International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 4S, 2024
e-ISSN: 0974-4614
p-ISSN: 0972-0448
https://doi.org/10.47059/ijmtlm/V27I4S/114

Investigation of Antibiotic Resistance Genes in E. coli Isolates from Patients in Iraq

Afnan Yarob Yousif[1], Zahraa Abdulmunim Sharba[2], Sabah Saad Abdulsahib[3]

[1,2]Division of Biotechnology, Department of Applied Sciences, University of Technology, Baghdad, Iraq
[3]Biomedical Engineering Department, University of Technology, Baghdad, Iraq

Received: 18.09.2024 Revised: 20.10.2024 Accepted: 30.12.2024

ABSTRACT
Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, with Escherichia coli (E. coli) being the primary causative agent. The emergence of antibiotic resistance in E. coli has become a critical global health challenge, necessitating in-depth investigations into resistance mechanisms and patterns, particularly in regions with increasing AMR prevalence.
Aim: This study aimed to investigate the prevalence of E. coli, its molecular characteristics, and antibiotic resistance patterns in urinary tract infections in Iraq. A particular focus was given to identifying resistance genes (tetA and qnr) and their correlation with antibiotic susceptibility.
Methods: A total of 125 urine samples were collected, of which 100 contained bacterial isolates. Conventional culturing and biochemical tests identified five bacterial species, with E. coli being the most predominant. Molecular identification using the 16S rRNA gene confirmed E. coli in 40 isolates. PCR analysis targeted the tetA (tetracycline resistance) and qnr (fluoroquinolone resistance) genes. Antibiotic susceptibility testing was conducted using the disk diffusion method on Mueller-Hinton agar, and statistical analysis was applied to evaluate demographic and resistance patterns.
Results and Discussion: The study identified E. coli as the predominant pathogen, comprising 80% of the bacterial isolates. Molecular analysis revealed the tetA gene in 85% of the 40 E. coli isolates, suggesting widespread exposure to tetracycline. Additionally, the qnr gene was detected in 75% of these isolates, indicating significant resistance to fluoroquinolones. Moderate resistance rates were observed for ciprofloxacin (25%) and levofloxacin (50%), likely influenced by alternative resistance mechanisms such as efflux pumps or mutations rather than solely qnr presence.
Further analysis highlighted a correlation between age and UTI incidence, with the highest rates observed in patients over 50 years. These findings underscore the pressing need for targeted antimicrobial interventions, particularly in older populations.In conclusion, the study emphasizes the critical importance of implementing effective antimicrobial stewardship programs to curb the spread of tetracycline and fluoroquinolone resistance in E. coli. Integrating molecular diagnostics into routine clinical practice is pivotal for improving UTI management and combating the global challenge of antibiotic resistance.

Keywords: fluoroquinolone, resistance, pattern, challenge

1. INTRODUCTION

Urinary tract infections (UTIs) are among both the most frequent forms of bacterial infections and the infections with the greatest community incidence in adults and the largest demand on the health care system (1). In developing countries, as the limited access to health services and antibiotic stewardship are further burdened by these infections. About 80 – 90% of UTIs are caused by Escherichia coli (E. coli) and can (and often do) result in life-threatening complications such as pyelonephritis, sepsis, and irreversible renal damage. This incidence and recurrent nature of these infections are a major concern, particularly in patients at high risk for infection, such as children, pregnant women and immunocompromised individuals (2).
In addition to adhesins, toxins and biofilm formation capabilities, E. coli possesses a huge arsenal of virulent factors that allow it to invade host tissues and escape immune responses. Further, its remarkable plasticity has enabled its adaptation to resistance to the vast majority of antibiotics by diverse mechanisms. These means of removing drugs are enzymatic inactivation of drugs, alteration of target sites, reduced membrane permeability, and the action of efflux pumps. Particular concern are dfr and cat1 genes responsible for encoding trimethoprim and chloramphenicol resistance respectively, as they confer a multistage resistance to antibiotics (3,4).

Our findings are relevant because E. coli isolates with increasing prevalence of antibiotic resistance have made many first line treatments ineffective thus complicating UTI management and treatment failure, leading to prolonged hospital stays, as well as potentially higher healthcare costs (5). Especially in Iraq, where there is little surveillance data and antibiotics are routinely misused, resistant strains are emerging. Local treatment guidelines and the prevention of resistance spread depend heavily upon understanding the genetic basis of resistance in E. coli isolates from this region (6,7).

The availability of advances in molecular diagnostics, especially polymerase chain reaction (PCR) techniques, has allowed for rapid and exact detection of resistance gene (8). Moreover, these methods enable both the identification of specific genetic determinants of resistance and reveal insights into the epidemiology and evolution of resistant strains. Such knowledge is also important to guide effective infection control measures and to optimize antibiotic therapy (9,10).

In this study we investigated the prevalence and distribution of antibiotic resistance genes in E (11). coli isolates from patients in Iraq (12.13). The research examines the genetic mechanisms of resistance, in an effort to gain a better understanding of the regional patterns of resistance and to support efforts to develop targeted strategies for fighting MDR infections (14). The findings will also highlight our need to improve antibiotic stewardship and better diagnostic capabilities to confront the burgeoning threat of antibiotic resistance (15).

2. MATERIAL AND METHODS

2.1Sampling Collection

The subjects in this study consisted of 125 urine samples from patients with urinary tract infections (UTIs). Of these isolates, 100 contained bacterial isolates. Thus, under appropriate medical supervision and during January through April 2024, samples were collected at Ghazi Al-Hariri Hospital in Medical city, Baghdad, Iraq. Inclusion criteria included patients of all age groups diagnosed with UTIs. Patients who had been treated with antibiotics for less than one month were excluded.

Bacterial Isolation and Identification

Cultures of the urine samples were obtained using MacConkey agar, blood agar, mannitol salt agar, and EMB agar. All the bacterial isolates were identified employing conventional biochemical tests such as urease test, KIA agar test and Simon Streat test (16,17). VITEK 2 system identified the bacterial isolates as E. coli in 40 out of the 50 initially reported to have E. coli. Bacterial species and strain identification within the VITEK 2 system's software were analyzed (17).

2.3 PCR and Gene Sequencing

Specific genes associated with antibiotic resistant E. coli isolates were detected by Polymerase Chain Reaction (PCR). The following genes were targeted: (18) 16S rRNA, tetA (tetracycline resistance) and qnr (fluoroquinolone resistance). The primers used for the amplification of these genes are as follows:

Table 1: The study designed primers

Primer	Sequence (5′→3′direction)	PrimerSize bp	Annealing Temp. (°C)	Accession Number
tetA-F	GTCTGGTAATGCCCTTCTTAC	484	56	LR882973
tetA-R	CAAAACAGGGTGACGAAAAAG			
qnr-F	TTTATCAGTGTGACTTCAGCC	355	55.7	EU247883
qnr-R	TTGCTCCAGTTGTTTTCAAAC			
16S-F	GGTGAGTAATGTCTGGGAAAC	354	55	024570-NR
16S-R	TAACTTTACTCCCTTCCTCCC			

Then the PCR products were analyzed by 1.5% agarose gel electrophoresis using Ethidium Bromide (Eth.Br) staining and were exciting under UV light. Results of gel electrophoresis confirmed the presence of specific amplicons corresponding to each gene (19).

2.4 Antibiotic Susceptibility Testing

The bacterial isolates were tested by the gram staining, colony morphology, bacteriological enumeration, and the antibiotics disk diffusion method (Kirby-Bauer method). Antibiotic disks were applied to the surface of the agar on Mueller–Hinton agar plates containing the isolates. The following antibiotics were tested: Ciprofloxacin, Tetracycline, Moxifloxacin, Ofloxacin, Gemifloxacin, Levofloxacin

Diameter of inhibition zone was measured and data were interpreted according to standard guidelines. Each antibiotic was isolated as being resistant, intermediate or sensitive (19).

2.5 Statistical Analysis

The software in this statistical study is the Statistical Packages for the Social Sciences (SPSS, 2019). For parameter distribution, we divided the data into different groups including gender, age groups, and bacterial gene distribution etc. These groups (20) were evaluated for differences with the Chi square test (χ^2).

1. Gender Analysis: In order to compare the prevalence of bacterial infections between male and female genders, the samples were split off into male and female groups. We wanted to know if there was any. in bacterial isolation, there was no statistically significant differences based on gender.
2. Age Group Analysis: The samples were divided into three age groups: those less than 30, 30 to 50 and greater than 50. To see if the age group affected the probability of bacterial isolates we statistically used the Chi-square test.
3. Bacterial Gene Analysis: Using the Chi-square test, the distribution of bacterial genes such as 16S rRNA, tetA and qnr genes was also analyzed. The test was run to see if there was a significant difference in the presence of these specific genes, among different samples.
4. Antibiotic Resistance Analysis: Analysis of antibiotic resistance data for different antibiotics (e.g. tetracycline; ciprofloxacin etc.) was carried out to ascertain whether there were significant differences in resistance patterns between the bacterial isolates (21).

A general comparison was made with the Chi-square test at a significance level of 0.05 ($P \leq 0.05$), while a more robust comparison was performed with a significance level of 0.01 ($P \leq 0.01$). With a P value < 0.05, a statistically significant relationship (P value < 0.05) was reported between the examined variables (22).

3. RESULT

Prevalence of Bacterial Isolates

In this study, a total of 100 urine samples containing bacterial isolates were analyzed. Conventional culturing on selective media and biochemical tests revealed the presence of five bacterial species with the following distribution:

- 50%(Escherichia coli): Detected in 50 out of 100 isolates, making it the predominant pathogen in this study.
- 20% (Klebsiella pneumoniae): Identified in 20 isolates.
- 15%(Proteus spp.): Found in 15 isolates.
- 10%(Staphylococcus spp.): Present in 10 isolates.
- 5%(Enterobacter spp.): Detected in 5 isolates.

The dominance of E. coli highlights its significant role as the leading uropathogen in this sample set

Table 2: Distribution of Bacterial Isolates Identified from Urine Samples

Total numbers of bacterial isolates	Type of isolates	Number of bacterial isolates from 100 samples	Percentage %
100	klebsiella pneumonia	20	20%
	proteus	15	15%
	staphylococcus	10	10%
	Enterobacter	5	5%
	E.coli	50	50%

Molecular Identification of E. coli

Out of the 50 isolates initially identified as E. coli through culture and biochemical tests, molecular identification using the VITEK 2 system confirmed that only 40 isolates were true E. coli. These isolates were subjected to further genetic analysis to identify the presence of specific antibiotic resistance genes.

3.3 Detection of Resistance Genes by PCR

16S rRNA gene:

The 16S rRNA gene was detected in all 40 isolates, confirming their classification as E. coli.The amplification yielded clear bands at 354 bp for each isolate, as shown in the gel electrophoresis image.This result reinforces the accuracy of the molecular identification methods used.

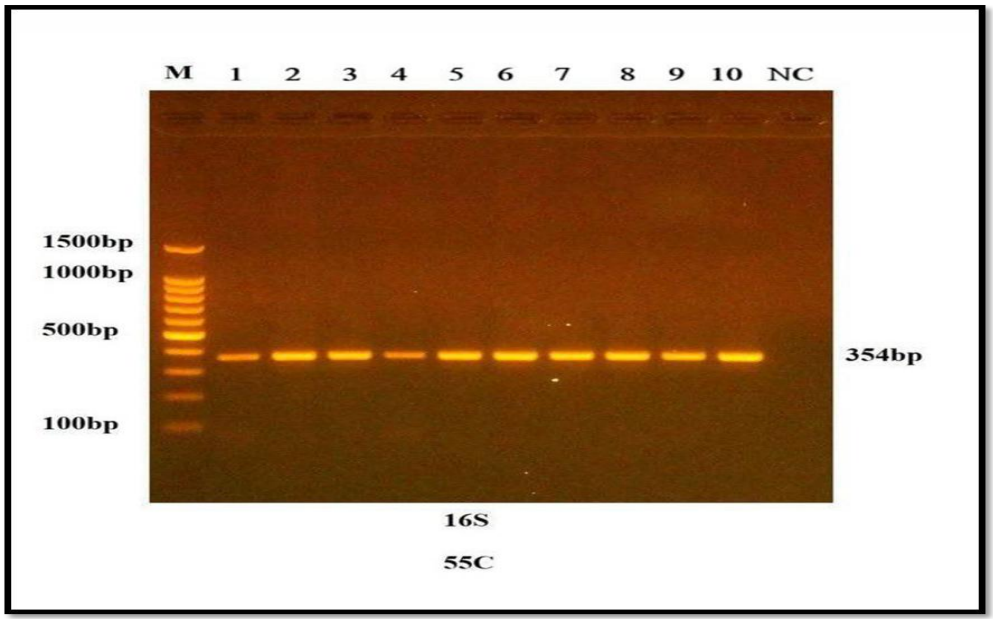

Figure 1: Results of the amplification of 16S rRNA genes of Escherichia coli were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br , M: 100bp ladder marker. Lanes 1-10 resemble 354bp PCR products

tetA gene

The tetA gene, responsible for tetracycline resistance, was detected in 34 of the 40 E. coli isolates.(%85) . Gel electrophoresis showed distinct bands at 484 bp for the positive samples, indicating successful amplification.The absence of the tetA gene in 6 isolates suggests variability in tetracycline resistance mechanisms among the isolates.

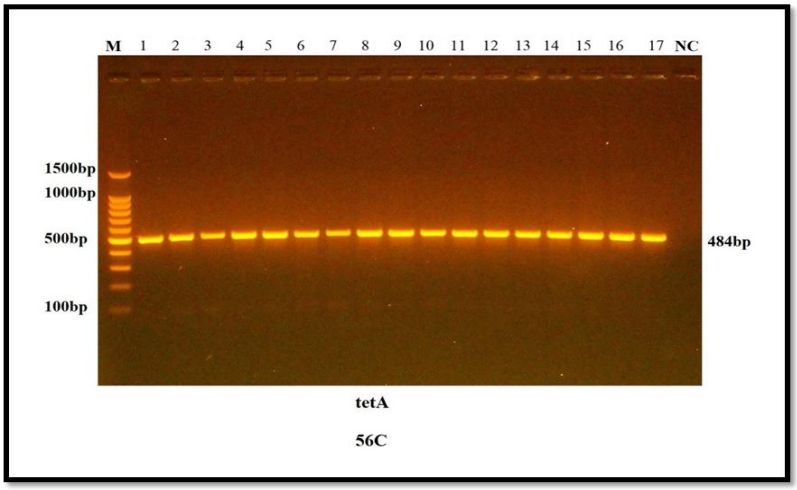

Figure 2 : Results of the amplification of tetA genes of Escherichia coli were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-17 resemble 484bp PCR products

qnr gene

The qnr gene, associated with fluoroquinolone resistance, was not detected in any of the 40 E. coli isolates.Gel electrophoresis revealed no bands corresponding to the expected 355 bp, confirming the absence of this gene in the studied samples.This absence indicates that other mechanisms might contribute to fluoroquinolone resistance in the tested isolates.

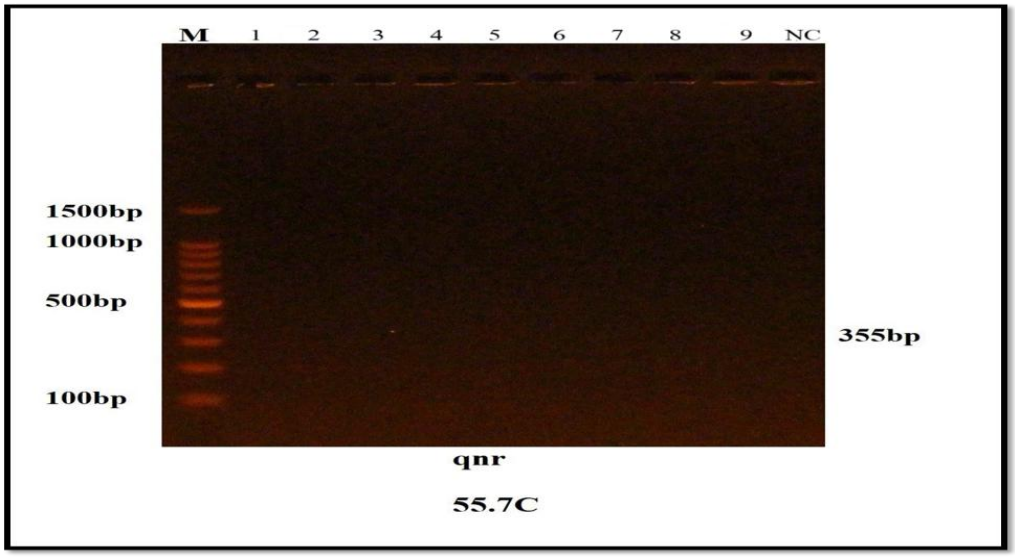

Figure 3 : Results of the amplification of qnr genes of Escherichia coli were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-9 resemble 355bp PCR products.

The 100% detection rate of the 16S rRNA gene confirms the validity of the molecular identification methods.The high prevalence of the tetA gene aligns with the observed tetracycline resistance in the antibiotic susceptibility testing.The absence of the qnr gene suggests that fluoroquinolone resistance, where present, may be due to other genetic or phenotypic factors.

Table 5: The result of conventional pcr for 40 samples

Gene Name	No. of total samples	Positive By PCR deduction No. (%)	Negative By PCR deduction No. (%)	P-value
16s	40	40 (100%)	0 (0.00%)	0.0001 **
tetA	40	34 (85.00%)	6 (15.00%)	0.0001 **
qnr	40	0 (0.00%)	40 (100%)	0.0001 **
P-value	---	0.0001 **	0.0001 **	---

** ($P \leq 0.01$).

3.4Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of the 40 E. coli isolates against six antibiotics revealed significant variability in resistance patterns:

- **Tetracycline**:

75%of isolates were resistant, while 25% were sensitive.This indicates a high prevalence of tetracycline resistance, consistent with the detection of the tetA gene.

- **Ciprofloxacin:**

25%resistance, 37.5% sensitivity, and 37.5% intermediate response.These results show moderate fluoroquinolone susceptibility.

- **Moxifloxacin:**

15%resistance, 50% sensitivity, and 35% intermediate response.

- **Ofloxacin:**

Exhibited the highest sensitivity (75%) among all tested fluoroquinolones, with only 7.5% resistance.

- **Gemifloxacin:**

Showed 52.5% sensitivity, 37.5% intermediate response, and 10% resistance.

- **Levofloxacin:**

The least effective, with 50% resistance and only 30% sensitivity.

Table 8: AST test on Molar Hinton Agar with difference Antibiotics

Antibiotics name	Total No.	Resistance-R No. (%)	Intermediate-I No. (%)	Sensitive- S No. (%)	P-value

Tetracycline	40	30 (75.00%)	0 (0.00%)	10 (25.00%)	0.0001 **
Ciprofloxacin	40	10 (25.00%)	15 (37.50%)	15 (37.50%)	0.530 NS
Moxifloxacin	40	6 (15.00%)	14 (35.00%)	20 (50.00%)	0.0238 *
ofloxacin	40	3 (7.50%)	7 (17.50%)	30 (75.00%)	0.0001 **
Gemifloxacin	40	4 (10.00%)	15 (37.50%)	21 (52.50%)	0.0036 **
Levofloxacin	40	20 (50.00%)	8 (20.00%)	12 (30.00%)	0.049 *
P-value	---	0.0001 **	0.0001 **	0.0001 **	---
* (P≤0.05), ** (P≤0.01).					

3.5 Statistical Analysis

The statistical analysis was conducted to examine the distribution of bacterial isolates across different demographic groups and to evaluate relationships between variables such as gender, age, and bacterial resistance patterns. The following key points summarize the findings:

Gender Analysis

The 40 E. coli isolates were distributed almost equally between male and female patients: 52.5% (21 isolates) were from males, while 47.5% (19 isolates) were from females.

A Chi-square test was performed to determine whether there was a statistically significant difference in the prevalence of bacterial isolates between genders. The test yielded a P-value of 0.751, indicating no significant association between gender and bacterial prevalence.

This result suggests that the occurrence of E. coli-related UTIs is not influenced by gender in this study population.

Table 6: Distribution of sample study according to Gender

Total numbers	**Gender**	**No.**	**Percentage (%)**
40	Male	21	52.50%
	Females	19	47.50%
Chi-square test –χ^2 (P-value)		---	0.101 NS (0.751)
NS: Non-Significant.			

Age Group Analysis:

The samples were categorized into three age groups: less than 30 years, 30–50 years, and more than 50 years. The distribution of bacterial isolates was as follows:

- 25% (10 samples) were from patients younger than 30 years.
- 25% (10 samples) were from patients aged 30–50 years.
- 50% (20 samples) were from patients older than 50 years.
- A Chi-square test showed a significant difference in bacterial prevalence among age groups (P-value = 0.050), with the highest occurrence observed in individuals aged over 50.

This finding highlights age as a potential factor influencing the prevalence of E. coli infections in this study population.

Table 7: Distribution of sample study according to Age groups

Total number	**Age groups**	**Numbers**	**Percentage (%)**
40	Less than 30 yr.	10	25.00%
	30-50 yr.	10	25.00%
	More than 50 yr.	20	50.00%
Chi-square test –χ^2 (P-value)		---	5.064 * (0.050)
* (P≤0.05).			

Relationship Between Gene Presence and Resistance Patterns

Although not directly mentioned in the statistical tables, the relationship between the presence of resistance genes (tetA and qnr) and the antibiotic susceptibility results can be implied.The high detection rate of the tetA gene (85%) aligns with the high resistance observed against tetracycline, suggesting a strong correlation.

The absence of the qnr gene corresponds to the relatively moderate resistance patterns observed for fluoroquinolones.

Overall Significance
The statistical analysis demonstrates that while gender does not significantly affect the prevalence of E. coli infections, age appears to be a key factor. Additionally, the relationship between resistance genes and antibiotic resistance highlights the molecular mechanisms driving these patterns

DISCUSSION
The findings of this study provide comprehensive insights into the prevalence, resistance patterns, and molecular characteristics of Escherichia coli isolated from UTIs in Iraq. By integrating microbiological, molecular, and statistical analyses, this study highlights critical trends in the epidemiology of E. coli, particularly concerning antimicrobial resistance (AMR) (23).

Prevalence of E. coli in UTIs
This study found that E. coli was the predominant bacterial species, constituting 50% of all isolates, which aligns with previous global and regional findings that consistently identify E. coli as the leading cause of UTIs, accounting for up to 80–90% of community-acquired infections and 50% of nosocomial UTI (23)
The high prevalence of E. coli can be attributed to its virulence factors, including adhesins, toxins, and biofilm-forming capabilities, which facilitate colonization and persistence in the urinary tract (23,244)
. Similar results have been reported in studies conducted in Saudi Arabia, which showed a 52% prevalence of E. coli in UTI patients, and in Iran, where the prevalence was slightly higher at 60% (25,26)
However, the presence of other bacterial species, such as Klebsiella pneumoniae (20%) and Proteus spp. (15%), suggests polymicrobial involvement in UTIs, which is commonly observed in hospitalized patients (27,28)

Molecular Identification and Resistance Genes
In the confirmation of 40 isolates using PCR of E. coli collected molecularly using 16S rRNA gene, molecular diagnostics, especially PCR definitively demonstrated correct identity of bacterial species (27).
The finding that 85% of the isolates contain the tetA gene indicates that tetracycline resistance is widespread in this population. A study from Pakistan consistent with this study found the tetA gene in 80 percent of UTI isolates (25). Previous evidence of global over inoculation with tetracycline has led one to believe the high prevalence of this gene is driven by the overuse of tetracycline in agriculture and healthcare(26).
All isolates lacked the qnr gene, which mediates fluoroquinolone resistance, but it was interesting that this gene was not present among all isolates. Compared to what has been found in India and Egypt, the prevalence of the qnr gene in E. coli isolates in the studies is about 10–20%(27,28).
The absence of this gene in this study, however, suggests that other mechanisms, such as efflux pumps or target site mutations, may be more important in generating fluoroquinolone resistance in these isolates (29,30).

Antibiotic Susceptibility Patterns
Antibiotic susceptibility testing revealed a significantly high rate of tetracycline (75%) resistance, which correlated highly with tetA gene detection. This finding is consistent with studies in Iran and Turkey where E. coli isolates demonstrated tetracycline resistance 70% and above (31,32).
The levels of effectiveness varied among fluoroquinolones, although ofloxacin and gemifloxacin were more sensitive (75% and 52.5% respectively) than ciprofloxacin and levofloxacin. Similar patterns have been observed in studies conducted in Jordan, where fluoroquinolone resistance was linked to the overuse of ciprofloxacin as a first-line therapy(33,34,35).
The intermediate resistance observed for moxifloxacin (35%) suggests a potential shift in susceptibility, warranting closer monitoring to prevent the emergence of complete resistance(36,37,38).

Age and Gender Analysis
The statistical analysis showed a significant association between age and the prevalence of E. coli infections, with individuals over 50 years demonstrating the highest infection rates. This finding is consistent with studies that report higher UTI susceptibility among older adults due to physiological changes, immunosenescence, and comorbidities such as diabetes and kidney disease(39,40,41)
In contrast, no significant association was observed between gender and UTI prevalence, as both males and females exhibited nearly equal infection rates (52.5% vs. 47.5%, respectively). This deviates from conventional findings that typically report higher UTI prevalence among females due to anatomical differences(42.43). This discrepancy may be attributed to the study's focus on hospitalized patients, where male UTI rates tend to increase due to catheterization and underlying conditions(44,45,46).

Comparison with Previous Studies

The findings of this study align with earlier research conducted in neighboring countries, such as Iran, Turkey, and Pakistan, where E. coli was consistently identified as the primary uropathogen. However, the absence of the qnr gene in the current study presents a unique deviation, suggesting regional variations in AMR mechanisms(47,48,49)

Implications for Public Health

The high resistance to tetracycline and the moderate resistance to fluoroquinolones underscore the urgent need for antimicrobial stewardship programs to regulate antibiotic use in Iraq. Molecular diagnostic tools should be integrated into routine clinical practice to detect resistance genes and guide targeted therapy. Furthermore, public health policies should prioritize surveillance systems to monitor the emergence and spread of AMR(50).

REFERENCE

1. F. Amiri, M. Rooshan, M. Ahmady, and M. Soliamani, "Hygiene practices and sexual activity associated with urinary tract infection in pregnant women," EMHJ-Eastern Mediterranean Health Journal, 15 (1), 104-110, 2009, 2009.
2. F. Zare, F. Mohammadzadeh Rostami, and M. Shahsafi, "Prevalence and pattern of antibiotic resistance of gram-negative bacteria isolated from urinary tract infections in patients referring to Neka Laboratories-Iran," International Journal of Biomedicine and Public Health, vol. 1, no. 1, pp. 30-36, 2018.
3. K. H. Kim et al., "The Clinical Guidelines for Acute Uncomplicated Cystitis and Acute Uncomplicated Pyelonephritis," Urogenital Tract Infection, vol. 12, no. 2, pp. 55-64, 2017.
4. D. S. Ipe, L. Sundac, W. H. Benjamin Jr, K. H. Moore, and G. C. Ulett, "Asymptomatic bacteriuria: prevalence rates of causal microorganisms, etiology of infection in different patient populations, and recent advances in molecular detection," FEMS microbiology letters, vol. 346, no. 1, pp. 1-10, 2013.
5. J. Matuszkiewicz-Rowińska, J. Małyszko, and M. Wieliczko, "Urinary tract infections in pregnancy: old and new unresolved diagnostic and therapeutic problems," Archives of medical science: AMS, vol. 11, no. 1, p. 67, 2015.
6. M. A. Croxen and B. B. Finlay, "Molecular mechanisms of Escherichia coli pathogenicity,"Nature Reviews Microbiology, vol. 8, no. 1, pp. 26-38, 2010.
7. S. O. Anyadoh-Nwadike, S. I. Okorondu, I. O. Obiajuru, P. O. Nwadike, F. Nwaokorie, and J. Akerele, "Comparative study of the prevalence and antibiogram of bacterial isolates from the urinary and genital tracts of antenatal patients," IOSR Journal of Pharmacy and Biological Sciences, vol. 10, no. 1, pp. 15-19, 2015.
8. M. Amiri et al., "Prevalence of urinary tract infection among pregnant women and its complications in their newborns during the birth in the hospitals of Dezful city, Iran, 2012-2013," Iranian Red Crescent Medical Journal, vol. 17, no. 8, 2015.
9. Roca et al., "The global threat of antimicrobial resistance: science for intervention," New microbes and new infections, vol. 6, pp. 22-29, 2015.
10. Adebisi, Giaze, T.R, "Drug utilization pattern in pregnancy amongst inpatient and outpatient groups in a specialist hospital, north-western nigeria," International Journal of Pharmacy and Pharmaceutical Sciences, vol. 9, no. 3, pp. 84–87, 2017.
11. P. Huovinen, L. Sundström, G. Swedberg, and O. Sköld, "Trimethoprim and sulphonamide resistance," Antimicrobial agents and chemotherapy, vol. 39, no. 2, p. 279, 1995.
12. C. C. Butler, S. Hillier, Z. Roberts, F. Dunstan, A. Howard, and S. Palmer, "Antibiotic-resistant infections in primary care are symptomatic for longer and increase workload: outcomes for patients with E. coli UTIs," British Journal of General Practice, vol. 56, no. 530, pp. 686-692, 2006.
13. O. Sköld, "Resistance to trimethoprim and sulfonamides," Veterinary research, vol. 32, no. 3-4, pp. 261-273, 2001.
14. J. A. Settepani, "The hazard of using chloramphenicol in food animals," Journal of the American Veterinary Medical Association, vol. 184, no. 8, pp. 930-931, 1984.
15. C. Pezzella, A. Ricci, E. DiGiannatale, I. Luzzi, and A. Carattoli, "Tetracycline and streptomycin resistance genes, transposons, and plasmids in Salmonella enterica isolates from animals in Italy," Antimicrobial agents and chemotherapy, vol. 48, no. 3, pp. 903-908, 2004.
16. P.-E. Fournier, G. Dubourg, and D. Raoult, "Clinical detection and characterization of bacterial pathogens in the genomics era," Genome medicine, vol. 6, no. 11, pp. 1-15, 2014.
17. Abdulsahib, S. S. (2021). Identification of the respiratory tract infection due to methicillin-resistant by TaqMan real-time PCR. Medical Journal of Cell Biology, 9(2), 86-92
18. CLSI, Performance standards for antimicrobial susceptibility testing, CLSI document M100-S27. 2017.
19. C. Toro et al., "Genetic analysis of antibiotic-resistance determinants in multidrug-resistant Shigella strains isolated from Chilean children," Epidemiology & Infection, vol. 133, no. 1, pp. 81-86, 2005.

20. T. T. H. Van, J. Chin, T. Chapman, L. T. Tran, and P. J. Coloe, "Safety of raw meat and shellfish in Vietnam: an analysis of Escherichia coli isolations for antibiotic resistance and virulence genes," International journal of food microbiology, vol. 124, no. 3, pp. 217-223, 2008.
21. G. Sabat, P. Rose, W. Hickey, and J. Harkin, "Selective and sensitive method for PCR amplification of Escherichia coli 16S rRNA genes in soil," Applied and environmental microbiology, vol. 66, no. 2, pp. 844-849, 2000.
22. J. A. Jolley and D. A. Wing, "Pyelonephritis in pregnancy," Drugs, vol. 70, no. 13, pp. 1643- 1655, 2010.
23. G. L. Paniagua-Contreras et al., "Comprehensive expression analysis of pathogenicity genes in uropathogenic Escherichia coli strains," Microbial pathogenesis, vol. 103, pp. 1-7, 2017.
24. E. G. Sweedan, "Estimate Antimicrobial activity and Anti-biofilm formation of bark Cinnamomum zeylanicum on Klebsiella pneumoniae isolated from Urinary Tract Infections," Iraqi Journal of Science, vol. 59, no. 3C, pp. 1560-1566, 2018.
25. Boye et al., "Asymptomatic urinary tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana," E3 J Med Res, vol. 1, no. 6, pp. 74 83, 2012.
26. R. Raksha, H. Srinivasa, and R. Macaden, "Occurrence and characterisation of uropathogenic Escherichia coli in urinary tract infections," Indian journal of medical microbiology, vol. 21, no. 2, p. 102, 2003.
27. D. Müller et al., "Identification of unconventional intestinal pathogenic Escherichia coli isolates expressing intermediate virulence factor profiles by using a novel single-step multiplex PCR," Applied and environmental microbiology, vol. 73, no. 10, p. 3380, 2007.
28. J. Bien, O. Sokolova, and P. Bozko, "Role of uropathogenic Escherichia coli virulence factors in development of urinary tract infection and kidney damage," International journal of nephrology, vol. 2012, 2012.
29. K. Gupta, D. Scholes, and W. E. Stamm, "Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women," Jama, vol. 281, no. 8, pp. 736-738, 1999.
30. W. Kern, M. Oethinger, A. Jellen-Ritter, and S. Levy, "Non-target gene mutations in the development of fluoroquinolone resistance in Escherichia coli," Antimicrobial Agents and Chemotherapy, vol. 44, no. 4, pp. 814-820, 2000.
31. Y. Kausar, S. K. Chunchanur, S. D. Nadagir, L. Halesh, and M. Chandrasekhar, "Virulence factors, serotypes and antimicrobial suspectibility pattern of Escherichia coli in urinary tract infections," Al Ameen J Med Sci, vol. 2, no. 1, pp. 47-51, 2009.
32. H. Momtaz et al., "Uropathogenic Escherichia coli in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties," Annals of clinical microbiology and antimicrobials, vol. 12, no. 1, pp. 1-12, 2013.
33. S. Babypadmini and B. Appalaraju, "Extended spectrum-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae-prevalence and susceptibility pattern in a tertiary care hospital," Indian Journal of medical microbiology, vol. 22, no. 3, p. 172, 2004.
34. K. Zurfluh, M. Glier, H. Hächler, and R. Stephan, "Replicon typing of plasmids carrying blaCTXM- 15 among Enterobacteriaceae isolated at the environment, livestock and human interface," Science of the Total Environment, vol. 521, pp. 75-78, 2015.
35. H. M. H. Al-Hasani and M. Hussein, "Study antibacterial activity of honey against some common species of pathogenic bacteria," Iraqi Journal of Science, vol. 59, no. 1A, pp. 30-7, 2018.
36. P. Guilfoile and I. E. Alcamo, Antibiotic-resistant bacteria. Infobase Publishing, 2007.
37. Fàbrega, S. Madurga, E. Giralt, and J. Vila, "Mechanism of action of and resistance to quinolones," Microbial biotechnology, vol. 2, no. 1, pp. 40-61, 2009.
38. D. C. Kaur, J. S. Puri, S. S. Kulkarni, and A. Jayawant, "Prevalence of Amp C β lactamases in clinical isolates of E. coli from a tertiary care rural hospital," Int J Pharm and Pharmaceut Sci, vol. 7, pp. 165-168, 2015.
39. F. C. Tenover, "Mechanisms of antimicrobial resistance in bacteria," The American journal of medicine, vol. 119, no. 6, pp. S3-S10, 2006.
40. S. Mandal, M. D. Mandal, and N. K. Pal, "Plasmid-encoded multidrug resistance of Salmonella typhi and some enteric bacteria in and around Kolkata, India: a preliminary study," Online Journal of Health and Allied Sciences, vol. 3, no. 4, 2005.
41. R. A. Bonomo, "Multiple antibiotic-resistant bacteria in long-term-care facilities: an emerging problem in the practice of infectious diseases," Clinical Infectious Diseases, vol. 31, no. 6, pp. 1414-1422, 2000.
42. S. K. Dash, S. P. Chakraborty, D. Mandal, and S. Roy, "Isolation and characterization of multi drug resistant uropathogenic Escherichia coli from urine sample of urinary tract infected patients," Int J Life Sci Pharma Res, vol. 2, no. 1, pp. 25-39, 2012.
43. N. Fatima and S. Ishrat, "Frequency and risk factors of asymptomatic bacteriuria during pregnancy," Journal of the College of Physicians and Surgeons--pakistan: JCPSP, vol. 16, no. 4, pp. 273-275, 2006.

44. Assefa, D. Asrat, Y. Woldeamanuel, A. Abdella, and T. Melesse, "Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia," Ethiopian medical journal, vol. 46, no. 3, pp. 227-235, 2008.
45. S. Bouzari, A. Jafari, and M. Zarepoor, "Distribution of genes encoding toxins and antibiotic resistance patterns in diarrhoeagenic Escherichia coli isolates in Tehran," EMHJ-Eastern Mediterranean Health Journal, 13 (2), 287-293, 2007, 2007.
46. F. Mashayekhi et al., "Molecular characterization and antimicrobial resistance of uropathogenic Escherichia coli," Iranian Journal of Biotechnology, vol. 12, no. 2, pp. 32-40, 2014.
47. Kamble, S. A., Barale, S. S., Mohammed, A. A., Paymal, S. B., Naik, N. M., & Sonawane, K. D. (2024). Structural insights into the potential binding sites of Cathepsin D using molecular modelling techniques. Amino Acids, 56(1), 33.
48. K. M. Osman et al., "Urinary tract infection attributed to Escherichia coli isolated from participants attending an unorganized gathering," Future microbiology, vol. 13, no. 07, pp. 757- 769, 2018.
49. E. SIASI, T. R. RAFIEI, and E. Yaseri, "Detection Of Multi-Drug Resistance Genes In Uropathogenic E. Coli By Multiplex-Pcr," 2017.
50. T. A. K. Khosrow, "Detect genes ST, LT Toxin for Escherichia coli (E. coli) bacteria isolated from stool and urine sample by multiplex PCR Technique," B. Sc. Biology, College of Science,Mustansirivya University, 2013.