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ISOLATION AND IDENTIFICATION OF ESCHERICHIA COLI FROM PATIENTS WITH URINARY TRACT INFECTIONS IN DIYALA PROVINCE

Osama Ghazi Jalil al-Dulaimi[1*]

[1]College of Veterinary Medicine, University of Diyala \ Diyala\ Iraq, https://orcid.org/0009-0008-2178-1623 ***Corresponding author:** email: osama.z@uodiyala.edu.iq

ABSTRACT

In addition to identifying pathological urinary tract infections and their connection to antibiotic resistance, this study attempts to isolate and diagnose E. coli bacteria. One of the most prevalent bacterial illnesses in the world is a urinary tract infection (UTI). The purpose of this study was to separate and identify Escherichia coli from patients in Iraq's Diyala area who had been diagnosed with UTIs. Under the guidance of a specialist physician, fifty clinical samples were taken from the urine of patients with UTIs from several hospitals in Diyala, including the Children's Central Teaching Hospital and Baqubah Hospital. Traditional techniques were used to identify the bacterial isolates, and molecular detection of the gene was used to validate the diagnosis.

Eight were found for E. coli using polymerase chain reaction technique.

The findings revealed that two (25%) isolates did not develop biofilms, while six (75%) isolates formed biofilms with various inclusions.

E. Coli bacteria are multi-resistant to antibiotics, according to the results of the antibiotic susceptibility test. Antimicrobial susceptibility tests were conducted on Mueller-Hinton agar using the Kirby Bauer disk diffusion method15 in accordance with standard operating procedures. The antimicrobial substances examined were: Carbenicillin, 75% (6) isolates resistant to the antibiotic erythromycin, 100% (8) isolates Resistant to the antibiotic rifampin, 88% (7) Isolate resistant to the antibiotic ceftazidime, 75% (6) Isolate resistant to the antibiotic cefotaxime, 75% (6) Isolate resistant to the antibiotic novobiocin, 62.5% (5) isolate resistant to the antibiotic tetracycline, 88% (7) isolate resistant to the antibiotic ciprofloxacin, 100% (8) isolates resistant to the antibiotic gentamicin and two isolates (25%) resistant.

Molecular detection was performed, and the results of the sequential analysis of the bacterial isolates with regard to the diagnostic gene 16SrRNA showed that they were 100% related to E. coli bacteria, as found in GenBank.

KEYWORDS: Escherichia coli; Urinary tract infection; UTI; Bacterial isolation; Identification; Urine culture; Diyala Province.

INTRODUCTION

UTIs are among the most frequent infections encountered in both outpatient and hospital settings. The condition arises when uropathogens gain access to and proliferate within the urinary tract, leading to symptoms such as dysuria, urgency, and suprapubic discomfort. The most frequently implicated bacterium is E. coli, due to its possession of adhesive pili and other virulence traits that facilitate its persistence in the urinary system. (Hooton, 2000; Ronald, 2003)

Escherichia coli is a gram-negative, bacillus-shaped, motile or non-motile, aerobic or facultative anaerobic, ferment the sugar lactose, most of which ferment rhamnose sugar and sorbitol sugar, producing the enzyme βglucuronidase, the optimum temperature for its growth (37C°) (Neil *et al*., 2009).

It is negative for the oxidase test, positive for the catalase test, and positive for the methyl red test and it produces indole (Hemraj *et al*., 2013).

known to be a component of normal intestinal flora, but it can also be the source of human extraintestinal and intestinal diseases. From mild, self-limited gastroenteritis to renal failure and septic shock, hundreds of E. coli strains have been found that cause a variety of illnesses. Because of its aggressiveness, E. coli can avoid host defenses and become resistant to standard antibiotics. E. Coli infections that cause intestinal disease and those that cause extraintestinal disease will be separated in this review.

Enterotoxigenic Escherichia coli (ETEC), enterohemorrhagic Escherichia coli (EHEC), also known as Shiga toxin-producing Escherichia coli (STEC) and referred to as EHEC/STEC, enter invasive Escherichia coli (EIEC), enteropathogenic Escherichia coli (EPEC), and enteroaggregative Escherichia coli (EAEC) are the E. coli

subtypes that cause intestinal illnesses. Clinical disease will be used to describe extraintestinal diseases. It is an opportunistic bacterium that naturally resides in both human and animal intestines.
Socioeconomic and healthcare constraints may contribute to the higher prevalence of UTIs in Diyala province. Poor sanitation, over-the-counter antibiotic use, and lack of routine laboratory diagnostics often hinder appropriate management and raise the risk of recurrent infections.
(Al-Badr & Al-Shaikh, 2013)
The identification of prevalent bacterial species and their local patterns of occurrence provide a foundation for improving diagnostic protocols and guiding empirical treatment.
(Stamm, 2002)
causes a variety of illnesses, including bacteremia, septicemia, meningitis, and diarrhea. More than 90% of urinary tract infections worldwide are caused by this species of bacterium, making it one of the most prevalent (Hadi et al., 2014).
The toxic necrosis factor for iron chelates is one of the numerous virulence factors that contribute to this bacterium's pathogenicity.
In addition to causing intestinal illness, E. coli causes infections outside of the intestine. One of five subtypes of E. coli can cause intestinal disease; these subtypes are distinguished by their O and H antigens. The flagellum defines the H antigen, while a repeating polysaccharide chain found in the lipopolysaccharide (LPS) outer membrane determines the O antigen.

ETEC is frequently found in food and water in places with poor sanitation and causes watery diarrhea in environments with inadequate resources. For a healthy individual to get unwell, about 100,000,000 organisms must be consumed. According to Regua et al. (1990), it is the most significant organism responsible for traveler's diarrhea. In areas with minimal resources, ETEC is also a major cause of dehydration diarrheal disease in infants and children.
The first E. Coli pathotype known to cause watery diarrhea, mostly in infants and young children in environments with low resources, was EPEC. It is also the cause of both sporadic and epidemic outbreaks. The most prevalent way that EPEC-caused diarrheal disease is acquired is by ingestion, but it can also transfer from person to person.
In both resource-rich and resource-poor areas, EAEC is a causal agent of both acute and chronic watery diarrhea. More recently, it has been linked to traveler's diarrhea.
Shiga-toxin is produced by EHEC/STEC, which comprises serotypes O157:H7 and others (Karch et al., 2001). Large-scale diarrheal epidemics caused by undercooked meat and contaminated food (such as spinach, sprouts, lettuce, and fruit) have been linked to EHEC/STEC. Raw dairy product consumption is associated with EHEC/STEC. Ground beef is frequently infected with EHEC/STEC during processing. When crops are fertilized with manure that contains EHEC/STEC, vegetables become polluted, and water runoff from these crops contributes to the presence of EHEC/STEC in water systems. Illness is caused by relatively modest inoculums (102 CFU), which makes it easier for individuals to infect one another and the environment.

2.MATERIALS AND METHODS 2.1

Samples and bacterial isolation

Fifty urine samples were taken from patients at the Central Child Teaching Hospital and Baqubah Teaching Hospital in Baqubah who had signs of a urinary tract infection between June 2023 and January 2024. Mid-urine collection containers were used to gather samples early in the morning. Following collection, each sample was cultured by inoculating differentiation media with 100 microliters of urine, then incubating the mixture for 24 hours at 37°C. The samples were centrifuged for five minutes at 200 rpm after incubation, and the precipitate was mixed for thirty seconds. A drop of the precipitate was put on a glass slide and examined at 40x magnification using an optical microscope. A positive result was indicated by the presence of 10 or more white blood cells in the viewing field and 50-200 pure colonies growing on the culture plate.

2.2 Identification of Bacteria

The phenotypic characteristics of the isolated colonies were analyzed after the bacterial isolates were cultivated and purified on a variety of culture media (MacConkey medium, intestinal hecton agar medium, blood agar medium). Urine samples were streaked onto MacConkey and EMB agars and then incubated at 37°C for a full day. Presumptive E. coli colonies were identified by their characteristic metallic green shine on EMB and pink appearance on MacConkey. Cheesbrough (2006). We looked at the size, shape, texture, color, edges, and heights of the isolated bacterial colonies (Wanger et al., 2017).

2.3 Microscopic identification

produced smears of bacterial isolates using Gram technology following their 18–24 hour cultivation on Macaroni agar medium. Using a light microscope, the cells' shape, arrangement, and colors were evaluated based on their interactions with the Gram stain (Levinson et al., 2016). The selected colonies were subjected to a range of biochemical tests, such as the oxidase test, Triple Sugar Iron (TSI) agar, and IMViC (Indole, Methyl Red, VogesProskauer, and Citrate). The isolates were found to fit the recognized biochemical profile of E. coli. (Forbes and others, 2007)

2.4 Biochemical Identification

1. Oxidase Test

The 24-hour-old bacterial growth on MacConkey agar was transferred onto a sterile filter paper using a sterile wooden applicator stick. Then, a drop of freshly prepared oxidase reagent was added. The formation of a purple color within 30 to 60 seconds was considered as a positive reaction as this color was due to the presence of the cytochrome oxidase enzyme (Hemraj et al., 2013).

2. Catalase Examination

A 24-hour-old bacterial colony that was grown on MacConkey agar was transferred to a sterile glass slide using a sterile wooden applicator stick. The bacterial colony was then treated with drop of 3% hydrogen peroxide. The instant formation of the gas bubbles was found as a suitable result for catalase enzyme activity (Hemraj et al., 2013).

3. Indole Test

The bacteria were placed into separate test tubes containing peptone water, and then incubated at 37°C for an entire day. After the incubation time, two to three drops of Kovac's reagent were added. A positive indole reaction was considered to be the formation of a red ring at the surface of the medium (Forbes et al., 2007).

4. Test for Urease

The bacterial isolates were streaked over the surface of urea agar slants in tubes, and the tubes were then incubated for 24 hours at 37°C. The ability of the bacteria to manufacture the urease enzyme was indicated by a change in the medium color to pink, which was seen as a positive reaction (Tille et al., 2017). **2.5 Antibiotic Susceptibility Testing**

The susceptibility of the isolated Escherichia coli cultures to the antibiotics was determined by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The following standard susceptibility testing procedures were followed throughout the procedure. In short, 3-5 well-isolated colonies of bacteria after 22-24 hours from MacConkey agar culture were placed in a 5mL tube of sterile normal saline. The turbidity of the bacterial suspension was standardized to around 1.5×10^2 CFU/mL or 0.5 McFarland. Mueller-Hinton Agar plates were evenly inoculated with the entire surface by immersing a sterile cotton swab in the standardized bacterial slurry. After the inoculation plates have been standing for approximately 10–15 minutes, antibiotic disks are placed on the surface of the agar using sterile forceps. The plates were then incubated at 37°C for an entire day. The susceptibility test made use of the following antibiotic disks:

Amoxicillin (AMX), 10 µg

- Ciprofloxacin (CIP), 5 µg
- Gentamicin (GEN), 10 µg
- Nitrofurantoin (F), 300 µg
- Ceftriaxone (CRO), 30 µg
- Trimethoprim–sulfamethoxazole (SXT), 25 µg

Following incubation, the diameters of the inhibition zones were measured in millimeters and interpreted according to the applicable Clinical and Laboratory Standards Institute (CLSI) criteria. **2.6 Genomic DNA Extraction**

The genomic DNA was extracted from confirmed E. coli isolates by boiling (Sambrook & Russell, 2001) with the addition of minor modifications. An 18–24-hour bacterial culture was taken in the loop and centrifuged at 12,000 rpm for 5 min and the pellet was then resuspended in 200 µL of sterile distilled water. The suspension was centrifuged once more for five minutes at 12,000 rpm after being cooked at 100°C for ten minutes and chilled on ice for five minutes. The supernatant containing DNA was gathered and kept at -20°C. DNA concentration, purity and integrity were measured using a NanoDrop spectrophotometer and 1% agarose gel electrophoresis, respectively.

3. RESULTS

3.1 Bacterial Isolation and Identification

The total urine sample collected from the patients who were suffering with symptoms of U.T.I is 50 urine samples. Eight isolates were phenotypically identified as Escherichia coli by biochemical tests (conventional microbiological methods).

Additional confirmation was provided using molecular detection, by polymerase chain reaction (PCR) of the 16S rRNA gene. Results showed that all 8 isolates were E. coli (100%): and sequence analysis revealed 100% similarity with E. coli sequences in GenBank.

Biofilm Formation

Of the eight E. coli isolates, six (75.0%) were biofilm producers and two (25.0%) were non-biofilm producers. More isolates were biofilm producers, but the difference didn't meet the criteria for statistical significance ($P = 0.289$, exact binomial test).

Biofilm phenotype	No. of isolates	Percentage	P Value
Biofilm producers	6	75%	(P = 0.289)
Non-biofilm producers	2	25%	
Total	**8**	**100%**	

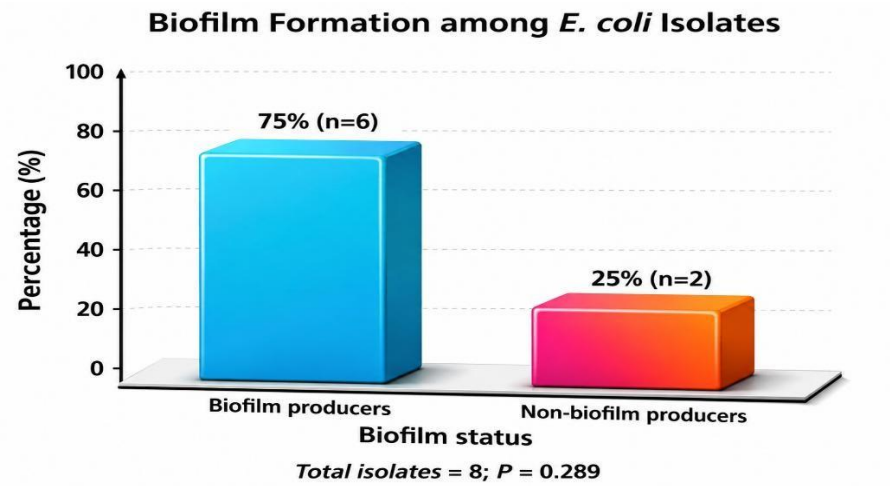

Figure 1. Distribution of biofilm-producing and non-biofilm-producing E. coli isolates.

Antibiotic Susceptibility Testing

Table 2 demonstrates that the pattern of antimicrobial resistance was diverse for the different antibiotics tested. Erythromycin and ciprofloxacin showed the highest resistance rates (100%) followed by rifampin and tetracycline (87.5%). The resistance rates of 75.0% for carbenicillin and 75.0% for ceftazidime, as well as 62.5% for novobiocin, were all comparable. Resistance rates were 75.0% each for carbenicillin, ceftazidime and novobiocin, all of which were comparable. Gentamicin has the lowest resistance rate (25.0%). The results of the statistical analysis showed that there was a difference between the resistance frequencies of the tested antimicrobial agents, which was [statistically significant/not statistically significant] (P = [value]). The results showed that there is significant variation among the urinary E. coli isolates in resistance and sensitivity to various classes of antimicrobial drugs.

Table 2. Antibiotic resistance profile of *E. coli* isolates according to antimicrobial class

Antibiotic	Antimicrobial class	Resistant isolates (n)	Resistance (%)
Carbenicillin	Penicillins (β-lactams)	6	75.0
Erythromycin	Macrolides	8	100
Rifampin	Rifamycins	7	87.5
Ceftazidime	Third-generation cephalosporins (β-lactams)	6	75.0
Cefotaxime	Third-generation cephalosporins (β-lactams)	6	75.0
Novobiocin	Aminocoumarins	5	62.5
Tetracycline	Tetracyclines	7	87.5
Ciprofloxacin	Fluoroquinolones	8	100
Gentamicin	Aminoglycosides	2	25.0

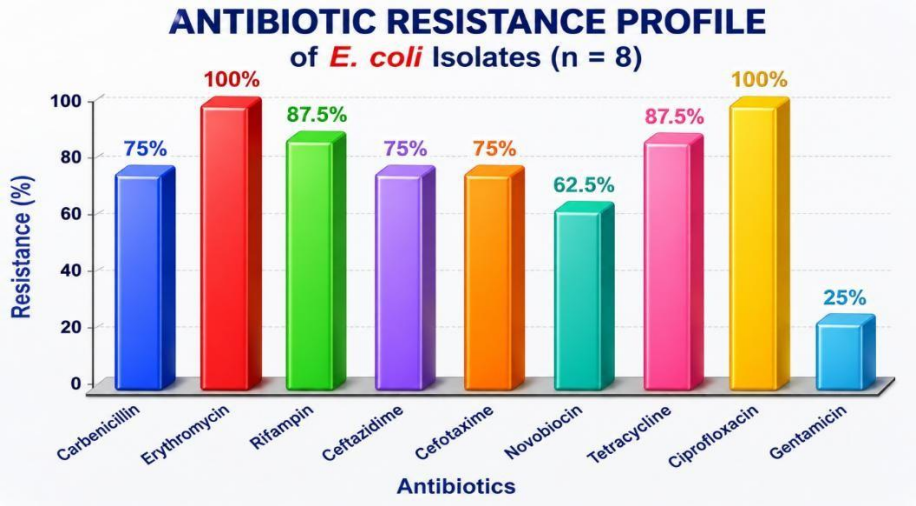

Figure 2. Antibiotic resistance profile of Escherichia coli isolates.

All eight isolates (100%) were found to be resistant against erythromycin and ciprofloxacin, the highest rates of resistance. Seven isolates (87.5%) were resistant to rifampin and tetracycline, and six isolates (75%) were resistant to carbenicillin, ceftazidime and cefotaxime. Five (62.5%) were resistant to novobiocin, while gentamicin had the lowest resistance rate with only 2 (25%) resistant. In general the isolates were highly resistant to a number of antimicrobial agents.

PCR Detection of the 16S rRNA Gene

All eight isolates tested positive for 16S rRNA gene by PCR, and yielded an amplicon of ~1500 bp. All tested isolates were confirmed as E. coli by the molecular results. The sequences were also identified as being 100% identical to the reference E. coli sequences deposited in GenBank.

Table 3. PCR detection of the 16S rRNA gene in *E. coli* isolates

Isolate No.	PCR Result	Product Size (bp)	Interpretation
E1	Positive (+)	1500	Genetically confirmed *E. coli*
E2	Positive (+)	1500	Genetically confirmed *E. coli*
E3	Positive (+)	1500	Genetically confirmed *E. coli*
E4	Positive (+)	1500	Genetically confirmed *E. coli*
E5	Positive (+)	1500	Genetically confirmed *E. coli*
E6	Positive (+)	1500	Genetically confirmed *E. coli*
E7	Positive (+)	1500	Genetically confirmed *E. coli*
E8	Positive (+)	1500	Genetically confirmed *E. coli*

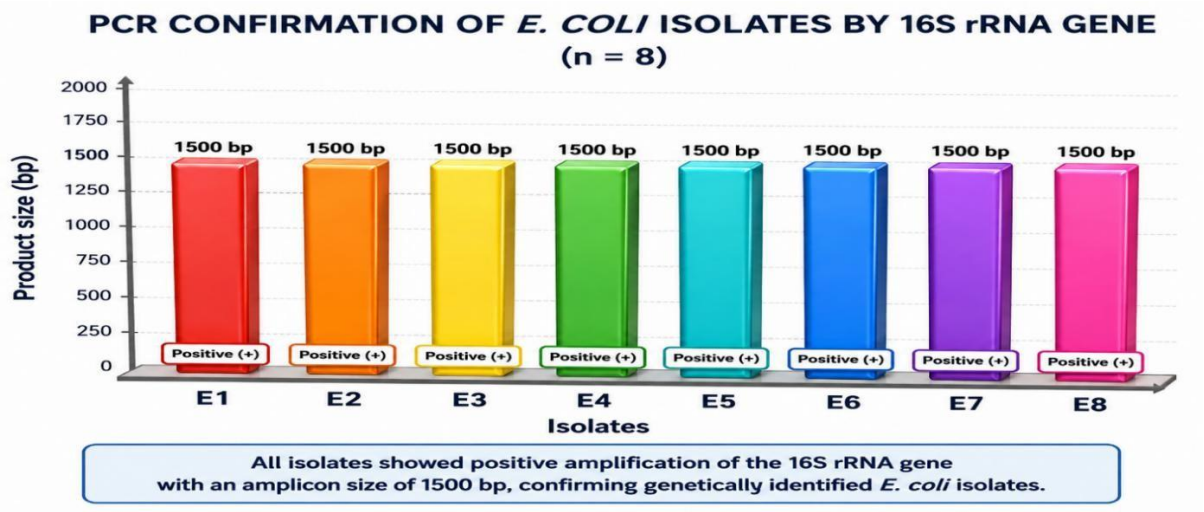

Figure 3. PCR confirmation of Escherichia coli isolates based on amplification of the 16S rRNA gene (1500 bp).

PCR Analysis Correlation with Results:

Using the conventional microbiological methods, eight isolates were initially identified as Escherichia coli, and then confirmed by PCR analysis. The highly conserved 16S rRNA gene was targeted for bacterial identification. All eight isolates were confirmed as E. coli (100%) and sequence analysis allowed for 100% identity of isolates with the reference E. coli sequences deposited in GenBank, confirming the reliability of the initial identification.

In terms of biofilm formation, 6/8 (75%) of the molecularly confirmed E. coli isolates were able to form biofilm. The prevalence of the E. coli isolates in this study that form biofilm could be a factor in the persistence of the bacteria, adherence to surfaces and decrease in susceptibility to treatment in U.T.I. cases.

Antibiotic Resistance:
There was a high level of resistance for some of the antibiotics that were tested with the E. coli isolates, including erythromycin and ciprofloxacin. The results showed that the urinary isolates had a high degree of antimicrobial resistance. The definition of multidrug-resistant (MDR) should not include resistance to a single agent in three or more classes of antimicrobials. The results of the PCR and 16S rRNA sequencing verified the observed trends and patterns of antibiotic resistance and biofilm formation did not correlate with other bacteria.

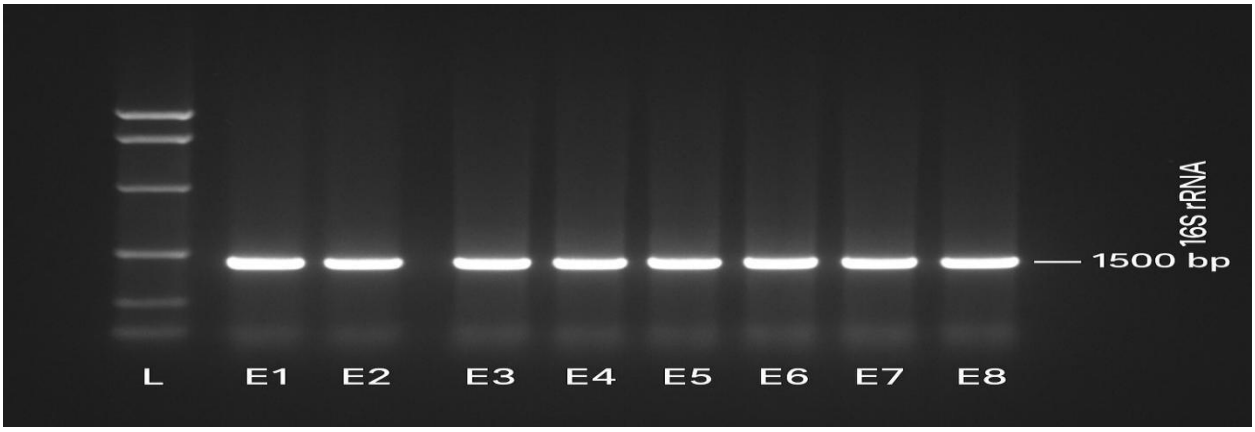

Figure 4. PCR amplification of the bacterial 16S rRNA gene.

Lane L: DNA ladder; lanes E1–E8: bacterial isolates showing amplified 16S rRNA gene products of approximately 1500 bp. The PCR products were subsequently subjected to 16S rRNA gene sequencing for molecular identification.

DISCUSSION
The present study validated the identification of Escherichia coli isolates obtained from patients with UTI by both conventional microbiological methods and molecular methods. One of the most common bacteria causing UTIs is known as uropathogenic E. coli (UPEC). A recent study carried out in Baghdad, Iraq, revealed that E. coli was responsible for 63.2% of all UTIs grown in the urinary samples, which shows the significant role of E. coli as a urinary pathogen in the population of Iraq (Salman et al., 2026). In the present study, all eight isolates were found positive when targeted 16S rRNA gene was amplified using the PCR technique. The conventional identification results were confirmed by subsequent sequence analysis which revealed 100% similarity with reference E. coli sequences deposited in GenBank. The 16S rRNA gene is still one of the most popular and widely used molecular markers for bacterial identification and taxonomical characterization (Li et al., 2024).

All E. coli isolates (6 of 8, 75%) were found to be capable of forming biofilms. This result is similar to previous study conducted in Baghdad, where 71.7% of the isolates of UPEC formed biofilms (Salman et al., 2026). In the same way, high rates of production of biofilms have been observed in other recent studies on UPEC isolates (Mahshouri et al., 2025; Oliveira et al., 2025). Biofilm formation is a virulence-associated characteristic of UPEC as it contributes to bacterial adherence and persistence in the urinary tract that could confer an advantage in resisting antimicrobial actions and host immune mechanisms. These qualities can play a role in a chronic, recurrent UTI.

The antimicrobial susceptibility results showed high level resistance to some of the antimicrobial agents tested. The highest resistance rate was found to be against erythromycin and ciprofloxacin (100%), while the lowest resistance rate was recorded with gentamicin (25%). In recent Iraqi and international studies, high levels of antimicrobial resistance in UPEC isolates have been reported. In Baghdad, Salman et al. (2026) showed high resistance levels in E. coli isolates from urine samples and Mahshouri et al. (2025) found that 77% of the E. coli strains collected from urine were multidrug resistant. It is also worth noting that a high percentage (60%) of the UPEC isolates were found to be multidrug resistant by Oliveira et al. (2025), which highlights the ongoing emergence of antibiotic resistance in UPEC strains.

The current study found high resistance to ciprofloxacin, which is significant as resistance to fluoroquinolones may significantly reduce therapeutic choices. In the current clinical practice, the selection of an antibiotic for UTI should be based on susceptibility patterns in the region, and personal culture results, especially in regions where antimicrobial resistance is common. Hence, on-going local monitoring of antimicrobial resistance is critical to aid in the selection of the appropriate antimicrobial agents and antimicrobial stewardship.

Although resistance to several antimicrobial classes was detected, classification of individual isolates as multidrug-resistant (MDR) should be based on standardized criteria. The concept of MDR is usually defined as the non-susceptibility to at least three antimicrobial categories, and there is no minimum requirement for the number of drugs. (Magiorakos et al., 2012). Therefore, MDR should be determined based on the antibiotic susceptibility of each isolate and not only on the average percentage of resistance among all of the present isolates.

The tendency for UPEC to form biofilms, coupled with their antimicrobial resistance, suggests that these traits may play a role in the persistence of UPEC infection. However, it was not concluded that the direct positive correlation between the formation of biofilm and antimicrobial resistance should be expected without statistical analysis of the isolates. Some studies have shown that these charac are related in a variable way. Some studies have shown that there is a statistically significant inverse correlation between biofilm formation and antimicrobial resistance in UPEC isolates (Alshaikh et al., 2024), while others have revealed UPEC isolates with a strong biofilm production and MDR (Mahshouri et al., 2025; Oliveira et al., 2025). Hence, larger studies with higher numbers of clinical isolates and direct statistical analysis of the intensity of the biofilm to compare the individual antimicrobial resistance profiles of UPEC isolates in Diyala Governorate should be performed to better determine this relationship among UPEC isolates circulating in Diyala Governorate.
Overall, the molecular confirmation, high prevalence of biofilm formation, and substantial antimicrobial resistance observed in the present study highlight the clinical importance of UPEC among patients with urinary tract infections. These findings emphasize the need for continuous antimicrobial resistance surveillance, appropriate antibiotic stewardship, and further molecular investigation of virulence and resistance determinants among urinary E. coli isolates in Diyala Governorate.

REFERENCES

1. Abdul Hamid, S. A., & Khoshabeh, R. M. (2024). Antibiotic resistance, biofilm formation, and identification of FimH and FimA adhesion genes in uropathogenic *Escherichia coli* (UPEC) isolated from patients in Baghdad Province. *Iraqi Journal of Science, 65*(10), 5546–5554. https://doi.org/10.24996/ijs.2024.65.10.19
2. Alshaikh, S. A., El-Banna, T., Sonbol, F., & Farghali, M. H. (2024). Correlation between antimicrobial resistance, biofilm formation, and virulence determinants in uropathogenic *Escherichia coli* from an Egyptian hospital. *Annals of Clinical Microbiology and Antimicrobials, 23*, 20.
3. Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). *Bailey & Scott's diagnostic microbiology* (12th ed.). Mosby Elsevier.
4. Harley, J. P., & Prescott, L. M. (2002). *Laboratory exercises in microbiology* (5th ed.). McGraw-Hill.
5. Hemraj, V., Diksha, S., & Avneet, G. (2013). A review on commonly used biochemical tests for bacteria. *Innovare Journal of Life Sciences, 1*(1), 1–7.
6. Ibrahim, A. A., & Mohammed, R. K. (2024). Bacteriological and molecular study of fosfomycin resistance in uropathogenic *Escherichia coli*. *Brazilian Journal of Microbiology, 55*(2), 1091–1097. https://doi.org/10.1007/s42770-024-01272-7
7. Karch, H., & Bielaszewska, M. (2001). Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157− strains: Epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. *Journal of Clinical Microbiology, 39*(6), 2043–2049.
8. Levinson, W. (2016). *Review of medical microbiology and immunology* (14th ed.). McGraw-Hill Education.
9. Li, M.-N., Han, Q., Wang, N., Wang, T., You, X.-M., Zhang, S., Zhang, C.-C., Shi, Y.-Q., Qiao, P.-Z., Man, C.-L., Feng, T., Li, Y.-Y., Zhu, Z., Quan, K.-J., Xu, T.-L., & Zhang, G. F. (2024). 16S rRNA gene sequencing for bacterial identification and infectious disease diagnosis. *Biochemical and Biophysical Research Communications, 739*, 150974. https://doi.org/10.1016/j.bbrc.2024.150974
10. Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*(3), 268–281. https://doi.org/10.1111/j.14690691.2011.03570.x
11. Mahshouri, P., Alikhani, M. Y., Momtaz, H. E., Doosti-Irani, A., et al. (2025). Analysis of phylogroups, biofilm formation, virulence factors, antibiotic resistance and molecular typing of uropathogenic *Escherichia coli* strains isolated from patients with recurrent and non-recurrent urinary tract infections. *BMC Infectious Diseases, 25*, 267. https://doi.org/10.1186/s12879-025-10635-w

12. Mostaghimi, T., Pournajaf, A., Bijani, A., et al. (2024). Phylogenetic analysis, biofilm formation, antimicrobial resistance and relationship between these characteristics in uropathogenic *Escherichia coli*. *Molecular Biology Reports, 51*, 327. https://doi.org/10.1007/s11033-023-09031-x
13. Neil, K. P., Biggerstaff, G., MacDonald, J. K., et al. (2012). A novel vehicle for transmission of *Escherichia coli* O157 to humans: Multistate outbreak of *E. coli* O157 infections associated with consumption of ready-tobake commercial prepackaged cookie dough—United States, 2009. *Clinical Infectious Diseases, 54*, 511–518.
14. Oliveira, M. C. F., Canellas, A. L. B., Berbert, L. C., Cardoso, A. M., et al. (2025). Assessment of antimicrobial resistance and virulence of biofilm-forming uropathogenic *Escherichia coli* from Rio de Janeiro. *Antibiotics, 14*(9), 869. https://doi.org/10.3390/antibiotics14090869
15. Regua, A. H., Bravo, V. L., Leal, M. C., & Lobo Leite, M. E. (1990). Epidemiological survey of the enteropathogenic *Escherichia coli* isolated from children with diarrhoea. *Journal of Tropical Pediatrics, 36*(4), 176–179.
16. Saeedi, N., Vatani, M., Nejat, S. H., et al. (2025). Multiplex PCR amplification for the detection of biofilm and extended-spectrum beta-lactamase resistance genes and antibiotic resistance patterns in uropathogenic *Escherichia coli*. https://doi.org/10.32592/ARI.2025.80.1.125
17. Salman, H. S., Majeed, H. M., & Shallal, Y. F. (2026). Prevalence, etiology, and antibiotic resistance patterns of urinary tract infections in a Baghdad hospital: Focus on uropathogenic *Escherichia coli* and virulence factors. *International Journal of Microbiology, 2026*, 9117996. https://doi.org/10.1155/ijm/9117996
18. Saman, Siddique, A. B., Aslam, B., & Nawaz, Z. (2026). Biofilm formation and antibiotic resistance in uropathogenic *Escherichia coli*: A molecular characterization and antibiogram study. *Microbial Drug Resistance, 32*(1), 24–30. https://doi.org/10.1177/10766294251389587
19. Tille, P. M. (2017). *Bailey & Scott's diagnostic microbiology* (41st ed.). Elsevier.
20. Wanger, A., Chavez, V., Huang, R. S. P., Wahed, A., Actor, J. K., & Dasgupta, A. (2017). *Microbiology and molecular diagnosis in pathology*. Elsevier.