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Research Article

Characterization of Virulence Factors and Two-Component Regulatory System Genes in Uropathogenic *Escherichia coli* Isolates from Young Women with Urinary Tract Infections

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Abstract:

Background: Urinary tract infections are one of the most common bacterial infections, caused mainly by *Escherichia coli*. Uropathogenic *Escherichia coli* (UPEC) possess virulence factors that can worsen infections, partly regulated by the BarA/UvrY two-component system, which controls genes associated with adhesion, motility, and biofilm formation through small RNAs and carbon metabolism regulators.

Objectives: To determine the pathogenicity and some genotype features of *Escherichia coli* from urine specimens collected from young women patients and assess their antibiotic resistance.

Methods: Between September and November 2024, 200 urine samples were collected from young women aged 18 to 30 who had urinary tract infections, from a number of major hospitals in Baghdad. Identification of bacteria was confirmed using differential and chromogenic media, followed by biochemical confirmation with the VITEK 2 Compact System. Antimicrobial susceptibility was tested using the VITEK 2 Compact System. The ability of *Escherichia coli* to form biofilm, swarm, and produce hemolysin was evaluated. Conventional PCR methods were used to detect the prevalence of the two-component system genes in *Escherichia coli*.

Results: The isolation rate of *Escherichia coli* from urine samples was 44.5%, yielding 89 isolates. High resistance to cefotaxime (82.5%) was observed, while all isolates were fully sensitive to meropenem, amikacin, and tigecycline. A substantial 85% of isolates produced β-lactamase, and 2.5% displayed both β-lactamase production and polypeptide resistance mechanisms. Production of hemolysin was seen in 48.3% of isolates, while 34.8% showed swarming motility. Biofilm formation varied, with 13.4% being moderate producers, 29% being weak producers, and 57.3% not producing. Genotypic analysis of 16 representative isolates revealed the presence of the *BarA* and *UvrY* genes.

Conclusion: This study highlighted the critical role of UPEC in urinary tract infections among young women, marked by significant cefotaxime resistance. Despite the uniform presence of the *BarA* and *UvrY* genes, isolates displayed diverse virulence profiles, underscoring the complex interplay between resistance and pathogenicity.

Keywords: Antibiotic resistance; *BarA-UvrY*; PCR; *Escherichia coli*; UPEC;Virulence factors.

Introduction:

Urinary tract infections (UTIs) are a common bacterial illness that impacts women, presenting a 50% lifetime risk. Recurrent urinary tract infections every six months or three years negatively impact the quality of life with their burden of misery, pain, and systemic illness (1). Approximately 400 million UTIs are reported annually worldwide, resulting in over 200,000 fatalities, establishing UTIs as one of the most prevalent bacterial diseases and significant contributors to antibiotic consumption (2). Uropathogenic *Escherichia coli* (UPEC) is recognized as the leading cause of UTI, accounting for approximately 85% of uncomplicated cases (3). The virulence factor of UPEC strains encompasses adhesive factors, toxins, iron uptake systems, and other factors (4). Adhesions allow bacteria to withstand urine and establish intracellular bacterial communities. These communities circumvent Toll-like receptor 4 ejection, multiply within the uroepithelium, and infect adjacent cells, leading to persistence in the urinary system (5). UPEC, which readily forms biofilms, is more persistent and challenging to treat due to the biofilm's inherent capacity to protect cells against antibiotic treatment (6). The first interaction with host cells, mediated by surface adhesions, which are important virulence factors, is an important means by which the pathogen achieves the ability to generate a biofilm. In addition, the transfer of virulence factors and antibiotic

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resistance genes may happen more easily among bacteria in a biofilm (7). The surface motility of *Escherichia coli* (*E.coli*), termed swarming, is defined by its dependence on flagella. These swarming cells exhibit the expression of virulence genes, an upregulation of supramolecular structures, and increased resistance to phagocytosis and drugs. Flagella are essential for swarming motility, and interestingly, pili also play a role in this process (8). It is widely believed that 95% of such infections are ascending infections, indicating that the infection starts with colonization of the periurethral region, subsequently migrating up the urethra into the bladder, and potentially extending to the ureters and kidneys (9). Numerous UPEC isolates have been identified as secreting soluble toxins, notably α- hemolysin (HlyA), and a significant association exists between HlyA production by UPEC and the severity of UTIs. α-hemolysin generated by UPEC has been documented to induce cellular toxicity and urothelial injury (10). Genome sequencing revealed ~ 30 two-component systems in *E.coli*; four *E.coli* sensor proteins, ArcB, EvgS, TorS, and BarA, contain D1 and H2 domains along with H1. The BarA forms a two-component system with the UvrY response regulator protein. UvrY activates *csrB/csrC* RNAs that sequester CsrA, preventing mRNA repression. The Csr system strongly regulates carbon metabolism. The loss of the *BarA* or *UvrY* genes in *E.coli* greatly reduces the bacterial competitive growth potential in relation to the carbon source of the medium. Mutations in the Csr system of *E.coli* lead to several phenotypic changes, such as alterations in motility, adhesion, and biofilm production (11).

This study aimed to uncover the predominant bacterial species responsible for UTIs and evaluate their resistance to frequently prescribed antibiotics. Moreover, it sought to investigate the capabilities of *E.coli* isolates in forming biofilms, swarming, and exhibiting hemolytic activity and its association with the *BarA/UvrY* genes. By understanding these dynamics, this study has the potential to inform better treatment strategies and combat antibiotic resistance in UTIs.

Subjects and methods:

Samples collection: A total of 200 urine samples of young women aged between 18 and 30 years diagnosed with UTIs were collected utilizing transport swabs from Al-Karama Hospital, Al-Yarmouk Teaching Hospital, Al-Shaheed Ghazi Al-Hariri Hospital, and Teaching Laboratories in the Medical City during the period from September to November 2024. Women who had used antibiotics within the past two weeks, as well as those who underwent medical treatment or had underlying medical conditions, were excluded. The samples were inoculated onto MacConkey agar, Eosin Methylene Blue (EMB) agar, HiCrome™ UTI agar, and MUG Nutrient agar in a laboratory environment under aseptic conditions. The samples were thereafter incubated at 37°C for 24 hours (12-15). Ethical approval for the study was granted by the Ethics Committee of Baghdad College of Science (Approval No. CSEC/1124/011).

Identification of Bacterial Isolates and Evaluation of Antibiotic Susceptibility Patterns Using the VITEK 2 Compact System: Bacterial strains were identified with the VITEK2 compact system following the manufacturer's guidelines. To establish a turbidity of 0.5, we introduced several bacterial colonies into glass vials according to the McFarland standard. Using negative pressure, the bacterial suspension was transferred into a cassette, and the sample was loaded into the VITEK-2 compact system. The cassette underwent incubation for 12 hours to complete the antimicrobial susceptibility testing and biochemical reaction. The data were analyzed using the custom software designed for the VITEK-2 compact system to identify various species and strains of bacteria, in addition to determining susceptibility results.

Hemolysis assay: Hemolysis testing was performed on blood agar with 5% sheep blood. Incubation was performed overnight at 37°C, followed by storage at 4°C for 16–24 hours to improve hemolysis detection on the plates with colonies. Hemolysin was detected by complete lysis of erythrocytes around the bacterial colonies (16).

Biofilm formation assay: Biofilm formation of *E.coli* isolates was assessed by the two methods: Congo red agar (CRA) growth and microtiter plate assay. All isolates were tested for their biofilm formation by CRA method, which is a qualitative test. Strains were streaked onto Congo red agar and grown for 24 h at 37°C. Biofilm producers would form black colonies (with a dry, crystalloid morphology) which is indicative of exopolysaccharide (EPS) production that contributes to the structural integrity and adherence capacity of the biofilm matrix, whereas non-biofilm-forming isolates would form pink and smooth colonies (17). Quantitative analysis was performed using the standard 96-well U-shape microtiter plate assay. Bacterial suspensions were cultured in tryptic soy broth for 24 hours at 37°C. Then, 100 µl of bacterial suspension adjusted at 0.5 McFarland were mixed with tryptic soy broth with 1% of sucrose. Then 200 µl of the mixture were added in triplicate to microtiter wells and incubated for 24 hours at 37°C, and after followed by the aspiration of non-adherent cells, the wells were washed and fixed with 200 µl of methanol. After incubation, the wells were stained with 0.1% crystal violet for 15 minutes and then washed with 200 µl of ethanol for 10 minutes. The biomass of biofilm was determined as the optical density (OD) at 630 nm with a microplate reader. The bacterial biofilm-producing power is determined as strong, moderate, or weak based on OD cut off = OD

average negative control group + 3 x standard deviation (SD) negative control group's ODs. The biofilm intensity was divided into four states:1) OD ≤ ODc= non-biofilm-former. 2) ODc < OD ≤ 2 × ODc = weak biofilm-former. 3) 2 × ODc < OD ≤ 4 × ODc = moderate biofilm-former. 4) OD >4 × ODc = strong biofilm-former (18), (19).

Swarming assay: For this experiment, modified Swarming agar was utilized to assess bacterial motility, following the identical protocol outlined in study (20).

DNA extraction: UPEC DNA was extracted via the Presto small gDNA bacteria kit (Geneaid Biotech Ltd, Taiwan), thereafter evaluated by using gel electrophoresis to detect the presence of genes.

Molecular detection of *BarA* and *UvrY* genes by single plex PCR: This study utilized the species-specific primers BarA and UvrY for UPEC. The primers were provided by Macrogen in lyophilized form, subsequently dissolved in nuclease-free water to create the stock solution, and then diluted to get the working concentration. Subsequently, single-plex PCR was employed to detect these genes in UPEC isolates obtained from patients with UTIs. The *BarA* and *UvrY* genes were identified using primers specifically designed in this study, as detailed in the accompanying Table 1. These primers were created using the Primer 3 program. The *BarA* product size was 279 bp, amplifying a segment of this gene, whereas the *UvrY* product size was 125 bp, amplifying a segment of the *UvrY* gene. The PCR conditions for *BarA* and *UvrY* are summarized as follows: 1) An initial denaturation at 95°C for 3 minutes (1 cycle), 2) Denaturation at 95°C for 30 Seconds, 3) Annealing at 55°C for 30 Seconds, 4) Extension at 68°C for 1 minutes, and 5) Final extension at 68°C for 5 minutes (Steps 2–4 were followed for 35 cycles)

.

Table 1: Primer Sequences Used for Amplification of *BarA* and *UvrY* Genes

Target gene	Forward 5'⟶3'	Reverse 5'⟶3'	References
BarA	CAGAAATAATCGGCGTGCGG	GTGCCAGCATTATTGAGCCG	Designed in the current study
UrvY	AAAAGTCGTCGGTGAGGCAT	TTTTACGCGTCGCCTCAAGA	

Statistical Analysis: The Statistical Package for the Social Sciences (SPSS) version 2019 was used for data analysis. Cochran's Q test and Chi-square / Goodness-of-Fit test were used to determine the significance of associations between categorical variables. Statistical significance was assessed was determined and tested at two probability levels ($P \leq 0.01$and $P \leq 0.05$).

Results:

Isolation and characterization: Out of 200 urine specimens from young women there were 89 isolates of *E.coli*, with a prevalence of 44.5%. *E.coli* was carefully confirmed and verified using specific and differential culture media, which have high specificity. Colonies grew pink to red in color on MacConkey agar, consistent with lactose fermentation. EMB agar showed colonies with a green metallic sheen, characteristic of UPEC strains. The identification of *E.coli* was probable by the formation of distinct purple colonies on HiCrome™ UTI Agar, and the presence of β-glucuronidase activity was established by the green-blue fluorescence under ultraviolet light on MUG Nutrient Agar. These findings were visually documented, as shown in Figure 1, revealing the probability of *E.coli* presence. Forty UPEC isolates were obtained from a total of eighty-nine confirmed isolates. The biochemically verified isolates were then processed with the VITEK-2 Compact System, where it was confirmed that all 40 were *E.coli*, with one sample identified as *E.coli* O157.

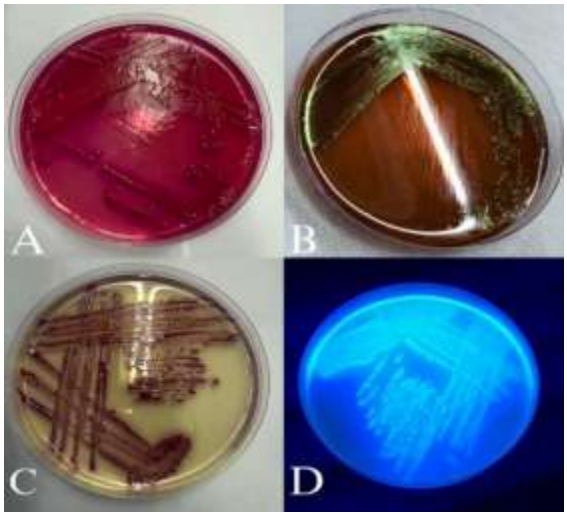

Figure 1: Differential and confirmatory media for identification of *E.coli*. A) MacConkey Agar, B) EMB Agar, C) HiCrome™ UTI Agar, D) MUG Nutrient Agar.

Antimicrobial susceptibility test using VITEK 2 compact system: Resistance to cefotaxime was the most common (82.5%), followed by 25% for ceftazidime, 15% for ciprofloxacin, 12.5% for trimethoprim, 10% for cefepime, 5% for ampicillin, 2.5% for colistin, and 2.5% for piperacillin. The antibiotics with 100% sensitivity included meropenem, amikacin, and tigecycline. Additionally, ceftazidime-avibactam and ceftolozane, along with imipenem, demonstrated sensitivity, as did gentamicin and piperacillin, which showed specified yields of 97.5%.

gentamicin, in conjunction with trimethoprim and ampicillin exhibited a sensitivity of 82.5%. piperacillin demonstrated sensitivity, with cefepime at 22.5%, ceftazidime at 27.5%, cefotaxime at 17.5%, and ciprofloxacin at 20%. Additionally, certain antibiotics exhibited varying levels of sensitivity, with colistin demonstrating an efficacy of 97.5%. This was followed by ciprofloxacin at 65%, ceftazidime at 47.5%, and ampicillin at 12.5%. Statistical analysis revealed that the differences in antibiotics susceptibility were highly significant ($P \leq 0.01$), as shown in Table 2. This illustrated a differential sensitivity and resistance based on bacterial isolates. Most bacterial isolates demonstrated the presence of the extended-spectrum β-lactamase enzymes (ESBL), with 85% producing β-lactamases and 2.5% exhibiting the enzymes that β-lactamase production in combination with polypeptide resistance mechanisms.

Table 2: Antimicrobial Susceptibility Profiles of Clinical Isolates Across Multiple Drug Classes Using the VITEK 2 Compact System

Antibiotics	Resistant	Intermediate	Sensitive	P-value
Ampicillin	2 (5%)	5 (12.5)	33 (82.5%)	0.0001
Piperacillin	1 (2.5%)	0 (0%)	39 (97.5%)	0.0001
Cefotaxime	33 (82.5%)	0 (0%)	7 (17.5%)	0.0001
Ceftazidime	10 (25.0%)	19 (47.5%)	11 (27.5%)	0.0001
Ceftazidime- Avibactam	0 (0%)	0 (0%)	40 (100%)	0.0001
Ceftolozane	0 (0%)	0 (0%)	40 (100.0%)	0.0001
Cefepime	4 (10.0%)	27 (67.5%)	9 (22.5%)	0.0001
Imipenem	0 (0%)	0 (0%)	40 (100.0%)	0.0001
Meropenem	0 (0%)	0 (0%)	40 (100.0%)	0.0001
Amikacin	0 (0%)	0 (0%)	40 (100.0%)	0.0001
Gentamicin	1 (2.5%)	0 (0%)	39 (97.5%)	0.0001
Ciprofloxacin	6 (15.0%)	26 (65.0%)	8 (20.0%)	0.0001
Tigecycline	0 (0%)	0 (0%)	40 (100.0%)	0.0001
Colistin	1 (2.5%)	39 (97.5%)	0 (0%)	0.0001
Trimethoprim	5 (12.5%)	0 (0%)	35 (87.5%)	0.0001
P-value	0.0001	0.0001	0.0001	---

Detection of Hemolysin Production: Hemolysin production in *E.coli* isolates was assessed using blood agar assays to determine hemolytic activity. Out of 89 *E.coli* cultures identified, 43 (48.3%) demonstrated distinct hemolytic activity, suggesting the production of hemolysin, while 46 (51.7%) were non-hemolytic. The difference between hemolysin-producing and non-producing isolates was not statistically significant ($P > 0.05$), Figure 2 and Table 3.

Figure 2: Phenotypic Evaluation of Hemolysin production on Blood Agar: A) Non-hemolytic *E.coli* isolate (intact erythrocytes with no visible hemolysis surrounding the bacterial colonies), and B) Hemolytic *E.coli* isolate (clear zones of hemolysis around the colonies)

Table 3: Prevalence of Hemolytic Activity, EPS Production, and Swarming Ability Among UPEC Isolates.

Virulence factors	Producer	Non- producer	P-value
Hemolytic activity	43 (48.3%)	46 (51.7%)	0.751 NS
EPS Production (CRA)	62 (69.7%)	27 (30.3%)	0.0002
Swarming ability	31 (34.8%)	58 (65.2%)	0.0042

Biofilm formation by *E.coli*: Biofilm formation was observed in 62 of the 89 isolates with varying degrees of biofilm formation under the same experimental conditions. Sixty-two (69.7%) isolates showed the typical dry, black, crystalloid colonies on CRA, suggesting EPS production, whereas 27 (30.33%) gave rise to pink colonies with a smooth appearance, indicating that they were not able to synthesize the EPS, as demonstrated in Figure 3 and Table 3. The difference

in the proportion between EPS-producing and non-producing isolates was statistically significant ($P \leq 0.01$). The 62 EPS-positive culture isolates were further evaluated by the 96-well microtiter plate method, wherein 12 (19.4%) were found to be moderate biofilm producers, 26 (41.8%) weak, and 24 (38.7%) non-producers. These distribution patterns also demonstrated statistically significant differences among the three categories ($P \leq 0.05$), as illustrated in Figure 3 and Table 4.

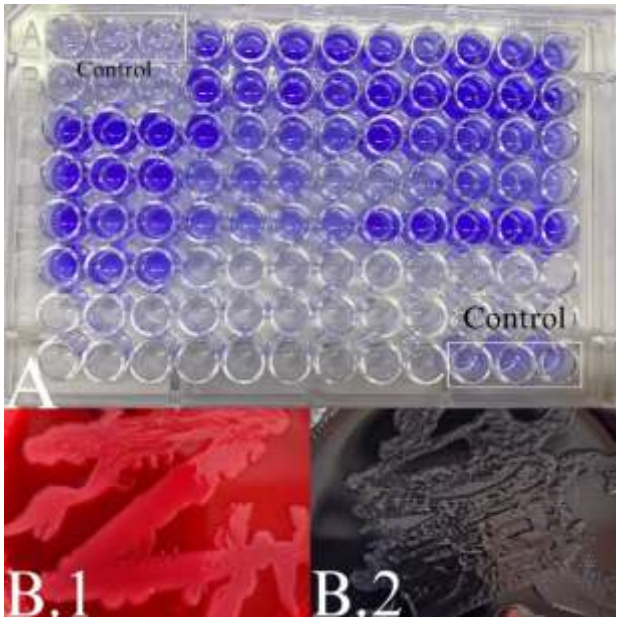

Figure 3: Assessment of Biofilm Formation Using Quantitative and Qualitative Approaches: (A) Quantitative using the 96-well U-bottom microtiter plate assay. (B) Qualitative CRA. 1) Non-biofilm-forming strains (pink colonies with smooth or slightly crystalline surfaces). 2) Biofilm-producing isolates (black colonies with a dry, crystalline morphology).

Table 4: Distribution of Biofilm Intensity Levels in UPEC Isolate

Biofilm formation	Biofilm intensity
Moderate	12 (19.4%)
Weak	26 (41.9%)
No producer	24 (38.7%)
Total	62 (100.0%)
P-value	0.0498

Swarming motility by *E.coli*: Of the 89 isolates, 31 (34.8%) were able to swarm on swarming medium with differed form, Figure 4 and Table 3.

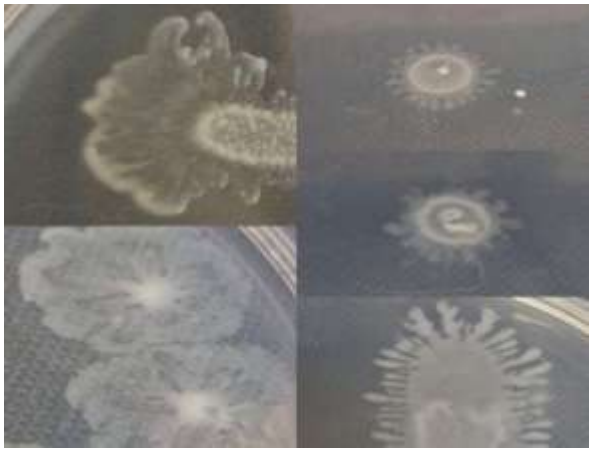

Figure 4: Swarming patterns of *E.coli* on semi-solid agar following inoculation onto plates after incubation at 37°C for 18–24 hours.

Detection of *BarA* and *UvrY* gene by PCR technique: Based on the virulence factors (swarming motility, biofilm formation, hemolysin production), and the VITEK-based antimicrobial susceptibility test results, 16 isolates of 89 were selected to undergo further analysis by conventional PCR, the *BarA-UvrY* genes in all isolates as demonstrated in Figure 5 and Table 5.

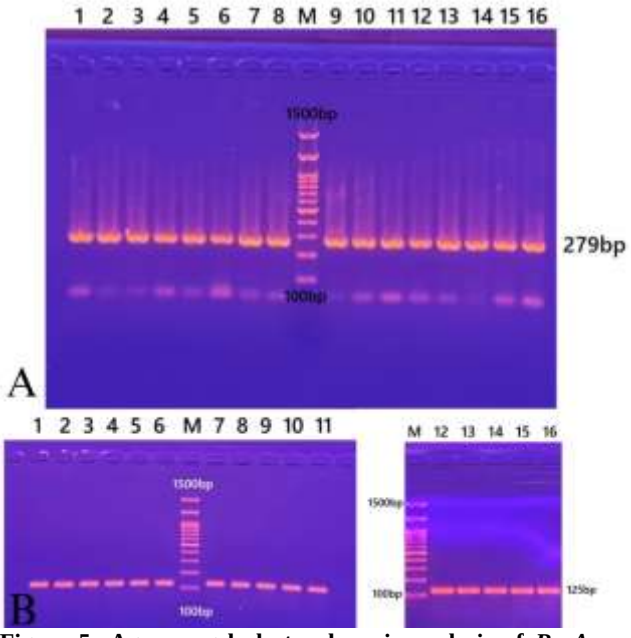

Figure 5: Agarose gel electrophoresis analysis of *BarA* and *UvrY* gene amplification: A) Electrophoretic separation performed on a 1.5% agarose gel in 1× TBE buffer at 150 V for 30 minutes. A distinct band at 279 bp confirms the successful amplification of the *BarA* gene. B) Amplification of the *UvrY* gene produced a clear band at 125 bp, corresponding to the expected amplicon size and verifying the specificity of the PCR reaction.

Table 5: Phenotypic and Genotypic Characterization of UPEC Isolates: Selected Virulence Traits and ESBL Production

Isolates	*BarA-UvrY*	Biofilm Intensity (Microtiter plate)	Swarming ability	Hemolysin Production	ESBL Production
1	+	Moderate	-	+	+
2	+	No producer	-	+	+
3	+	No producer	+	+	+
4	+	No producer	+	+	+
5	+	Moderate	+	+	+
6	+	Moderate	-	-	+
7	+	Moderate	-	-	+
8	+	Weak	+	+	+
9	+	Moderate	+	-	+
10	+	No producer	+	+	+
11	+	Moderate	+	+	+
12	+	No producer	+	-	+
13	+	Moderate	+	-	+
14	+	No producer	+	+	+
15	+	Moderate	-	+	+
16	+	Weak	-	-	+

Discussion:

This study contributes further phenotypic and genotypic characterization of *E.coli* isolates from the clinical samples, emphasizing the multifactorial virulence and antibiotic resistance dynamics for the organism. Continued clinical relevance of *E.coli*, including the predominance of UPEC as a urogenital pathogen in sexually active women, is confirmed by a 44.5% *E. coli* isolation rate among the full cohort, including a singular O157 strain. In contrast, a previous study conducted in Iraq (21) showed isolation of 57 out of 120 culture-positive specimens of *E.coli*, which corresponds to 47.5% of the overall positive cultures obtained from the same number of 200 clinical samples. The observed 3% variation in isolation rates (44.5% vs. 47.5%) may be attributable to normal sampling variation. These include differences between patient populations (age, sex, and underlying medical conditions) that are known to modify the prevalence and distribution of UPEC, the most common pathogen in reproductive-age people, and the leading edge can be explained by physiological susceptibility combined with more frequent sexual activity, as previously noted in a study from China (22). In addition, regional differences in antimicrobial utilization and temporal trends in antimicrobial therapy significantly affect the selection pressure in microbial communities. All of these may appear to evolve, leading to a significant change in uropathogen dominance over time, either to promote or inhibit species of bacteria.

Most bacterial isolates demonstrated the presence of the β-lactamase enzyme and significant resistance to cefotaxime. There were important signs of antibiotic resistance concerning our bacterial isolates that corresponded with previous studies, whereas some did not. Comparatively, reported *E.coli* resistance rates in previous studies vary. For instance, a study conducted in Iraq by Al-Jubouri *et al.* (23) found that resistance to ciprofloxacin was 47%, cefepime 72%, ampicillin 100%, and gentamicin 67%, revealing systematically higher resistance rates than those of our study, except for ampicillin, which was much lower in our cases. in another local study by Marwa *et al.* (24), piperacillin and cefotaxime resistance was reported at 78.4% and ceftazidime at 76.6%, suggesting increased resistance rates in comparison to our findings, which highlighted only 25% ceftazidime resistance. Other studies also demonstrated variability in resistance patterns, a study by Younus *et al.*. from the same region (25) which continues to emphasize gaps, especially in the resistance levels for trimethoprim and gentamicin, which were reported at 96.8% and 82.3%. On the contrary, our data showed much lower rates of resistance for these antibiotics, with trimethoprim resistant at 12.5% and gentamicin resistance at 82.5%. In a study conducted by AbdulHameed *et al.* (26), ciprofloxacin resistance was recorded at 59.7%, which is higher than the 15% resistance found in our study. This points to a significant difference in resistance patterns among various bacterial isolates and locations. The study by Al-Azawi *et al.* (27) shows an amikacin resistance rate of 18.8%, which differs from ours, where amikacin showed full susceptibility. There is also gentamicin resistance from the study by Abbood (28), which, at 28%, is lower than the 67% reported as resistance in the study by Al-Jubouri *et al.* (23) but is more consistent with ours, where gentamicin was effective at 82.5%. The study by Arif *et al.* in Pakistan (29) mentions colistin resistance in 19.9% of isolates, which is significantly higher than 2.5% from our study, perhaps suggestive of differences in resistance patterns due to geography, clinical setting, or even study design. Interestingly, the study by Zakia *et al.* in Indonesia (30), found that 89% of *E.coli* isolates were ESBL producers, and this was comparable to our findings, where 85% of the bacterial isolates were found to be β-

lactamase producers. There are differences in the analysis of resistance phenotypes from similar studies that show a high degree of variability by location and time over the last decade, suggesting that local antimicrobial stewardship, as well as differences in the underlying populations, has a significant effect.

In this study, 48.3% of isolates exhibited hemolytic activity, reinforcing hemolysin as a major virulence factor in UPEC disease pathogenesis. Our findings are consistent with previously published reports, including a study conducted in South Korea (31), which indicate that between 40% and 58% of UPEC isolates can produce hemolysin. The percentage of hemolysin-positive organisms we identified is similar to results from other research, reinforcing the idea that hemolysin is a significant virulence factor in clinical *E.coli* isolates.

The current investigation revealed that EPS production on CAR was noted in 69.7% of *E.coli* isolates, indicating a significant possibility of biofilm formation. This outcome aligns with the results of a study conducted by Hussein *et al.* in Iraq (17), which found a comparable prevalence of 71%. The ability to visually identify EPS-producing colonies through darkly pigmented or black colony morphology makes CRA a useful phenotypic assay for detecting biofilm-forming ability. CRA has a high concordance rate with a previous study, demonstrating its value as a rapid, effective, and practical screening method for biofilm production, especially in clinical and environmental microbiology. The findings here do not align with a previous study conducted by Saied *et al.* (32), which highlighted a notable difference; this investigation failed to show the ability for strong biofilm formation. Additionally, the distribution of moderate and weak biofilm producers has shifted: this study identified 41.8% as weak producers, compared to 58% in earlier research, while moderate biofilm producers accounted for 19.35% here, down from 22% previously. The differences in biofilm thickness can be linked to various factors, such as the unique abilities of isolates to develop biofilms. The capacity is affected by various surface determinants, including the type of fimbriae 1, flagella, and F-pilus (33).

Demonstrated swarming motility by 34.8% of isolates, supports expression of motility-associated virulence traits, and possible roles in colonization and dissemination. The swarming incidence of 34.4% observed in the current study is higher than that of a previous study conducted by Kaittan *et al.* (20), which found an incidence of 27.3%. It is likely that several biological and experimental differences explain the differences in the swarming percentages of our study compared to the previous study, despite the fact that we used similar materials and experimental conditions. It could be due to differences in bacterial strain adaptation, fluctuations in humidity or temperature, or little deviations in media preparation. In addition, the density of the bacterial population and their physiological states at the time of inoculation may play a role in swarming efficacy and thus account for the disparity seen in the literature. The bacteria behaved as typical chemotaxis organisms, moving toward nutrient through the agar's pores to the extent possible, utilizing local resources to support their expansion (34).

Conventional PCR analysis confirmed the presence of the *BarA-UvrY* genes in all 16 selected UPEC isolates. This observation is in strong agreement with the findings of a previous study conducted in in the United States by Zere *et al.* (35), which demonstrated through comparative genomic analysis that the BarA–UvrY two-component system is highly conserved across γ-proteobacteria, including nearly all *E.coli* strains, UPEC among them, with very few exceptions. The evolutionary conservation of this regulatory network indicates its functional significance in the fitness of bacteria, as it allows these UPEC strains to respond to metabolic cues and adapt to various environmental conditions. Interestingly, the presence of the *BarA-UvrY* genes was not restricted to isolates of any particular virulence trait pattern or diversity, as exemplified by its detection in isolates with disparate virulence profiles, such as isolate No. 16 (biofilm formation only) and isolate No. 5 (multiple virulence phenotypes). This suggests that the BarA-UvrY system is functionally relevant, regardless of the quantity or combination of expressed virulence factors. These observations are consistent with a previous study conducted by Mitra *et al.* (36) that had illustrated that the absence of the *UvrY* gene results in dramatically decreased expression of several virulence traits, such as β-hemolysin, biofilm formation, and swarming motility, underscoring the involvement of the BarA–UvrY system in the global regulation of various pathogenic properties. Combined, these findings are consistent with a multi-faceted regulatory network extending from *BarA-UvrY*, which contributes to pathogenesis but may mediate additional genetic or environmental signals. It is imperative that future research concentrate on the specific downstream effects of the Bar A-UvrY system in UPEC. The full scope of its regulatory network needs to be clearly defined.

Conclusion:

This study highlighted the critical role of UPEC in urinary tract infections among young women, which is marked by significant cefotaxime resistance. Despite the uniform presence of the *BarA* and *UvrY* genes, isolates displayed diverse virulence profiles, underscoring the complex interplay between resistance and pathogenicity.

Limitation:

This study had several limitations, including a focused sample size and demographics (young women from specific hospitals), and analysis of a subset of isolates

for genotypic characterization.These limitations highlight the need for further research to expand upon these findings.

Authors' declaration:
We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical considerations' Approval-Ethical Clearance: The project was approved by the local ethical committee of (College of Science, University of Baghdad, according to the code number (CSEC/1124/011).
Conflict of Interest: None
Funding: No specific grant from a public, private, or nonprofit funding organization was obtained for this study.
Data availability: Upon reasonable request, the corresponding author will make the data sets generated and/or analyzed during the current work available.

Authors' contributions:
Study conception & design: (May Talib Flayyih). Literature search: (Ali Hazim Jabbar). Data acquisition: (Ali Hazim Jabbar). Data analysis & interpretation: (Ali Hazim Jabbar), Manuscript preparation: (Ali Hazim Jabbar). Manuscript editing & review: (Ali Hazim Jabbar).

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توصيف لعوامل الضراوة وجينات نظام التنظيم ثنائي المكونات في عزلات الإشريكية القولونية الممرضة للجهاز البولي المعزولة من شابات مصابات بعدوى المسالك البولية

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الخلاصة:

الخلفية: تعد التهابات المسالك البولية من أكثر أنواع العدوى البكتيرية شيوعا، وتعزى بشكل رئيسي إلى الإصابة ببكتيريا الإشريكية القولونية. وتمتلك سلالات الإشريكية القولونية الممرضة للمسالك البولية عددا من عوامل الفوعة التي تسهم في تفاقم الحالة السريرية لهذه الالتهابات. ومن أبرز المنظومات التنظيمية المسؤولة عن ضبط التعبير الجيني لعوامل الفوعة هذه، منظومة BarA/UvrY ثنائية المكونات، والتي تتحكم في عدد من الجينات المرتبطة بالالتصاق، والحركة، وتكوين الأغشية الحيوية، من خلال تنظيم عدد من الحمضيات النووية الصغيرة والمنظمات المسؤولة عن أيض الكربون.

الأهداف: تهدف هذه الدراسة إلى تقييم درجة الفوعة وبعض الخصائص الجينية لعزلات بكتيريا الإشريكية القولونية المعزولة من عينات بول تم جمعها من نساء شابات، بالإضافة إلى دراسة أنماط مقاومتها للمضادات الحيوية.

المنهجية: في أواخر عام 2024، تم جمع مئتي عينة بول من نساء شابات تتراوح أعمارهن بين 18 و30 عاما، ممن يعانين من التهابات في المسالك البولية. وقد جرى تأكيد التعرف على البكتيريا باستخدام الأوساط الزرعية التفريقية والكروموجينية، تلاها التأكيد البيوكيميائي بواسطة نظام VITEK 2 Compact كما تم اختبار الحساسية تجاه المضادات الحيوية باستخدام النظام ذاته. وتم تقييم قدرة عزلات الإشريكية القولونية على تكوين الأغشية الحيوية، حركة العج، وفعالية تحلل الدم. كما استخدمت تقنيات تفاعل البوليمراز المتسلسل التقليدي للكشف عن مدى انتشار جينات نظام الاستجابة ثنائي المكونات في عزلات الإشريكية القولونية.

النتائج: بلغت نسبة عزل بكتيريا الإشريكية القولونية من عينات البول 45%، أي ما يعادل 89 عزلة. أظهرت العزلات مقاومة عالية للسيفوتكسم بنسبة 82.5%، بينما كانت جميعها حساسة تجاه الإروبينيم، والأميكاسين، والتيجيسيكلين. بينت النتائج أن 85% من العزلات كانت منتجة لإنزيمات البيتا-لاكتاماز، و2.5% منها امتلكت آليات مقاومة مزدوجة. تم رصد فعالية تحلل الدم في 48.3% من العزلات، وأظهرت 34.8% منها حركة العج. أبدت 19.35% إنتاجا متوسطا و41.9% إنتاجا ضعيفا للغشاء الحيوي، في حين لم تظهر 38.7% أي قدرة على إنتاجه. التحليل الجيني لـ 16 عزلة ممثلة كشف عن وجود جينات *BarA* و*UvrY* في جميع العينات.

الإستنتاج: تسلط هذه الدراسة الضوء على الدور الحاسم للإشريكية القولونية الممرضة للمجاري البولية في التهابات المسالك البولية لدى الشابات، والتي تميزها مقاومة كبيرة للسيفوتاكسيم. وعلى الرغم من وجود جينات *BarA* و*UvrY* بشكل موحد، أظهرت العزلات أنماط ضراوة مختلفة، مما يؤكد على التفاعل المعقد بين المقاومة والقدرة الإمراضية.

الكلمات المفتاحية: المقاومة للمضادات الحيوية; *BarA-UvrY*; تفاعل البوليمراز المتسلسل; الإشريكية القولونيةUPEC; ;عوامل الفوعة.