

Molecular Identification and Antimicrobial Resistance Profiles of *Escherichia coli* Isolated in Urine Cultures from Shamiya State Hospital, Iraq

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Abstract: *Background:* *Escherichia coli* is the leading causative agent of urinary tract infections worldwide and represents a major public health concern due to its increasing resistance to commonly used antimicrobial agents. Rapid molecular identification, together with the characterization of antimicrobial resistance profiles, is essential for effective patient management and infection control. *Objective:* This study aimed to molecularly identify *E. coli* isolates obtained from urine cultures at Shamiya State Hospital, Iraq, and to determine their antimicrobial resistance patterns to support appropriate therapeutic strategies. *Methods:* Urine samples were collected from patients attending Shamiya State Hospital, Iraq, and processed using standard microbiological procedures. Bacterial isolates presumptively identified as *Escherichia coli* were obtained through culture on selective and differential media and confirmed by conventional biochemical tests. Molecular identification was performed by polymerase chain reaction (PCR) targeting species-specific genetic markers. Antimicrobial susceptibility testing was carried out using the disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. *Results:* The isolates comprised 29% *E. coli*, 21% *Staphylococcus aureus*, 18% *Klebsiella pneumoniae*, 12% *Proteus mirabilis*, 16% *Enterococcus faecalis*, and 4% *Pseudomonas aeruginosa*. Among the resistance genes, the CTX-M gene exhibited the strongest resistance profile, while the TET-A and TEM genes showed weaker resistance. The QNR-B gene demonstrated the weakest resistance compared to the CTX-M gene among all isolates. In addition, the demographic characteristics of the individuals from whom the *E. coli* strains were isolated were recorded. The results obtained contributed to the development of clinical strategies for the prevention and management of uropathogenic *E. coli* (UPEC) infections in the Shamiya region.

Keywords: CTX-M, *Escherichia Coli*, Qnr-B, Shamiya-Iraq, TET-A, TEM.

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INTRODUCTION

Urinary tract infection (UTI) is primarily caused by the colonization of the uroepithelium by microorganisms from the colon and perianal region flora. Bacteria, most of which are Gram-negative, cause UTI. *Escherichia coli* ranks first among Gram-negative bacteria, followed by other Gram-negative bacteria such as *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, as well as Gram-positive bacteria such as *Enterococcus faecalis* and *Staphylococcus aureus* (Hooton and Stamm, 1997).

Some *Escherichia coli* strains may exhibit pathogenic properties due to various virulence factors they possess. For this reason, pathogenic *E. coli* strains are among the most common pathogenic bacteria in infectious diseases. Pathogenic *E. coli* strains of the urinary system are referred to as uropathogenic *E. coli* (Johnson and Russo, 2005).

Identifying resistance factors in pathogenic bacteria is crucial for the effective management and treatment of these pathogens. The resistance problem, which has developed as a result of widespread antibiotic use, has become a significant issue in the treatment of

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urinary tract infections. Inadequate infection control measures and the increased use of antibiotics in animals are other contributing factors to the rise in resistance. It is essential to understand regional resistance patterns and conduct studies for this purpose, particularly to ensure the correct selection of antibiotics for empirical treatment (Chomarat, 2000).

In this study, *Escherichia coli* strains isolated from urine samples of patients admitted to Shamiya State Hospital (Iraq) will be identified using conventional and automated microbiological procedures. The antimicrobial susceptibilities of the identified *E. coli* strains will be determined by conventional microbiological methods. The presence of Tet-A, Qnr-B, Ctx-M, and TEM resistance genes in the identified *E. coli* strains will be investigated by PCR. The demographic characteristics of individuals from whom resistant *E. coli* strains were isolated using automated microbiological systems and PCR methods will also be identified. The results obtained will contribute to the development of clinical strategies for the prevention and management of *E. coli* infections causing UPEC in the Shamiya region.

MATERIALS AND METHODS

Specimens Collection and Urine Culture

From September to December 2023, 157 samples were randomly collected from AL-Shamiya General Hospital in the city of Al-Shamiya, Al-Qadisiyah Governorate, Iraq. The protocol for specimen collection was carried out following the directions in Bailey & Scott's diagnostic microbiology textbook (Tille, 2015).

The samples were collected in sterilized containers. Midstream urine specimens were inoculated onto blood agar, MacConkey agar, and cysteine lactose agar, then incubated overnight at 37°C. Bacterial growth was presumptively identified on Chromogenic agar based on the color and morphology of the colonies, as illustrated by the manufacturer. Colonies on MacConkey agar were also examined. To confirm the identity of the isolates, they were subjected to standard identification protocols, including Gram staining, motility testing, catalase testing, oxidase testing, and other relevant biochemical analyses (Biji *et al.*, 2017).

Isolation and Identification

Taking into account the microscopic examination, the morphological characteristics of the culture media, and the biochemical tests, *E. coli* was identified. Moreover, the identification was performed following the guidelines of Bailey and Scott. The Gram stain is a type of differential stain that allows microbiologists to observe the morphology and cell structure of organisms while also distinguishing between the two most common chemical cellular structures found in bacteria (Garrity *et al.*, 2004).

Polymerase Chain Reaction

The kits that were used in the present research: DNA Extraction kit (Favorgen Biotech, Taiwan), Gram stain kit (Hi media, India) and ViteK-2 system cards (BioMérieux, France). The polymerase chain reaction (PCR) materials include GoTaq® G2 Green Master Mix, a DNA ladder, primers (forward and reverse), a DNA template, and nuclease-free water. The gene regions and primer sequences used were presented: *CTX-M* F: ATGTGCAGYACCAGTAA, R: CCGCTGCCGGTYTTATC (Tabi 2018). *TEM*: F: TCAACATTTTCGTGTCGCCC, R: AACTACGATACGGGAGGGCT (Yang *et al.*, 2020). *QNR-B*: F: GATCGTAAAAGCCAGAAAGG, R: ACGATGCCTGGTAGTTGTCC (Herrera *et al.*, 2021). *TET-A*: F: GGTTCACTCGAACGACGTCA, R: CTGTCCGACAAGTTGCATGA (Boroumand *et al.*, 2021). The PCR mixture was prepared as follows: 5 µl of Master Mix, 2.5 µl each of forward and reverse primers, 5 µl of DNA template, and 5 µl of deionized water.

Analytical Profile Index

The Analytical Profile Index (API) 20E test system confirmed the *E. coli* isolates identified through biochemical testing. This test was performed according to the manufacturer's instructions, which was analyzed using the API 20E analytical index. The Vitek2® system (BioMérieux, France) was used to identify all *Escherichia coli* isolates following the manufacturer's instructions. The standard inoculum for each test isolate.

Molecular Detection

A total of 300 µl of distilled water was inoculated with 3 to 5 fresh, pure *Escherichia coli* colonies from a MacConkey agar plate. The suspension was then heated in a water bath at 100°C for 20 minutes to lyse the cells. After heating, the sample was rapidly cooled on ice for 30 minutes and centrifuged at 8500 rpm for 10 minutes to remove any remaining cell debris. Finally, the DNA template was extracted from the supernatant (Omar *et al.*, 2014).

RESULTS

The study cohort comprised 157 patients. Bacterial growth was observed in 91 patients (58.0%), while 66 patients (42.0%) showed no growth. This difference was statistically significant ($p = 0.005$), leading to the rejection of the null hypothesis at the 0.05 significance level.

Among the 91 culture-positive cases, six bacterial species were identified. The most prevalent was *Escherichia coli* (28.6%, 26/91), followed by *Staphylococcus aureus* (20.9%, 19/91), *Klebsiella pneumoniae* (17.6%, 16/91), *Enterococcus faecalis* (16.5%, 15/91), *Proteus mirabilis* (12.1%, 11/91), and *Pseudomonas aeruginosa* (4.4%, 4/91) (Table 1).

The overall study population included 126 female (80.3%) and 31 male (19.7%) participants. Within the culture-positive group, 77 (84.6%) of the 91 patients were female and 14 (15.4%) were male. In contrast, the culture-negative group consisted of 49 females (74.2%) and 17 males (25.8%). *Escherichia coli* infections were more frequently identified in female patients (Table 2).

During the study period, patients were classified into three age groups. The first group (14–29 years) included 87 patients, representing 55.4% (87/157) of the total sample. The second group (30–49 years) comprised 46 patients (29.3%, 46/157), while the third group (50–79 years) consisted of 24 patients (15.3%, 24/157).

Table 1: Distribution of bacterial types

Bacterial types	Number of positive cultures	Percentage
<i>Escherichia coli</i>	26	29%
<i>Staphylococcus aureus</i>	19	21%
<i>Klebsiella pneumoniae</i>	16	18%
<i>Proteus mirabilis</i>	11	12%
<i>Enterococcus faecalis</i>	15	16%
<i>Pseudomonas aeruginosa</i>	4	4%

Table 2: Positive and negative infections of the total bacterial sample and positive *E. coli* infections according to sex

Case study	Total sample		Negative infections		Positive infections		<i>Escherichia coli</i>	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Number	31	126	17	49	14	77	6	20
Percentage	19.7%	80.3%	25.7%	74.3%	18.2%	81.8%	0.30%	0.70%

Table 3: Six cases of *Escherichia coli* infection by age group

Age group	Total sample		Six bacterial growth		<i>Escherichia coli</i>	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
(14-29)	87	55.4%	48	52.7%	11	42.3%
(30-49)	46	29.3%	25	27.5%	6	23.1%
(50-79)	24	15.3%	18	19.8%	9	34.6%

Table 4: Antibiotic susceptibility distributions of *Escherichia coli* cultures

Antibiotics	Number of Resistant <i>E. coli</i> culture	Percentage	Number of Intermediate <i>E. coli</i> culture	Percentage	Number of Sensitive <i>E. coli</i> culture	Percentage	Number of total <i>E. coli</i> culture
CFM	22	84.6%	3	11.6%	1	3.8%	26
AM	25	96.2%	0	0.00%	1	3.8%	26
AK	13	50.0%	9	34.6%	4	15.4%	26
CN	5	19.2%	3	11.6%	18	69.2%	26
CTX	25	96.2%	0	0.00%	1	3.8%	26
ATM	15	57.7%	1	3.8%	10	38.5%	26
CIP	17	65.4%	4	15.4%	5	19.2%	26
LEV	15	57.7%	4	15.4%	7	26.9%	26
IPM	3	11.6%	1	3.8%	22	84.5%	26
TET	26	100.0%	0	0.00%	0	0.00%	26
MRP	0	0.00%	0	0.00%	26	100.0%	26

Abbreviations: CFM: Cefixime, AM: Amoxicillin, AK: Amikacin, CN: Gentamycin, CTX: Cefotaxime, ATM: Aztreonam, CIP: Ciprofloxacin, LEV: Levofloxacin, IPM: Imipenem, TET: Tetracycline, MRP: Meropenem

Among patients with bacterial growth (n = 91), 48 cases were recorded in the first age group (14–29 years), accounting for 52.7% (48/91). In the second age group (30–49 years), 25 cases were observed (27.5%, 25/91), whereas 18 cases were documented in the third age group (50–79 years), representing 19.8% (18/91).

With respect to *Escherichia coli* infections (n = 26), 11 cases were identified in the first age group (42.3%, 11/26), 6 cases in the second age group (23.1%, 6/26), and 9 cases in the third age group (34.6%, 9/26). Overall, the highest number of *E. coli* infections was observed in the 14–29-year age group (Table 3).

In the present study, the antimicrobial susceptibility of *Escherichia coli* isolates was assessed

against 11 antibiotics (CFM, AM, AK, CN, CTX, ATM, CIP, LEV, IPM, TET, and MRP). The highest level of resistance was observed for TET, with a resistance rate of 100%, whereas the lowest resistance was recorded for IPM. Moreover, no resistance to MRP was detected among the isolates (Figure 1; Table 4).

Based on the results presented in Table 5, the CTX-M gene was detected in 80.8% (21/26) of the isolates, representing the highest prevalence among the resistance genes examined (Figure 2). Accordingly, CTX-M was identified as the most predominant resistance gene in the bacterial isolates analyzed in this study.

Table 5. Resistance and susceptibility of *E. coli* cultures across four gene loci.

<i>E. coli</i> Isolates	CTX-M	TEM	QNR-B	TET-A	<i>E. coli</i> Isolates	CTX-M	TEM	QNR-B	TET-A
<i>E. coli</i> (1)	(+)	(+)	(-)	(-)	<i>E. coli</i> (16)	(+)	(+)	(+)	(+)
<i>E. coli</i> (2)	(+)	(-)	(-)	(-)	<i>E. coli</i> (17)	(+)	(-)	(+)	(+)
<i>E. coli</i> (3)	(+)	(-)	(-)	(-)	<i>E. coli</i> (18)	(-)	(-)	(-)	(+)
<i>E. coli</i> (4)	(-)	(-)	(-)	(-)	<i>E. coli</i> (19)	(+)	(-)	(-)	(-)
<i>E. coli</i> (5)	(+)	(-)	(-)	(-)	<i>E. coli</i> (20)	(+)	(-)	(-)	(-)
<i>E. coli</i> (6)	(-)	(+)	(+)	(+)	<i>E. coli</i> (21)	(+)	(+)	(+)	(-)
<i>E. coli</i> (7)	(-)	(-)	(-)	(-)	<i>E. coli</i> (22)	(+)	(-)	(-)	(-)
<i>E. coli</i> (8)	(+)	(-)	(-)	(+)	<i>E. coli</i> (23)	(+)	(-)	(-)	(+)
<i>E. coli</i> (9)	(+)	(-)	(-)	(-)	<i>E. coli</i> (24)	(+)	(-)	(-)	(-)
<i>E. coli</i> (10)	(+)	(-)	(-)	(-)	<i>E. coli</i> (25)	(+)	(-)	(-)	(+)
<i>E. coli</i> (11)	(+)	(+)	(+)	(+)	<i>E. coli</i> (26)	(+)	(+)	(+)	(-)
<i>E. coli</i> (12)	(+)	(-)	(-)	(-)	Number and Percentage of Resistance (+)	21 (80.8%)	5 (19.2%)	7 (30.0%)	19 (70.0%)
<i>E. coli</i> (13)	(+)	(+)	(-)	(+)		6 (23.1%)	20 (76.9%)	9 (34.6%)	17 (65.4%)
<i>E. coli</i> (14)	(-)	(-)	(-)	(-)	Number and Percentage of Sensitive (-)	21 (80.8%)	7 (30.0%)	6 (23.1%)	9 (34.6%)
<i>E. coli</i> (15)	(+)	(-)	(-)	(-)					

Abbreviations: CTX-M: β -lactamases, known for their potent hydrolytic activity against cefotaxime.

TEM: β -lactamases, named after the first patient from whom it was isolated in Temoneria. TET-A: Associated with tetracycline resistance. QNR-B: Associated with fluoroquinolone resistance

The TET-A gene ranked second, with a prevalence of 34.6% (9/26) (Figure 3). Although detected in a substantial proportion of isolates, its prevalence was markedly lower than that of the CTX-M gene. The TEM gene ranked third, being identified in 30.0% (7/26) of the isolates (Figure 4), followed by the QNR-B gene, which ranked fourth with a prevalence of

23.1% (6/26) (Figure 5). Both the TEM and QNR-B genes exhibited considerably lower prevalence rates compared with CTX-M. Overall, these findings demonstrate that the CTX-M gene is the most prevalent and significant resistance gene among the bacterial isolates examined in this study.



Figure 1: Kirby-Bauer antibiotic susceptibility tests of *E. coli*. (R: Resistant, S: Sensitive). Cefixime (R), Amoxicillin (R), Amikacin (S), Gentamycin (R), Cefixime (R), Aztreonam (S), Ciprofloxacin (S), Levofloxacin (S), Imipenem (S), Tetracycline (R), Meropenem (S)

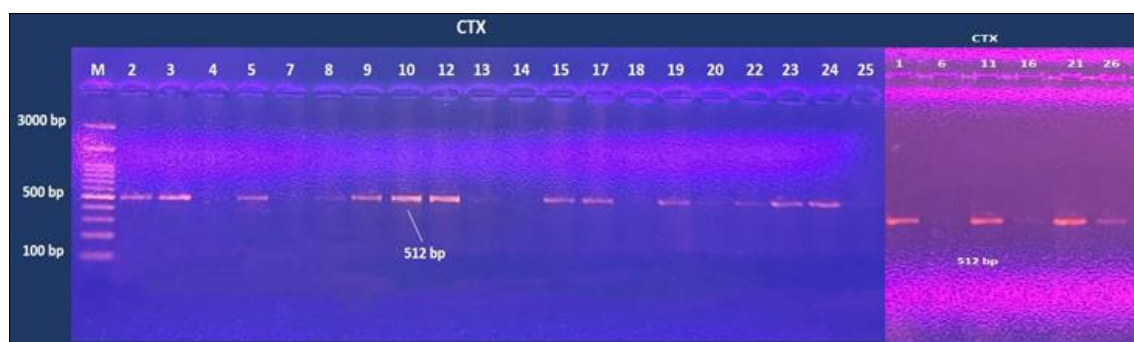


Figure 2: Agarose gel electrophoresis of the 512 bp CTX-M gene product amplified via conventional PCR from *E. coli* genomic DNA. Positive results are visible in lanes 1, 2, 3, 5, 8, 9, 10, 11, 12, 13, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25, and 26

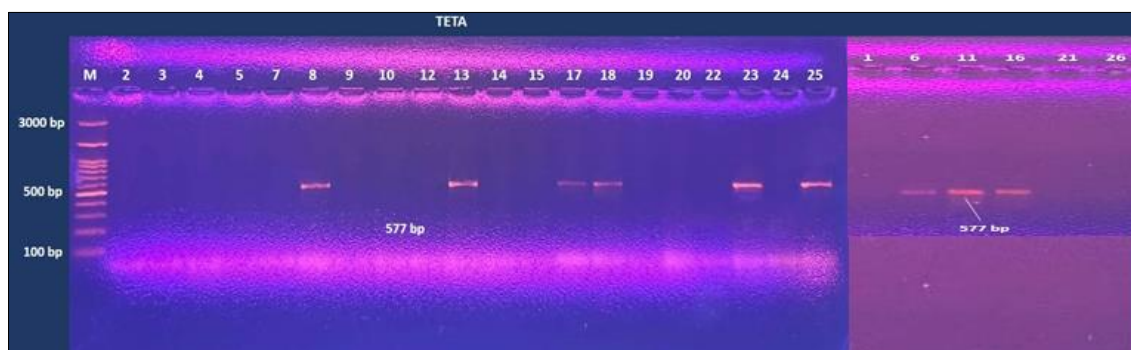


Figure 3: Agarose gel electrophoresis of the 577 bp TET-A gene product amplified via conventional PCR from *E. coli* genomic DNA. Positive results are visible in lanes 6, 8, 11, 13, 16, 17, 18, 23, and 25

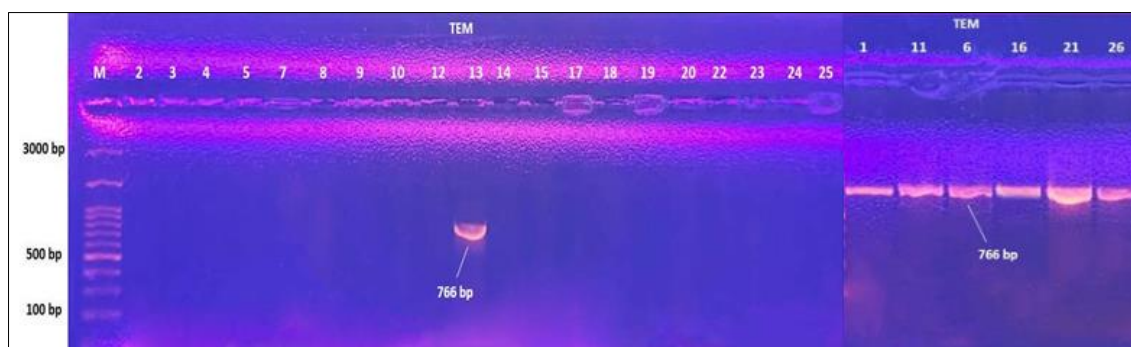


Figure 4: Agarose gel electrophoresis of the 766 bp TEM gene product amplified via conventional PCR from *E. coli* genomic DNA. Positive results are visible in lanes 1, 6, 11, 13, 16, 21, and 26

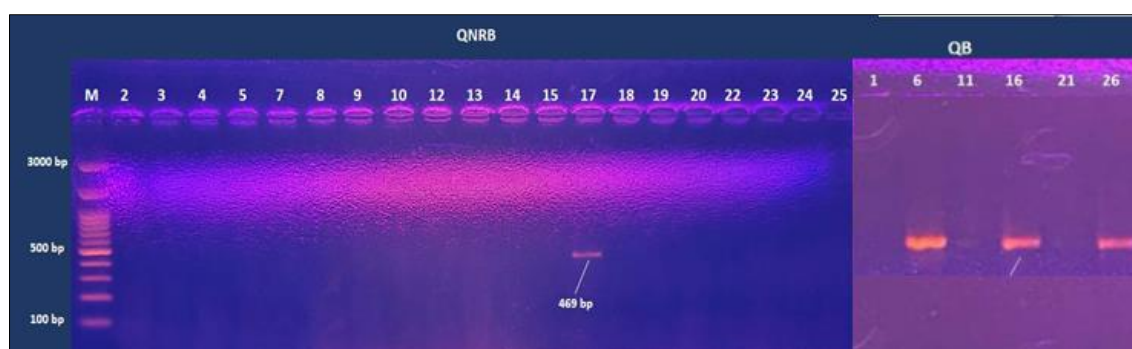


Figure 5: Agarose gel electrophoresis of the 469 bp QNR-B gene product amplified via conventional PCR from *E. coli* genomic DNA. Positive results are visible in lanes 6, 11, 16, 17, 21, and 26

DISCUSSION

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, affecting millions of individuals in recent years. UTIs can occur across all age groups and in both sexes; however, their prevalence is markedly higher in women, exceeding 50%. The spectrum of bacteria isolated from UTIs is broad and includes both Gram-positive and Gram-negative pathogens. Commonly reported uropathogens include *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Enterobacter* spp., and *Citrobacter* spp. Recent studies conducted in several Asian countries, as well as in Europe and the United States, have reported comparable epidemiological patterns for Gram-negative uropathogens (Flores-Mireles *et al.*, 2015).

Among these pathogens, *Escherichia coli* is the most frequently isolated species, followed by *Klebsiella* spp. UTIs occur more commonly in females, largely due to anatomical and physiological characteristics of the female urinary and reproductive systems. In particular, the close proximity of the urethral opening to the anal region facilitates bacterial colonization, thereby increasing susceptibility to infection (Tamalli *et al.*, 2013).

The data reveal a pronounced gender disparity, with females constituting 80.3% (126/157) of the total sampled cases in AL-Shamiya Hospital, Iraq. This skew is consistent across outcomes: females represented 74.3% of negative cultures, 81.8% of positive cultures, and notably, 0.70% of the *E. coli* infections, compared to 0.30% in males. The notably higher prevalence among females aligns with global epidemiological patterns for community-acquired urinary tract infections (UTIs), where anatomical factors, such as a shorter urethra, significantly increase female susceptibility (Flores-Mireles *et al.*, 2015). The finding that *E. coli* was isolated in a subset of positive cultures underscores its established role as the predominant uropathogen, responsible for a substantial majority of such infections in both community and hospital settings (Klein & Hultgren, 2020). The data from this single-center study thus highlight a patient demographic heavily weighted toward females and confirm the ongoing clinical relevance of *E. coli* in bacterial infections within this

regional context, suggesting that local prevention and empirical treatment guidelines should account for this demographic profile.

At AL-Shamiya Hospital, while the youngest cohort (14-29 years) constituted the majority of the total sample (55.4%) and positive bacterial growths (52.7%), the burden of confirmed *E. coli* infection was disproportionately higher in the oldest age group (50-79 years). Notably, this group, representing only 15.3% of the sample, accounted for 34.6% of all *E. coli* isolates. This shift in prevalence towards older adults aligns with established literature, which identifies age as a significant risk factor for complicated and healthcare-associated *E. coli* infections. Physiological changes, such as diminished mucosal immunity and increased comorbidities (e.g., diabetes, prostate enlargement), along with higher rates of hospitalization and instrumentation in this demographic, create a favorable environment for pathogenic *E. coli* (Nicolle, 2016). Conversely, the high frequency of positive cultures in the 14-29 age group is consistent with the peak incidence of uncomplicated community-acquired urinary tract infections (UTIs), particularly among sexually active young women (Foxman, 2014). The data suggest that while young adults present with symptomatic infections more frequently, the etiology in older patients is more likely to be attributed to *E. coli*, potentially indicating more severe or complicated infections that warrant targeted diagnostic and antimicrobial stewardship efforts in this vulnerable population.

The antibiotic susceptibility profile detailed in Table 4 reveals a critical state of multidrug resistance among *Escherichia coli* isolates at AL-Shamiya Hospital, severely limiting therapeutic options. Alarming high resistance rates were observed for first-line agents, including universal resistance to Tetracycline (100%) and near-universal resistance to Amoxicillin (96.2%) and Cefotaxime (96.2%). This pattern is consistent with the global proliferation of extended-spectrum beta-lactamase (ESBL)-producing *E. coli*, which confers resistance to penicillins and third-generation cephalosporins like cefotaxime. Of particular concern is the high resistance to fluoroquinolones (Ciprofloxacin, 65.4%; Levofloxacin, 57.7%),

historically used for empirical UTI treatment, mirroring worldwide trends driven by their extensive misuse (Lerminiaux & Cameron, 2019).

In stark contrast, the carbapenems Imipenem and Meropenem retained exceptional efficacy (84.5% and 100% susceptibility, respectively), establishing them as reliable last-resort agents. However, the emergence of even low-level resistance to Imipenem (11.6%) is a serious sentinel finding, hinting at the possible presence of carbapenemase genes and underscoring the urgent need for antimicrobial stewardship to preserve these vital drugs (Temkin *et al.*, 2014). The aminoglycosides Gentamicin (69.2% sensitive) and Amikacin (50.0% resistant) show variable efficacy, suggesting they may still play a role in tailored combination therapy. This local resistance map strongly argues against the empirical use of penicillins, cephalosporins, tetracyclines, and fluoroquinolones, advocating instead for culture-guided treatment and the rigorous implementation of infection control measures to curb the spread of resistant clones.

The genomic profiling of the 26 *E. coli* isolates reveals a significant burden of antibiotic resistance, particularly concerning β -lactamase production. The CTX-M gene was the most prevalent, appearing in 80.8% (n=21) of the samples. This finding aligns with global epidemiological shifts where CTX-M has supplanted TEM and SHV variants as the dominant ESBL genotype in Enterobacteriaceae (Bezabih *et al.*, 2021). The presence of CTX-M is clinically critical, as it confers high-level resistance to third-generation cephalosporins like cefotaxime, often complicating empirical treatment protocols.

In contrast, the TEM gene was identified in only 30.0% of isolates. While TEM-1 is traditionally associated with ampicillin resistance, its lower frequency relative to CTX-M suggests a highly evolved resistance profile within this specific cohort, likely driven by selective pressure from modern cephalosporin use. Furthermore, the data indicates a notable incidence of co-resistance: Fluoroquinolone Resistance: The QNR-B gene was present in 23.1% of isolates. Tetracycline Resistance: The TET-A gene showed a higher prevalence at 34.6%.

The co-occurrence of these genes—particularly in isolates like *E. coli* (11), (16), and (21) which tested positive for all four loci—highlights the rise of Multi-Drug Resistance (MDR). According to Partridge *et al.*, (2018), these resistance genes are frequently co-located on mobile genetic elements (plasmids), facilitating their rapid horizontal transfer between bacterial populations. The 76.9% sensitivity rate to QNR-B suggests that while fluoroquinolones may still be effective for a majority of these cases, the established presence of plasmid-mediated quinolone resistance (PMQR) genes poses a significant risk for future treatment failure.

CONCLUSION

Accordingly, *Escherichia coli* strains, the primary causative agents of urinary tract infections (UTIs), were isolated from urine samples collected from 157 patients who attended Shamiya State Hospital, Iraq, and were identified using both conventional and automated microbiological methods. The demographic characteristics, including age and sex, of the patients from whom *E. coli* strains were isolated were analyzed. The antimicrobial susceptibility profiles of the identified *E. coli* isolates were subsequently determined. In addition, the presence of the TET-A, QNR-B, CTX-M, and TEM resistance genes was investigated in the isolated strains. The findings of this study provide valuable insights that may support the development of effective prevention, management, and clinical strategies for infections caused by uropathogenic *E. coli* (UPEC) in the Shamiya region of Iraq.

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Author Contributions

This study is based on the Master's thesis of Ali Hameed Neamah ALHUSSEINI. The author was responsible for material preparation, data collection, analysis, and drafting the manuscript. All authors reviewed, edited, and approved the final version.

Competing Interests: The authors have no competing interests to declare.

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