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Molecular Epidemiology of ESBL-Producing Uropathogenic Escherichia Coli and its Association with Biofilm Formation among Women with UTIs in Irbid, Jordan

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Abstract
This cross-sectional study investigated the molecular epidemiology and biofilm-forming capacity of ESBL-producing Escherichia coli among women (18–50 years) diagnosed with UTIs in Najaf, Iraq. Out of the total 300 urine samples, 183 (61.0%) yielded E. coli, with 77 isolates (42.1%) phenotypically confirmed as ESBL producers. All the 70 available ESBL+ isolates underwent molecular and biofilm analysis; seven isolates were excluded due to low viability or failure to recover after cryopreservation. The PCR revealed blaCTX-M in 90% (63/70) of isolates, solely (58.6%) or combined with blaOXA (31.4%). The biofilm formation was assessed through the microtiter plate method. A significant majority (68.6%, 48/70) of ESBL+ isolates formed moderate-to-strong biofilms, compared to only 31.2% of non-ESBL isolates ($p < 0.01$, Chi-square). Notably, among ESBL+ isolates, strong biofilm formation was significantly more prevalent in married women (37.8%, 14/37) as compared to unmarried women (18.2%, 6/33) after adjusting for age and prior antibiotic use (adjusted OR = 3.12; 95% CI: 1.02– 9.56; $p = 0.046$). The near-equal distribution of ESBL+ infections between married (53%) and unmarried (47%) women indicated a broad community circulation beyond traditional risk factors. The dominant blaCTX-M prevalence aligned with regional patterns, which emphasized a unified resistance landscape. Based on the high resistance and biofilm prevalence, empirical utilization of nitrofurantoin is strongly recommended. This study highlighted the dual threat of genetic resistance and biofilm-mediated persistence, advocating for integrated molecular and virulence diagnostics in routine gynecological care to guide targeted therapy and infection control strategies in Jordan and similar settings.

Keywords: ESBL, Comparative Analysis, Virulence Diagnostics, Gynecological, Biofilm Formation

Introduction

Introduction Urinary tract infections (UTIs) represent one of the most prevalent bacterial infections globally, which imposes a significant burden on healthcare systems and alarming affecting patient quality of life, especially among women of reproductive age (Stamm & Norrby 2001, Odaa & Rasheed 2025) [16, 14]. Escherichia coli (E. coli) is the predominant etiological agent, which have been responsible for approximately 75-90% of uncomplicated community-acquired UTIs, which serves as a leading cause of infections which can ranged from simple cystitis to life-threatening pyelonephritis (Taneja *et al*. 2010) [17]. The management of these infections, once considered routine, has been profoundly complicated by the relentless global escalation of antimicrobial resistance (AMR), declared a critical threat to public health by the World Health Organization (WHO 2023) [21]. The most alarming resistance mechanisms in Gramnegative bacteria has been the production of ExtendedSpectrum β-Lactamases (ESBLs). These enzymes hydrolyzed and conferred resistance to a broad spectrum of vital antibiotics, which included penicillins, third- and fourth-generation cephalosporins, and aztreonam, severely limited effective oral therapeutic options (Bush *et al*. 1995) [6]. ESBL-producing E. coli (ESBL-EC) strains have been frequently associated with multidrug-resistant (MDR) phenotypes, which complicated treatment regimens and led to elevated morbidity, mortality, healthcare costs, and length of hospital stays (Lautenbach *et al*. 2001; Giske *et al*. 2008) [13, 11]. The epidemiology of ESBL-EC exhibited significant geographical variation, which was influenced by local antibiotic consumption patterns, infection control practices, and the clonal spread of high-risk strains (Pitout & Laupland 2008) [15]. The molecular landscape of ESBL resistance has been dominated by the _bla_CTX-M gene family, which has significantly replaced _bla_TEM and _bla_SHV types

worldwide (Bonnet *et al*. 2001) [5]. Recently, _bla_OXA variants, especially those within the _bla_OXA-48-like group, have emerged as significant contributors to βlactam resistance, which often exhibited co-carriage with other resistance determinants on mobile genetic elements, facilitating rapid dissemination (Woodford *et al*. 2006) [20]. A recent comprehensive study from neighboring Jordan by Alsheikh and his colleagues emphasized the severity of this issue in the Middle East. Their research investigated UTIs across multiple hospitals, which indicated an overall ESBL-EC prevalence of 18%, with the _bla_CTX-M gene present in a striking 98% of ESBL-positive isolates. The study also revealed significant regional disparities, with northern governorates which demonstrated the highest ESBL prevalence (50%), and identified a higher carriage of the _bla_OXA gene among female patients (Alsheikh *et al*. 2025) [3]. These findings highlighted a critical and dynamic resistance landscape in the region, which have been driven by the pervasive spread of specific genetic determinants. In Iraq, the healthcare infrastructure and antimicrobial resistance surveillance have faced severe challenges due to decades of conflict and instability. This has likely disrupted antibiotic stewardship programs, which as a result altered prescribing practices, and strained laboratory capacities for effective resistance monitoring. Consequently, there has been a pressing paucity of contemporary, high-resolution data on the molecular epidemiology of ESBL-EC which caused UTIs within the Iraqi population. The existing data were often fragmented, localized, or outdated, which have failed to provide a clear national picture of the predominant ESBL genotypes, their distribution, and associated risk factors. This gap in knowledge directly undermines clinical efficacy. In the absence of local, evidence-based guidelines, clinicians have been forced to rely on empirical therapy informed by international or regional data that may not accurately reflect Iraq's unique resistance ecology. As a result, leading to inappropriate antibiotic selection, initial treatment failure, and further amplification of resistance. Therefore, this cross-sectional study was designed to address these critical knowledge gaps by conducting a detailed molecular epidemiological investigation of ESBLEC isolated from women (aged 18-50 years) diagnosed with UTIs in Najaf, Iraq. The study aimed to achieve three primary objectives firstly to determine the prevalence of ESBL production among uropathogenic E. coli isolates in this demographic; secondly to characterize the molecular profile of key β-lactamase resistance genes (bla_CTX-M, bla_OXA) among the ESBL-positive isolates; and lastly to correlate these molecular findings with patient demographic data to identify potential risk factors. This research provided essential insights into the resistance mechanisms circulating in the community by integrating phenotypic confirmation with genotypic analysis. The findings are intended to guide the development of tailored empirical therapy guidelines, which could support antimicrobial stewardship initiatives, and contribute to the broader regional comprehension of AMR trends, ultimately aiding in the formulation of effective infection control and public health interventions in **Jordan**.

Materials and Methods

Materials and methods Study design and setting This cross-sectional study was conducted between January 2025 and November 2025 in Jordan. A total of 300 midstream urine samples were collected from women aged 18 to 50 years with clinically suspected urinary tract infections (UTIs) who were attending Al Zahrra Hospital for Maternity and Children. The demographic and clinical data included age, marital status, educational level, and prior antibiotic utilization were obtained using a standardized questionnaire. Inclusion and exclusion criteria The patients with typical UTI symptoms (dysuria, frequency, urgency, suprapubic pain) were included and had a positive urine culture which yielded Escherichia coli as the sole pathogen. The exclusion criteria comprised: known anatomical abnormalities of the urinary tract, indwelling urinary catheters, antibiotic therapy within the preceding two weeks, immunocompromised states, malignancies, and unwillingness to provide informed consent. Bacterial isolation and identification The urine samples were inoculated onto blood agar, MacConkey agar, and eosin methylene blue (EMB) agar (hi media) and incubated aerobically at 37 °C for a duration of 24 hours. The presumptive identification of Escherichia coli was based on colony morphology, Gram staining (Gram-negative rods), and standard biochemical tests: triple sugar iron (TSI) agar (acid/acid, gas production), indole production (positive), citrate utilization (negative), and methyl red/Voges–Proskauer (MR + VP –). Confirmatory identification using VITEK® 2 system The confirmatory identification of Escherichia coli isolates was performed utilzing the VITEK® 2 Compact system (bioMérieux, Marcy-L'Etoile, France) with Gram-negative (GN) identification cards, which strictly followed the manufacturer's instructions. A bacterial suspension equivalent to a 0.5 McFarland standard was prepared from fresh overnight cultures and automatically processed by the instrument. Antimicrobial susceptibility testing Antibiotic susceptibility was determined by utilizing the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (hi media) following CLSI 2025 guidelines. A bacterial suspension equivalent to 0.5 McFarland standard was prepared, inoculated onto agar plates, and the following disks (Liofilchem, Italy) were applied: amoxicillin-clavulanic acid (20/10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), gentamicin (10 µg), amikacin (30 µg), cephalexin (30 µg), cefuroxime (30 µg), co-trimoxazole (25 µg), meropenem (10 µg), doxycycline (30 µg), and nitrofurantoin (300 µg). After 18 to 24 h of aerobic incubation at 37 °C, inhibition zone diameters were interpreted based on the CLSI breakpoints. Isolates resistant to at least three different antimicrobial classes were classified as multidrug-resistant (MDR). Phenotypic confirmation of ESBL production The ESBL production was screened utilizing the double-disk synergy test. A disk of amoxicillin-clavulanic acid (20/10 µg) was placed at the center of a Mueller– Hinton agar plate, and disks of cefotaxime (30 µg), ceftriaxone (30 µg), and ceftazidime (30 µg) were placed 20 mm (edge-to-edge) from the central disk. The plates were incubated overnight at 37 °C. A clear enhancement (keyhole) of the inhibition zone between the clavulanate-containing disk and any cephalosporin disk indicated ESBL production. DNA extraction The genomic DNA was extracted from overnight broth cultures of ESBL-positive isolates utilizing the Wizard® Genomic DNA Purification Kit (Promega, USA) based on the manufacturer's instructions. Briefly, 1 mL of bacterial culture was centrifuged at 13,000× g for 2 minutes, and the pellet was resuspended in 600 µL of Nuclei Lysis Solution.

After the incubation at 80 °C for 5 minutes, 3 µL of RNase Solution was added, which was followed by incubation at 37 °C for 30 minutes. Protein Precipitation Solution (25 µL) was added, vortexed, and chilled on ice for 5 min. After centrifugation at 13,000× g for 3 min, the supernatant was transferred to a clean tube containing 600 µL of isopropanol. The DNA was pelleted by centrifugation, washed with 600 µL of 70% ethanol, air-dried, and rehydrated in 50 µL of DNA Rehydration Solution. The DNA concentration and purity were assessed utilizing a spectrophotometer and stored at – 20 °C until further utilization. Molecular characterization Out of the total of 77 phenotypically confirmed ESBL-producing isolates, out of which 70 were selected for molecular characterization (PCR) and biofilm formation assay, out of the 77 phenotypically confirmed ESBL-producing isolates, 70 were selected for molecular characterization and biofilm assay. Seven isolates were excluded due to low viability or failure to recover after cryopreservation, ensuring the accuracy and reliability of the laboratory findings. The selection criterion was based on higher biological activity, which assessed preliminarily by growth rate on culture media and PCR amplification efficiency in pilot tests, to ensure clear and interpretable molecular results. The seven excluded isolates demonstrated lower biological activity or did not meet the quality standards which were required for accurate molecular analysis. Importantly, the demographic and clinical distribution of these excluded isolates did not differ significantly from the studied isolates, which minimized the impact of this selection bias on the study's conclusions. Multiplex PCR (m-PCR) and primer selection The multiplex PCR was employed to detect blaCTX-M (816 bp) and blaOXA (550 bp) genes utilizing specific primers (Bonnet *et al*. 2001; Woodford *et al*. 2006) [5, 20]. The 25 µL reaction mixture included 2× PCR Master Mix (Promega, USA) and 10 pmol/µL of each primer. Thermal cycling and visualization An amplification was performed utilizing an Applied Biosystems Veriti Thermal Cycler with an optimized protocol: initial denaturation (95°C/5 minutes), 30 cycles of (95°C/30 seconds, 58°C/30 seconds, 72°C/45 seconds), and a final extension (72°C/10 minutes) as shown in (Table1). Amplicons were separated on 3% agarose gel, which were stained with Ethidium bromide, and visualized under UV light utilizing a Bio-Rad Gel Documentation System. The DNA fragments were sized utilizing a 100-bp Plus DNA ladder.

Table 1: The primer sequences and PCR conditions used in this study

Target Gene	Primer Name	Primer Sequence (5′ → 3′)	Amplicon Size (bp)	Ref.
*bla*CTX-M (universal)	CTX-M	F:ACCGCCG ATATCGTTG GT R:CGCTTTG CGATGTGC AG	816 bp	Bonnet et al. (2001)
*bla*OXA	OXA	F:GGTTAGT TGGCCCCCT TAAA R:AGTTGAG CGAAGAGG GGATT	550 bp	Woodf-ord et al. (2006)

Biofilm formation assay The biofilm-forming capacity was quantitatively assessed utilizing the crystal violet microtiter plate method which have been previously described (Christensen *et al*. 1985) [7]. Briefly, fresh bacterial cultures were adjusted to 0.5 McFarland standard in tryptic soy broth supplemented with 1% glucose. Aliquots of 200 µL were dispensed into sterile 96-well flat-bottom polystyrene microtiter plates and were incubated statically at 37 °C for 24 hours. After incubation, wells were gently washed three times with phosphate-buffered saline (PBS) to remove non-adherent cells. The adherent biofilms were fixed with methanol (15 minutes), air-dried, and stained with 0.1% crystal violet for 15 to 20 minutes at room temperature. AN excess stain was removed by washing with distilled water, and the plates were air-dried. The bound dye was solubilized with 200 µL of 95% ethanol or 33% acetic acid per well, and absorbance was measured at 570 nm (or 595 nm) utilizing an ELISA reader. The sterile broth served as a negative control. All the assays were performed in triplicate. Based on the optical density (OD) of the samples relative to the control, isolates were classified as non-producers, weak, moderate, or strong biofilm producers Statistical analysis The data were analyzed utilizing SPSS version 23 (IBM, USA). The categorical variables were expressed as frequencies and percentages. The associations between categorical variables were assessed utilizing the Chi-square test or Fisher's exact test. The logistic regression was performed to evaluate the relationship between demographic factors (marital status, age) and biofilm-formation strength among ESBL-positive isolates. A p-value < 0.05 was considered statistically significant.

Results

Bacterial Isolation and phenotypic characterization The total of 300 urine samples collected in Najaf; Escherichia coli was out of 183 (61.0%) recovered. The phenotypic screening confirmed that 77 isolates (42.1%) were ESBL producers. The distribution was nearly equal across marital status, as shown in Table 2.

Table 2: Distribution of E. coli and ESBL+ isolates based on marital status

Parameter	Number (n)	Percentage (%)
Total urine samples	300	100.0
Escherichia coli isolates	183	61.0
ESBL-producing *E. coli* isolates	77	42.1*

Bacterial isolation and identification An initial phenotypic identification revealed that 183 (61.0%) isolates were lactose-fermenting Escherichia coli, which was characterized by the appearance of bright pink, smooth, and circular colonies on MacConkey agar (Fig 1). This distinct morphological profile was attributed to the fermentation of lactose, which lowered the pH of the medium and caused the neutral red indicator to turn pink, a hallmark diagnostic characteristic for E. coli in clinical samples (Tille 2021) [18]. Antimicrobial susceptibility profile The data demonstrated that nitrofurantoin remained highly effective against uropathogenic Escherichia coli in Najaf, with an overall susceptibility rate of 89.1%. This high sensitivity has been a key finding, as it indicated that the local strains have not yet

developed significant resistance to this specific agent compared to other oral antibiotics like cephalosporins, as shown in Table 3.

Table 3: Antimicrobial susceptibility profile of uropathogenic *Escherichia coli* isolates

Antibiotic	Susceptible n (%)	Resistant n (%)
Nitrofurantoin	163 (89.1)	20 (10.9)

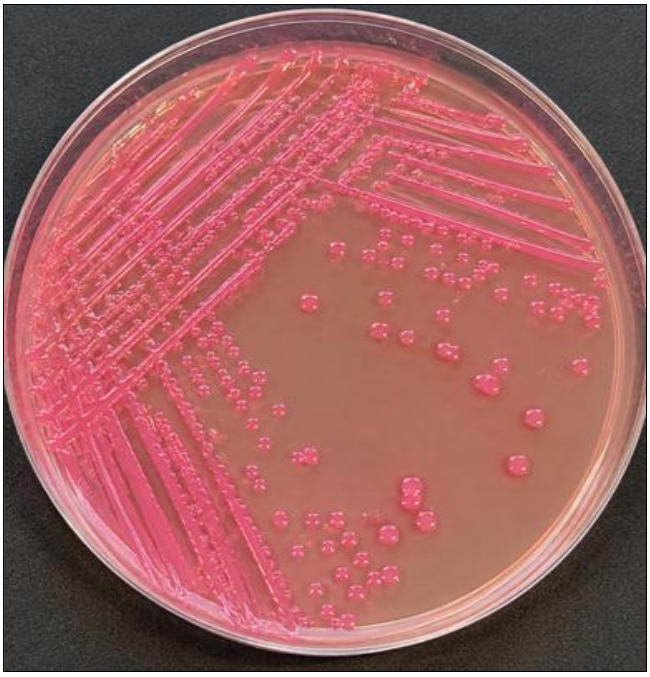

Fig 1: *Escherichia coil* on MacConkey agar. Typical lactose-fermenting colonies appear bright pink, smooth, circular, and convex due to lactose fermentation with acid production, which lowers the pH of the medium and causes the neutral red indicator to change color

Genotype correlation the results confirmed that the presence of _bla_CTX-M and _bla_OXA genes did not significantly reduce the effectiveness of the drug. Even in the most resistant group (isolates carrying both genes), the susceptibility remained remarkably high at 90.9%. This proved that nitrofurantoin has been a "robust" choice against multidrug-resistant (MDR) genotypes. The 113 isolates in this category included 106 phenotypically negative ESBL producers and 7 ESBLproducing isolates that were excluded from subsequent molecular analysis and biofilm assays due to insufficient biological activity. However, they were retained in the overall antimicrobial susceptibility profile to maintain clinical data integrity. Molecular profile of resistance genes A subset of 70 available ESBL+ isolates underwent PCR analysis (Fig 2). The _bla_CTX-M gene was found in 63 isolates (Table 5), which resulted in a prevalence of 90.0% among the tested cohort.

Table 4: Susceptibility of *Escherichia coil* isolates to Nitrofurantoin according to ESBL status and genotype

Bacterial category	Total number (n)	Susceptible N (%)	Resistant N (%)	Susceptibility rate (%)
E. coli isolates	183	163 (89.1)	20 (10.9)	89.1%
Non-ESBL producers *	113	98 (86.7)	15 (13.3)	86.7%
ESBL producers (Tested)	70	65 (92.8)	5 (7.2)	92.8%

* Non-ESBL producers were inferred based on the total *E. coli* isolates minus the number of ESBL producers.

Susceptible Resistant

Table 5: Genotypic Prevalence of blaCTX-M and blaOXA among ESBL-Producing *E. coli* Isolates (n = 70)

Genotype	Isolates (N)	Percentage (%)
blaCTX-M only	41	58.6
blaCTX-M + blaOXA	22	31.4
blaOXA only	4	5.7
Others / Undetermined	3	4.3
Total	**70**	**100.0**

Table 6: Comparison of Biofilm-Forming Strength between ESBL-Positive and ESBL-Negative *Escherichia coli* Isolates

Biofilm Category	ESBL+ (n = 70)	ESBL− (n = 106)	P-value
Non / Weak Producer	22 (31.4%)	73 (68.8%)	< 0.01
Moderate Producer	28 (40.0%)	25 (23.6%)	—
Strong Producer	20 (28.6%)	8 (7.5%)	—
Total (Moderate + Strong)	**48 (68.6%)**	**33 (31.2%)**	**< 0.01**

Association between marital status and strong biofilm formation among ESBL-producing isolates The total of 70 ESBL-producing Escherichia coli isolates were subjected to biofilm assay, out of which 20 isolates (28.6%) were classified as strong biofilm producers. The distribution of strong biofilm producers based on the marital status has been shown in Table 7. The 37 isolates recovered from married women, 14 (37.8%) exhibited strong biofilm formation, as compared to 6 of 33 (18.2%) from unmarried women. The univariate logistic regression analysis revealed that isolates from married women had a crude odds ratio (OR) of 2.74 (95% CI: 0.89–8.43, p = 0.078) for strong biofilm production relative to unmarried women. Although this association did not reach statistical significance in the unadjusted model, the effect size suggested a clinically meaningful difference warranting further adjustment for confounders. After the adjusting for potential confounders which included age group (18–30, 31–40, 41–50 years) and prior antibiotic use (yes/no) marital status emerged as a

significant independent predictor of strong biofilm formation. The adjusted odds ratio (AOR) was 3.12 (95% CI: 1.02–9.56, p = 0.046), which indicated that ESBLproducing isolates from married women were approximately three times more likely to form strong biofilms compared to those from unmarried women, after controlling for age and prior antibiotic exposure (Table 7). An age group and prior antibiotic use were not significantly associated with strong biofilm production in the multivariable model (p > 0.05 for all). The Hosmer–Lemeshow goodness-of-fit test yielded a non-significant result (chi-square = 5.78, p = 0.672), which indicated adequate model calibration. The Nagelkerke R2 value was 0.124, which suggested that 12.4% of the variance in strong biofilm formation was explained by the variables included in the model as shown in Fig 2.

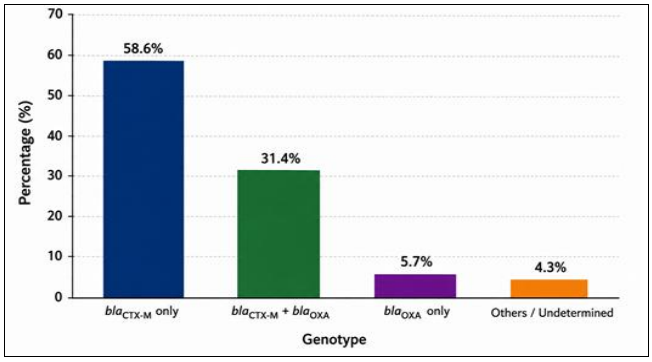

Fig 2: Distribution of ESBL resistance genotypes among ESBL-producing *Escherichia coil* isolates (n = 70)

Table 7: Multivariable Logistic Regression Analysis of Factors Associated with Strong Biofilm Formation among ESBL-Producing E. coli Isolates

Variable	Category	Adjusted Odds Ratio (AOR)	95% CI	P-value
Marital status	Married vs. Unmarried	3.12	1.02–9.56	0.046*
Age group	31–40 vs. 18–30 years	NS	—	>0.05
Age group	41–50 vs. 18–30 years	NS	—	>0.05
Prior antibiotic use	Yes vs. No	NS	—	>0.05

The strong biofilm formation was observed in 37.8% (14/37) of isolates from married women compared to 18.2% (6/33) from unmarried women. Married women had significantly higher odds of harboring strong biofilm-forming isolates after adjusting for age and prior antibiotic use (adjusted OR = 3.12; 95% CI: 1.02–9.56; p = 0.046).

Discussion

This study provided the first integrated molecular epidemiological analysis of ESBL-producing uropathogenic Escherichia coli isolated from women of reproductive age in Najaf, Iraq, with a specific focus on the association between resistance genotypes and biofilmforming capacity. The findings revealed three principal insights: firstly an overwhelming predominance of the blaCTX-M gene (90.0%) as the genetic driver of ESBL production; secondly a statistically significant synergy between ESBL production and moderate-to-strong biofilm formation (68.6% vs. 31.2%; p < 0.01); and thirdly a novel, independent association between marital status and strong biofilm phenotype among ESBL-positive isolates (adjusted OR = 3.12; 95% CI: 1.02–9.56; p = 0.046). These results have been discussed below in the context of local, regional and global literature. The detection of blaCTX-M in 90.0% of molecularly characterised ESBL-producing isolates confirmed that CTX-M-type enzymes were the dominant ESBL mechanism among community-acquired UTI isolates in this Jordan population. This figure aligned with earlier joudany reports. Al-Dahmoshi *et al*. (2020) [1] documented an 86.4% prevalence of blaCTX-M among ESBL-producing E. coli in Hillah, and Al-Mayahie and Al-Khafaji (2020) reported 88.2% in Baghdad. More importantly, the 90.0% closely aligned with the 98% prevalence recently reported in Jordan by Alsheikh *et al*. (2025) [3], which indicated a shared resistance gene pool across the region. This epidemiological convergence has been likely driven by the global dissemination of successful pandemic clones (e.g., ST131) and the horizontal transfer of plasmids which carried blaCTX-M-15. The co-carriage of blaCTX-M and blaOXA in 31.4% of isolates is a concerning phenomenon of gene stacking. Although OXA-enzymes possessed relatively weak hydrolytic activity against extended-spectrum cephalosporins, their association with mobile genetic elements may facilitated the persistence of multidrugresistant clones. The result that 68.6% of ESBL-producing isolates exhibited moderate-to-strong biofilm formation, compared with only 31.2% of non-ESBL isolates (p < 0.01), corroborated a well-established association between antimicrobial resistance and virulence. A global metaanalysis by Garousi *et al*. (2022) [10] reported that 82.35% of studies demonstrated a significant positive correlation between biofilm production and antibiotic resistance. The biofilm formation provided a physical barrier that limited antibiotic penetration and facilitated horizontal gene transfer within the biofilm matrix. The data therefore reinforced the concept that resistance and virulence were co-selected, and that phenotypic screening for biofilm production could serve as a surrogate marker for multidrug-resistant, high-risk clones. Based on the current knowledge, this has been the first study from Iraq which demonstrate a statistically significant association between marital status and biofilm phenotype among ESBL-producing E. coli. After adjustment for age and prior antibiotic use, the isolates from married women were three times more likely to be strong biofilm producers as compared to those from unmarried women (AOR = 3.12; 95% CI: 1.02–9.56; p = 0.046). The married women were more likely to experience pregnancy or use hormonal contraceptives, which modulated the vaginal environment and may favour colonization by uropathogens with enhanced adhesive capabilities. The frequent sexual intercourse can be a risk factor for UTI. The mechanical introduction of bacteria and microtrauma may exert selective pressure which favoured strains with superior biofilm-forming capacity for persistence. The recurrent antibiotic courses might enrich the vaginal reservoirs with biofilm-hyperproducing, multidrug-resistant clones. The high susceptibility of ESBL-producing isolates to nitrofurantoin (92.8%), which included those coharboring blaCTX-M and blaOXA (90.9%), which strongly supported its continued utilization as a first-line empirical agent for uncomplicated cystitis in this demographic (Bader *et al*. 2020; Emergency Care BC 2020) [4, 8]. In contrast, the poor

activity of orally administered cephalosporins (26 to 42%) and co-trimoxazole (30.1%) indicated the extensive dissemination of ESBL genes and associated resistance determinants (Bader *et al*. 2020; Emergency Care BC, 2020; Gallagher 2024) [4, 8, 9]. The differential activity between amikacin (86.3%) and gentamicin (60.1%) may be explained by the lower clinical utilization of amikacin in the community, which preserved its efficacy, or by the co-carriage of aminoglycoside-modifying enzymes with ESBL genes on the same plasmids, a phenomenon that has been shown to disproportionately affect gentamicin susceptibility (Haidar *et al*. 2016; WebMD Education, 2025) [12, 19]. These findings emphasized the requirement for regular antimicrobial susceptibility surveillance to guide empirical therapy and preserve remaining treatment options (Gallagher 2024; WebMD Education, 2025) [9, 19].

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