cellular stress response capacity. Although its precise molecular function remains incompletely characterized, the consistent upregulation of this gene in urinary isolates supports a model in which uropathogenic *E. coli* maintain a transcriptionally poised state to cope with multiple stressors encountered during urinary tract colonization.

Our finding of higher stress gene expression in urinary isolates contrasts with some previous reports that suggested intestinal strains might exhibit enhanced stress tolerance. However, those studies did not employ a paired-sample design, which may explain the discrepancy. The paired methodology of our study, comparing isolates from the same patients, provides greater confidence that the observed differences reflect true niche-specific adaptations rather than inter-strain genetic variation. This methodological strength has been emphasized in recent work demonstrating that paired sampling reveals adaptations not detectable in unpaired comparisons **(27)**.

This study has several limitations. First, although the paired-sample design strengthens internal validity, the sample size of 50 isolates from a single geographic region may limit broader generalization. Second, gene expression analysis was restricted to four selected stress-related genes rather than employing a whole-transcriptome approach. Third, transcript levels were measured at a single time point under standardized laboratory conditions, which may not fully capture dynamic expression patterns during infection. Fourth, the absence of protein-level data means we cannot confirm whether increased transcription translates to functional changes in chaperone activity. Future studies with larger sample sizes, whole-transcriptome analysis, and functional assays are recommended to confirm and expand these findings.

5. Conclusion

This investigation identifies a significant differential expression of the *bax, ibpA, ibpB*, and *cspH* genes between fecal and urinary *E. coli* isolates, whereas

biofilm-forming capacity remained consistent across sources. In summary, the present investigation elucidates a pronounced divergence in the transcriptomic profiles of *E. coli* isolates across distinct host anatomical niches. Our data demonstrate that urinary isolates exhibit significantly elevated expression levels of the *bax, ibpA, ibpB*, and *cspH* genes compared to fecal isolates. Elucidating the specialized role of these stress-response genes not only enhances our ability to accurately identify highly resilient pathogenic strains, but also facilitates the potential development of targeted, niche-specific therapeutic interventions. Such advancements are critical for optimizing the management of urinary tract infections and mitigating the global challenge of antimicrobial resistance.

6. Declarations

6.1 Acknowledgment

We would like to thank Kurdistan University of Medical Sciences and Research Deputy of Kurdistan University of Medical Sciences and Cellular & Molecular Research Center.

6.2 Ethical Considerations

This study was approved by the Ethics Committee of Kurdistan University of Medical Sciences, Kurdistan, Iran (Ethic approval code: IR.MUK.REC.1403.180). All research was performed following relevant guidelines according to the Declaration of Helsinki (https://www.wma.net/policies-post/wma-declaration-of-helsinki/). Written informed consent was obtained from all participants.

6.3 Authors' Contributions

AA designed and supervised the study. EA was responsible for data collection and doing experiments. RT performed data interpretation. KA and AT analyzed the statistical results of the study. AT wrote and edited the original draft. All authors approved the final version of the manuscript.

6.4 Conflict of Interests

This study does not include any conflict of interest for the authors.

6.5 Financial Support and Sponsorship

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6.6 Using Artificial Intelligence Tools (AI Tools)

All authors declare that there is no use of AI Tools in this study, including the writing of this manuscript.

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