KeAi CHINESE ROOTS GLOBAL IMPACT

International Journal of Innovation Studies

PHYLOGENETIC DIVERSITY AND ANTIBIOTIC RESISTANCE OF UROPATHOGENIC *ESCHERICHIA COLI* AMONG PATIENTS IN DIYALA

Dina N.A. Al-Obeidi[1], Hadi R.Rasheed Al-Taai[2], Mubarak K.I. [3]

[1,2,3] College of Science, Diyala University

dinanazar777@gmail.com

Abstract

Background and Objectives: *Escherichia coli* considered most important cause of Urinary tract the assessment of antimicrobial susceptibility is essential for establishing the extent of the issue and choosing suitable antimicrobial agents. The current study aimed to map the spread of UroPathogenic *E. coli* (UPEC) based on genetic profiles and identify resistance patterns among the isolates. The study also wanted to find the phylogenetic groups of *E. coli* in Baquba, Iraq, using the Claremont phylogenetic classification method and look at how resistant they were to antibiotics.

Materials and Methods: This study employed biological correlations to study the patterns of antibiotic resistance and the distribution of phylogenetic groups of 105 isolates of *E. coli* and the relationship between them, which were isolated from two hospitals in Diyala, Iraq.

Results: The results of phylogenetic analysis of *E. coli* isolates Phylogenetic group B2 was the most prevalent (40%), B1 and D (each (20%), and C and A (6.6%). Ampicillin showed highest resistance against antibiotics, while imipenem the lowest resistance .were the percentage equal to 95% and 54.3 % respectively. Group B2 showed the highest prevalence of antibiotic resistance, reaching 40%. Accordingly, 57 (54.3%) isolates were MDR and 40 (38.1%) were multidrug resistant. Furthermore, only 24 isolates (22.8%) were persistent and survived in stressful environments.

Conclusions. Our findings revealed a high prevalence of MDR *E. coli* isolates, with group B2 dominating. Such studies need to be done also in other regions to provide a greater understanding of the antibiotic resistance pattern and the prevalences of different phylogenetic groups

Keywords: Uropathogenic persistent *E.coli*, phylogrouping, antibiotic resistance.

Introduction:

Urinary tract infections are one of the most common infectious diseases worldwide, with an estimated 120-150 million cases each year, with significant morbidity and high medical costs (McCann *et al*., 2020). *E. coli*, a member of the Enterobacteriaceae family considered most often responsible for UTIs. These bacteria can adapt to their surroundings and have virulence factors that help them stay alive and cause disease (Gajdacs *et al*., 2021). UTIs account for 40% of all nosocomial infections and contribute to 50% of infections caused by bacteria, leading to greater illness and longer hospital stays (Asadi Karam *et al*., 2019). More than 90% of Community-Acquired UTIs (CA-UTI) and nearly 50% of Hospital-Acquired UTIs (HA-UTI) are caused by UPEC (Sheppard *et al*., 2018).

According to Zhou *et al*. (2023), many factors complicate development. These elements aid the virus's ability to endure and adapt in the urinary tract, evade the immune system, and remain there (Zhou *et al*., 2023). Persistent UPEC virulence factors include biofilm formation (Terlizzi *et al*. 2017). Curli fimbriae help the UPEC stick together, and ferric siderophores are crucial for survival. Blocking phagocytosis and antiserum activities, capsular polysaccharide synthesis K1 helps protect UPEC and avoid an immune response. (Kudinha, 2017).

More importantly, persistent UPEC had high rates of antimicrobial resistance (Pasillas Fabian *et al*. 2021). Multidrug-resistant (MDR) and extended-spectrum lactamase-producing *E. coli* are becoming more common in UTIs (Pasillas Fabian *et al*. 2021). Probably one of the important features of UPEC is their ability to grow intracellularly within the intracellular environment (Mann *et al*., 2017). The increasing prevalence of MDR-UPEC leads to 700,000 fatalities globally (Dutescu *et al*., 2021).

Most phylogenetic groups are B2 (Mahmoud *et al*., 2020). Many antibiotics, like β-lactams, fluoroquinolones, tetracycline's, and aminoglycosides, were becoming less effective (Masoud et *al*., 2021).El-Baz *et al*. (2022) report the discovery of many more MDR and XDR strains of UPEC, including many extended-spectrum lactamase producers (Ehsan *et al*., 2023). Persisters are different types of UPEC that can handle some drugs and help make MRD and XDR isolates (Keren *et al*., 2013). There are four main phylogenetic groups for UPEC clinical strains. These are A, B1, B2, and D. Genetic markers like chuA, yjaA, and the DNA fragment TspE4.C2 define these groups (Lee *et al.*, 2016). Four main phylogenetic groups are A, B1, B2, and D. These are further broken down into seven subgroups: A0, A1, B1, B22, B23, D1, and D2. These groups are linked to the pathogenicity of UPEC (Barzan *et al*., 2016). This study aimed to identify and collected E. coli (UPEC) isolates from urinary tract infections. Detection of persistent E.coli,and identify some virulence factors and antibiotic resistance and determining the phylogenetic of Escherichia coli (UPEC).

Materials and methods

Patients and methods:

This cross-sectional study was conducted in Diyala province, Iraq, from 1 December 2023 to 1 October 2024. A total of 355 urine samples were collected in sterile containers from patients complaining of UTI; 37 (35.2%) were males and 68 (64.7%) were females. The mean age ± SD of patients was 21.59 ± 15.07 years, ranging from 1 month to 70 years. They were allocated from Baquba Teaching Hospital, Al-Batoul Maternity Hospital, and healthcare centres. Urine samples were transported to the bacteriology laboratory as soon as possible using a cooling box. Streaking on the blood and MacConkey agar plate was done immediately and incubated for 24 hours at 37 °C. Identification of *E. coli* was done using standard morphological, cultural, biochemical, and molecular procedures (Alfinete *et al*., 2022). A total of 355 urine specimens were collected from patients with urinary tract infections (UTI) of all ages and sexes who were admitted to Baquba Teaching Hospital, Al-Batoul Maternity Hospital, and Teaching Laboratories between 1 December 2023 and 1 November 2024. The standard microbial and biochemical protocols, as previously described elsewhere, were used to identify UPEC isolates

Antibiotic susceptibility

The disc diffusion assay assessed the antibiotic susceptibility patterns in UPEC isolates. We purchased antibiotic discs of various names and concentrations from Turkey. The study involved the use of the following antibiotics: Ampicilin-Cloxaciline30 µg, Ampicilin20 µg, Amoxyline-clavulanic acid 30 µg, Ampicillin Amoxyline-Clavonic Acid 30 µg, Azithromycin 15 µg, Ciprofloxacin 10 µg, gentamicin (10 µg), Imipenem10 µg, Ofloxacine (5 µg), Piperacillin100 µg, Streptomycine10 µg, Trimathaprime Sulfamethazol 25 µg, Tetracycline (30 µg, nitrofurantoin (300 µg), We cultivated on Mueller-Hinton agar (MHA). Subsequently, they were transferred onto the sterile plates, which were then covered with antibiotic discs using aseptic techniques. The MHA plates then underwent a 24-hour incubation period at 37 °C. The inhibition zones were interpreted according to the guidelines of the Clinical & Laboratory Standard Institute (CLSI, 2024). Antibiotic sensitivity testing was assessed by a disc diffusion assay using 14 antibiotics and interpreted according to the guidelines of CLSI (2024). Classification of UPEC to MDS, MDR, and XDR was based on definitions (Magiorakos *et al*., 2012).

Molecular analysis

The Clermont *et al*. (2013) method evaluated the phylogroup distribution in UPEC isolates. Table 1 shows the primer sequences (Bioneer, Korea), the amplicon size in base pairs (bp), and the PCR conditions.

DNA extraction

Following the collection of 105 UPEC isolates, DNA was extracted using a commercial kit in accordance with the instructions supplied by the manufacturer, Geneaid (Presto TM Mini gDNA Bacterial Kit Protocol, Bioneer, Korea). Afterwards, specific DNA segments of interest were amplified using Polymerase Chain Reaction (PCR)

Phylogenic grouping is achieved through the use of quadruplex PCR.

Quadruplex PCR is a method for analyzing isolates based on the presence or absence of the four genes (arpA, chuA, yjaA, and TspE4.C2) Clermont *et al*. (2013). A 25-mL reaction mixture with 12.5 mL of the master mix, 1 mL of each primer (forward and reverse), and 4.5 mL of template DNA was used for multiplex PCR. We added distilled water to the final volume. Assay conditions of the PCR program were: 4 min at 94°C; 30 cycles of 5 sec at 94°C; 20 sec at 57°C for group E or 59°C for quadruplex and group C; 1 The amplification products were separated after 5 minutes at 72°C. After separating the amplification products, we placed the sample on a 1% agarose gel and stained it with ethidium bromide under a UV treanslumiator. The sequences of primers used are clarified in Table 1.

The study underwent an ethical review and received approval.

The study was approved by the Research Ethics Committee of the Department of Biology, University of Diyala. After obtaining permission from the Ministry of Health and Environment to collect samples for the current study, we isolated and initially diagnosed the isolates in the microbiology laboratories of Baqubah General Teaching Hospital, Al-Batoul Teaching Hospital, and Public Health Laboratories. We obtained permission from the study participants to collect socio-demographic information and conduct a study on the collected samples, all while respecting their privacy.

The details of employed primers were based on (Clermont *et al*., 2017) illustrated in table (1). Table (1): Details of the primers of genes used for phylogrouping of UPEC.

Gene	Primer name	Sequence (3'-5')	Product size/bp
chuA	chuA.F	ATGGTACCGGACGAACCAAC	288
	chuA.R	TGCCGCCAGTACCAAAGACA	
yjaA	yjaA.F	CAAACGTGAAGTGTCAGGAG	211
	yjaA.R	AATGCGTTCCTCAACCTGTG	
TspE4C2	TspE4C2.F	CACTATTCGTAAGGTCATCC	152
	TspE4C2.R	AGTTTATCGCTGCGGGTCGC	
Ace	AceK.F	AACGCTATTCGCCAGCTTGC	400
	ArpA1.R	TCTCCCCATACCGTACGCTA	
trpBA	trpBA.F	CGGCGATAAAGACATCTTCAC	489
	trpBA.R	GCAACGCGGCCTGGCGGAAG	

Phylogrouping of UPEC was based on the presence of 5 genes (Dadi *et al*., 2020). Patient privacy was respected by obtaining verbal consent from each participant. A full the Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference factors in study parameters. Least significant difference-LSD and T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

Statistical Analysis:

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference factors in study parameters. Least significant difference-LSD and T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

Results and Discussion:

Distribution of isolation categories

The results shown in table 2 showed the percentage of positive and negative bacterial growth from UTI patients, where 200 (56.4%) were positive and 155 (43.6%) were negative. *Escherichia coli* bacteria constituted the highest percentage of positive results, with 105 cases (52.5%) recorded. These results indicate that *Escherichia coli* bacteria is the main causative agent of this type of infection. The study showed that *E. coli* is the most common Gram-negative bacteria General urine examination were performed on each specimen, the colony-forming unit concentration per milliliter (cfu/ml) in the urine exceeded $1x10^5$, It has been categorized as a significant diagnostic criterion for urinary tract infection: intestinal bacteria, particularly *Escherichia coli*, mostly cause urinary tract infections (UTIs). The distribution of some sociodemographic features of UPEC isolates is shown in Table 2. According to age categories, the isolation rate was insignificantly higher among 11-20-year-olds.

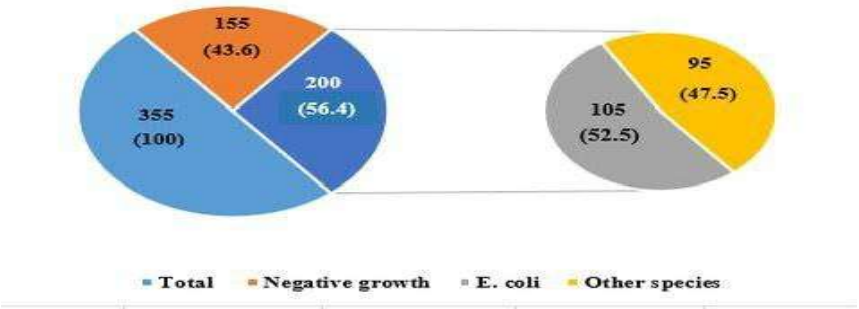

Figure (1): The percentage of bacterial isolates growth from urine Patients

Table (1): Biochemical test for (105) *E coli* isolates.

Biochemical tests									
Isolates	**Indole**	**MR**	**Motility**	**VP**	**Oxidase**	**Urease**	**TSI**	**Citrate utilization**	**Catalase**
E. coli	+	+	+/-*	-	-	-	**A/A-H2S+Gas**	-	+

***Most *E. coli* isolates are motile in motility test.**

Table (2): Distribution of some sociodemographic features of study participants.

Characteristics	**Description Range(min-max)**	**No.**	**(%)**	**χ^2 (*P-value)***
Age groups / year Mean ± SD =21.59 ± 15.07	<1	10	(9.5%)	16.805 ** (0.0001)
	1-10	20	(19.04%)	
	11-20	21	(20%)	
	21-30	25	(23.8%)	
	31-40	18	(17.1%)	
	41-50	7	(6.6%)	
	51-60	3	(3.3)	
	61-70	1	(0.9%)	
Gender M/F ratio= 1:1.84	Male	37	(35.2%)	9.152 ** (0.0025)
	Female	68	(64.7%)	
M/UM ratio =2:1	Married	70	(66.6%)	11.667 ** (0.0006)
	Unmarried	35	(33.3%)	
** (P≤0.01).				

The antibiotic sensitivity of all *E. coli* isolates against 14 drugs was investigated using a disc diffusion assay. The outcomes were interpreted according to the guidelines of CLSI (2024), as revealed in Table 3. There was a significantly increased resistance rate against Amoxacillin-Clavulenate 67(63.8), Ampicillin-cloxacillin92(87.6), Ampicillin98(93.3), Piperacillin90(85.7), Imipeneme1(0.95),Ceftriaxone82(78.09),Ciprofloxacin67(63.8),Ofloxacin64(60.95), Trimethoprim-sulfamethoxazole84(80), Tetracycline94 (89.5), Azithromycin66(62.8), Streptomycin29(27.6), and insignificantly resistant to Gentamycin45(42.8). There was significant sensitivity to imipenem and insignificant sensitivity to nitrofurantoin. For the detection of persistent UPEC isolates, all the identified 105 *E. coli* isolates were subjected to sudden genomic lysis stress and categorized according to (Huemer *et al.*, 2020). Only 24 (22.8%) isolates persisted and survived these circumstances. All data are shown in Table 5.

Table (3): The rate of persister isolates among the UPEC of the study.

Persistent	**No. (%)**	**Mean ± SD**
Produce	24 (22.8%)	0.228 ± 0.071
Non produce	81 (77.1%)	0.771 ± 0.419
Total	105 (100%)	--
Statistical	χ^2 =30.942 ** *P-value*= 0.0001 **	L.S.D. = 0.262 * *P-value*= 0.0387
* (*P*≤0.05), ** (*P*≤0.01).		

Table (4): Distribution of biofilm formation of *E.coli* isolates

Biofilm formation	**NO. %**	**Absorbency at 630 nm**	**Mean ± SD**
Strong	45 (42.8%)	0.115-1.127	0.428 ±0 .494 a
Moderate	20 (19.0%)	0.088-0.101	0.190 ± 0.392 b
Weak	40 (38.0%)	0.053-0.086	0.380 ±0.486 a
Statistical	χ^2 = 10.112 ** *P-value*= 0.0064	Control=0.088	L.S.D. = 0.1449 * *P-value*= 0.0287
Means having with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).			

Table (5): Relationship between biofilm formation and 15 persistent *E.coli* isolates

Biofilm	**Isolates no %**	**Persistent N%**
Strong	13 (86.67%)	10 (66.67%)
Moderate	2 (13.33%)	5 (33.33 %)
Chi-Square: χ^2 (*P-value*)	8.067 ** (0.0045)	3.922 * (0.0481)

* (P≤0.05), ** (P≤0.01).

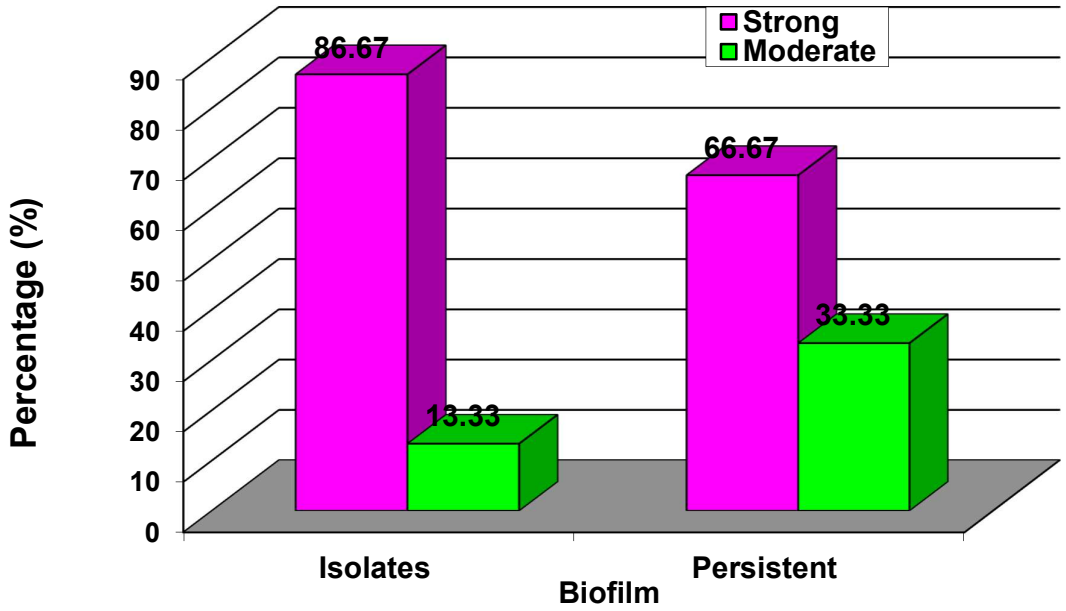

Figure 2: Relationship between biofilm formation and 15 persistent *E.coli* isolates

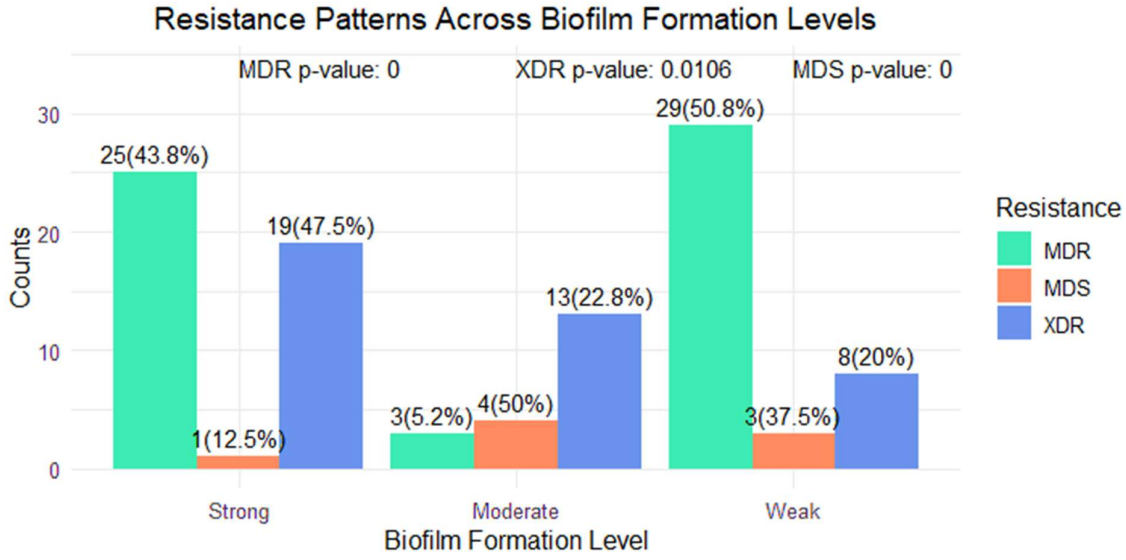

Figure (3): Resistance patterns across biofilm formation levels

Out of 24 persister isolates of UPEC, 15 were subjected for phylogrouping based on the detection of 5 genes including; chuA, *yja*A, *TspE*4C2, *Ace*, and *arp*AgC using the multiplex PCR. The outcomes of phylogrouping found that: 6 (40.0%) isolates were belonged to B2 phylogroup, 3(20%) were belonged to B1 phylogroup, 2 (13.3%) were belonged to C phylogroup, 3 (20%) were D phylogroup and 1 (6.7%) was belonged to A phylogroup. The *trpBA* gene was absence in all isolates. The *Ace* gene was detected in 5 (33.3%) isolates (E8, E53, E88, E92 and E12). The *chuA* gene was detected in 12 (80%) of the isolates and absence in the remaining three (E65, E8 and E88). Similarly, the *yjaA* gene was detected in 12 (80%)

isolates and absence in three isolates (E59, E53 and E12). The *TspE4C2* gene was detected in 12(80%) of isolates and absence in other three (E37, E57 and E12).All 105 *E. coli* isolates were exposed to sudden genomic lysis stress. Only 24/105 sub-isolates could persist and survive these isolates. The tolerant disc (TD) test can find bacteria that are resistant to antibiotics by combining the standard antibiotic disc test (step 1) with growth after glucose is added (step 2). The quick-kill method yielded the same result, identifying 24 out of 105 E. coli isolates as resistant, with a mean of 0.228 to 0.071. *P-value* = 0.0001. The present study studied biofilm production using ELISA (enzyme-linked immunosorbent). The first method found that the number of isolates capable of forming a biofilm is 65 with the ability to produce membranes, including 45 isolates with strong production and 20 isolates with moderate biofilm production. Ratio 0.048*and 0.001*, respectively, while 40 isolates could not produce biofilms. 65 (61.9%), 69.2% strong, and 30.7% moderate. In this study, 65 of 105 bacterial isolates (61.9%) produced biofilm using the ELISA method. These isolates could produce biofilm to different degrees when compared to the negative control, with 45 isolates (69.2%) producing strong biofilm and 20 isolates (30.7%) producing medium biofilm. In comparison, 40 bacterial isolates, at a rate of 26%, were weak in production,

Table (6): Distribution of phylogrouping outcomes of the 15 UPEC isolates.

Isolates	*trp*BA	*Ace*	*chu*A	*yja*A	*Tsp*E4C2	Phylogroup
E1 (37)	-	-	+	+	-	B1
E2 (17)	-	-	+	+	+	B2
E3 (24)	-	-	+	+	+	B2
E4 (101)	-	-	+	+	+	B2
E5 (64)	-	-	+	+	+	B1
E6 (10)	-	-	+	+	+	B2
E7 (59)	-	-	+	-	+	C
E8 (56)	-	-	+	+	+	B2
E9 (57)	-	-	+	+	-	B1
E10 (65)	-	-	-	+	+	D
E11 (8)	-	+	-	+	+	D
E12 (53)	-	+	+	-	+	C
E13 (88)	-	+	-	+	+	D
E14 (92)	-	+	+	+	+	B2
E15 (12)	-	+	+	-	-	A
+ Ve **-Ve**	0 (0%) 15 (100%)	5 (33.3%) 10 (66.7%)	12 (80%) 3 (20%)	12 (80%) 3 (20%)	12 (80%) 3 (20%)	---
P-value	0.0001 **	0.048 *	0.020 *	0.020 *	0.020 *	---

- **Phylogroup B1: Presence of *chu*A (+), *yja*A (+), and absence of *Tsp*E4C2 (−).**
- **Phylogroup B2: Presence of *chu*A (+), *yja*A (+), and *Tsp*E4C2 (+).**
- **Phylogroup C: Presence of *chu*A (+), *yja*A (−), and *Tsp*E4C2 (+).**
- **Phylogroup D: Absence or variability in *chu*A and positive *Tsp*E4C2.**
- **Phylogroup A: Absence of key markers like *chu*A and *Tsp*E4C2**

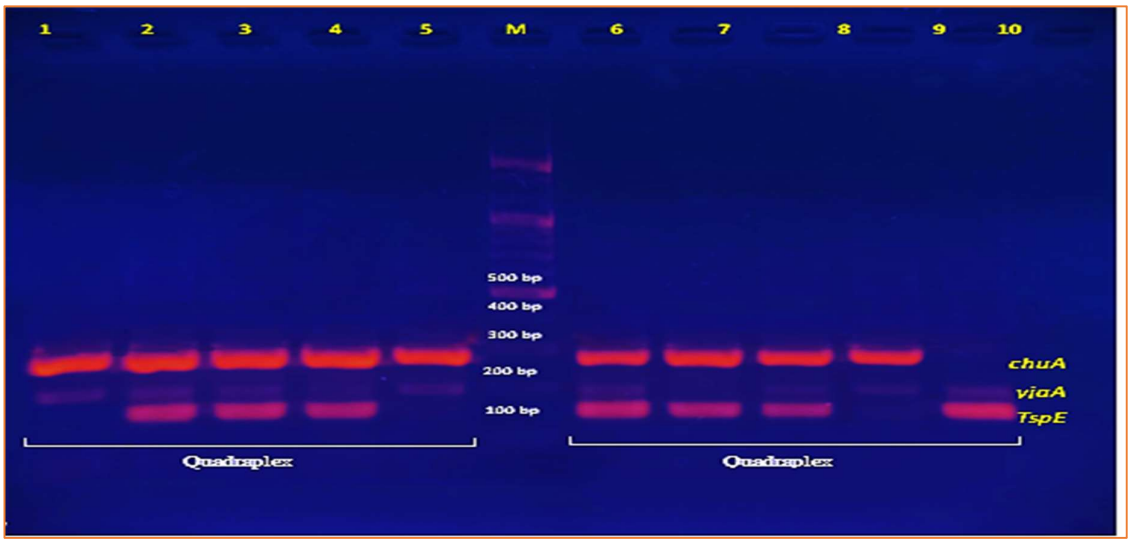

Figure (2): **Gel electrophoresis of amplified *chuA, yiaA, and TspE* gene of *Escherichia coli* isolates. Electrophoresis was done (1.5% agarose at 7 V for 1 h,** Lane M: DNA ladder marker (1000); lane 1,4 , 10 phylo-group --(- + + -); lane 2,3,4 , 7,8,9 , phylo-group -- (- + + +) ; lane 11 , phylo-group -- (- - + +) ; lane 12, N.C.(Negative control).

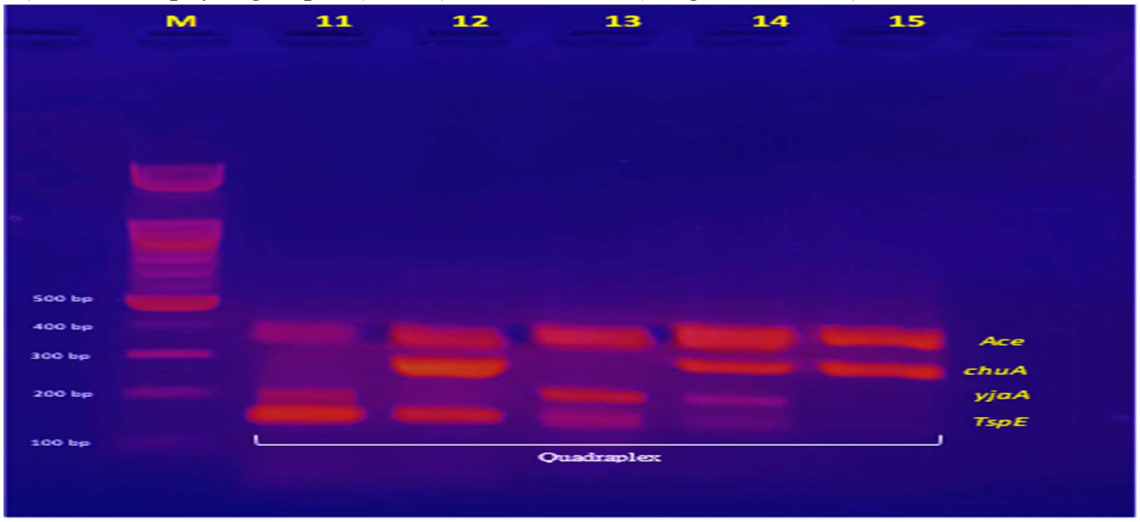

Figure (4**): Gel electrophoresis of amplified of *Escherichia coli* isolates. Electrophoresis was done (1.5% agarose at 7 V for 1 h,** Lane M: DNA ladder marker (1000); lane 2,4 phylogroup --(+ - ++); lane 3 phylogroup -- (+ + - +) ; lane 5 , phylo-group (++++) ; lane 6 phylogroup -- (++--);lane7, N.C.(Negative control).

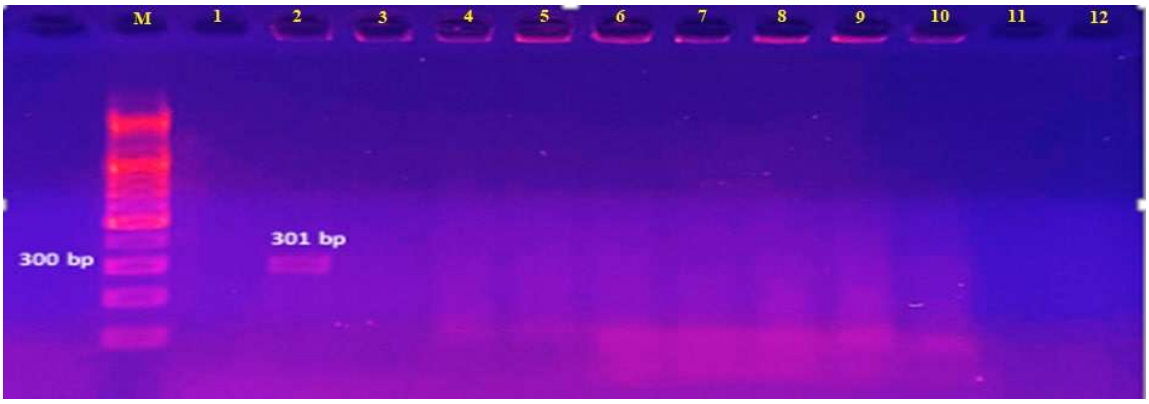

Figure (5): **Gel electrophoresis of amplified *arpA* gene of *Escherichia coli* isolates. Electrophoresis was done (1.5% agarose at 7 V for 1 h,** Lane M: DNA ladder marker (1000); lane 3 phylo-group E (Positive for $arpA\ gene$ with 301bp).

Discussion:

This study is important for three reasons: First, UTIs are very common worldwide, and E. coli is the most common bacteria that causes them, especially in women. UTIs are the most common bacterial infections, affecting 120–150 million people globally yearly (Zhou et al., 2023). Among nosocomial infections, UTIs are second only to respiratory tract infections, representing 24% of such cases in developing countries (Tandogdu and Wagenlehner, 2016). Second, many G+ and G-bacterial and fungal pathogens cause UTIs. Epidemiologically, uropathogenic E. coli (UPEC) are the main pathogenic agent for UTIs, accounting for about 75% of uncomplicated and 65% of complicated UTIs cases (Medina and Castillo-Pino, 2019). Without a doubt, UPEC can change physically, and they have a layer of viruses and toxins that help them stay alive and make people sick in UT (Carlini *et al*., 2021; Wojciuk *et al*., 2022). Thirdly, the current study addresses a critical issue in our community: the rapidly increasing prevalence of antibiotic resistance. The constantly emerging phenomenon of "superbugs" in clinical settings indicates that we are entering an era where traditional infection treatments are becoming increasingly ineffective (Chahine *et al*., 2022; Painuli *et al*., 2023). More seriously, adding to antibiotic resistance, persistent infections pose another major challenge and problem in managing bacterial infections (Gollan *et al*., 2019).). According to Shimoni *et al*. (2020).Previous research has yielded findings that align with the outcomes of our investigation. In France, Rossignol *et al*., (2017) found that between 70% and 80% of urine cultures yielded positive results for *E. coli*. It was observed according to a survey by Daoud *et al*. (2015), in Lebanon that *E. coli* was the predominant Gram-negative bacterial isolate; a urinary tract infection has been recorded in a documented range of 60.53% to 73.98%. A study conducted by Panagua-Contreras *et al*., (2017) in Mexico and Abernethy *et al*. (2017) in England found that 60.4% of the participants had the reported and differed from the results of the study by Ahumada-Kota *et al*., (2020), where the percentage reached 61.3%. On the other hand, the results were close to those of the study by Al-Naimi and Abbas (2016) in Iraq Baghdad, where the percentage reached 38.3%. As well as Vitek 2 system identification (Nmema *et al*., 2022). The distribution of some sociodemographic features of UPEC isolates is shown in Table 2. According to age categories, the isolation rate was insignificantly higher among 11-20-year-olds.

In the current study, the UTI rate was insignificantly higher in 21–30 years. In this issue, different studies reported different results; for instance, recurrent UTIs are a common problem in the elderly (Matthews and Lancaster, 2011). Most studies also agree that older people are more likely to get UTIs and that these infections are worse, causing more illness and death. This is because getting older greatly changes the host's physiology and immunity (Ligon *et al*., 2023)\. Some risk factors during adulthood can enhance UTIs, such as uncontrolled DM, hypertension, and chronic renal diseases (Geerlings *et al*., 2016). However, UTIs affect nearly one-third of children within one year of birth, and about 30% develop recurrent UTIs (Simoes *et al*., 2020).

The results also found that women had a significantly higher rate of UTIs. If you look at the urethra, it is shorter and closer to the rectum in women than in men, so this result makes sense and agrees with most other studies. Women are more likely to get sexually transmitted diseases. Basically, 20–30% of adult women with an initial UTI experienced a recurrence within 3–4 months (Terlizzi *et al*., 2017). Furthermore, estimates suggest that 40% of women experience at least one UTI during their lifetime (Micali *et al*., 2014), with 11% of women over 18 years experiencing a UTI episode annually (Foxman, 2014). After puberty, UTI risk increases and is frequently associated with sexual intercourse in adult women (Deltourbe *et al*., 2022). Also, women who have gone through menopause are more likely to get UTIs again because lower levels of oestrogen change the urogenital epithelium and microbiome in bad ways (Jung and Brubaker, 2019). The results of the current study found that the isolation rate of UPEC was 56.5%, as UPEC was globally causing around 85% of UTI cases (Medina and Castillo-Pino, 2019; Manges *et al*., 2019). Receiving antibiotics before collecting urine samples for culture may have contributed to this, as a high sample rate often results in negative growth. Additionally, the over-identification of UPEC likely occurred due to the successive use of four techniques for confirmation.

Our study showed that the isolated UPEC were only slightly sensitive to imipenem and not sensitive to nitrofurantoin. They were also significantly resistant to 12 other first-line therapeutic antibiotics. Most previous studies have found that UPEC is becoming less sensitive to antibiotics (Masoud *et al*., 2021; Abed *et al*., 2023; Nasrollahian *et al*., 2024). These results back this up. The rising rates of antimicrobial resistance probably result from irrational antibiotic policies, mainly because of inadequate broad-spectrum--- empirical therapies prescribed without urine cultures and antibiotic susceptibility testing (Adamus-Białek *et al*. 2018). Therefore, our results are in agreement with reports indicating elevated

References:

Abdul Hameed, H. and Abdul-Jabbar, A. Isolation, Identification and antibiotic resistance profile distribution of clinical *E. coli* in Iraqi patients. Eurasian Medical Research Periodical. 2022; 8: 116-21.

Adamus-Białek, W., Baraniak, A., Wawszczak, M., Głuszek, S., Gad, B., Wróbel, K., & Parniewski, P. (2018). The genetic background of antibiotic resistance among clinical uropathogenic Escherichia coli strains. *Molecular biology reports*, *45*(5), 1055-1065.

Abed AD, Mutter TY. Relationship between antimicrobial resistance and virulence factors in uropathogenic *Escherichia coli* isolates from Ramadi, Iraq: phenotype and genotype identification. Afr Health Sci. 2023; 23(3):486-96.

Al-Hasani, H. M., Al-Rubaye, D. S., and Abdelhameed, A. The emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) in Iraqi clinical isolates of *Escherichia coli*. J. Population Ther. Clin. Pharmacol. 2023; 30: 469-82.

Alkhulaifi ZM, Mohammed KA. Prevalence and molecular analysis of antibiotic resistance of *Pseudomonas aeruginosa* isolated from clinical and environmental specimens in Basra, Iraq. Iran J Microbiol. 2023; 15(1):45-54.

Alshaikh SA, El-Banna T, Sonbol F, Farghali MH. Correlation between antimicrobial resistance, biofilm formation, and virulence determinants in uropathogenic *Escherichia coli* from Egyptian hospital. Ann Clin Microbiol Antimicrob. 2024; 23(1):20.

Bumbangi, F. N., Llarena, A.-K., Skjerve, E., Hang'ombe, B. M., Mpundu, P., Mudenda, S., *et al*. (2022). Evidence of community-wide spread of multi-drug-resistant Escherichia coli in young children in Lusaka and Ndola districts, Zambia. Microorganisms, 2022; 10: 1684.

Carlini, F.; Maroccia, Z.; Fiorentini, C.; Travaglione, S. and Fabbri, A. Effects of the *Escherichia coli* Bacterial Toxin Cytotoxic Necrotizing Factor 1 on Different Human and Animal Cells: A Systematic Review. Int. J. Mol. Sci. 2021; 22:12610.

Chahine, E.B.; Dougherty, J.A.; Thornby, K.A. and Guirguis, E.H. Antibiotic approvals in the last decade: are we keeping up with resistance? Ann. Pharmacother. 2022; 56:441-62.

Chardavoyne, P.C. and Kasmire, K.E. Appropriateness of Antibiotic Prescriptions for Urinary Tract Infections. West J Emerg Med. 2020; 21(3):633-9.

Clermont, O.; Christenson, J.K.; Denamur E, Gordon DM. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylogroups. Environ Microbiol Rep. 2013; 5(1):58-65.

Dadi, B.R.; Abebe, T.; Zhang, L.; Mihret, A.; Abebe, W. and Amogne, W. Distribution of virulence genes and phylogenetics of uropathogenic Escherichia coli among urinary tract infection patients in Addis Ababa, Ethiopia. BMC Infect Dis. 2020; 20(1):108.

Deltourbe, L.; Lacerda Mariano, L.; Hreha, T.N.; Hunstad, D.A. and Ingersoll, M.A. The impact of biological sex on diseases of the urinary tract. Mucosal immunology 15 (2022). 10.1038/s41385-022-00549-0

Foxman, B. (2014). Urinary tract infection syndromes occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect. Dis. Clin. North Am. 28, 1-13.

Gatya Al-Mayahie, S.M.; Al-Guranie, D.R.; Hussein, A.A. and Bachai, Z.A. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic Escherichia coli phylogroup B2 isolates from outpatients in Wasit Province/ Iraq. PLoS One. 2022; 17(1):e0262984.

Geerlings, S.E. Clinical Presentations and Epidemiology of Urinary Tract Infections. Microbiol Spectr. 2016; 4(5).

Genao, L., and Buhr, G. T. Urinary tract infections in older adults residing in long-term care facilities. Ann. Longterm Care, 2012; 20: 33-8.

Giske, C. Contemporary resistance trends and mechanisms for the old antibiotics colistin, temocillin, fosfomycin, mecillinam and nitrofurantoin. Clin. Microbiol. Infect. 2015; 21: 899-905.

Gollan, B.; Grabe, G.; Michaux, C. and Helaine, S. Bacterial persisters and infection: past, present, and progressing. Annu. Rev. Microbiol. 2019; 73:359-85.

Jung, C. and Brubaker, L. The etiology and management of recurrent urinary tract infections in postmenopausal women. Climacteric. 2019; 22:242-9.

Kot, B. Antibiotic resistance among uropathogenic *Escherichia coli*. Pol. J. Microbiol. 2019; 68(4):403-415.

Kotłowski, R.; Grecka, K.; Kot, B. and Szweda, P. New Approaches for *Escherichia coli* Genotyping. Pathogens. 2020; 9(2):73.

Ligon, M.M.; Joshi, C.S.; Fashemi, B.E.; Salazar, A.M. and Mysorekar, I.U. Effects of aging on urinary tract epithelial homeostasis and immunity. Dev Biol. 2023; 493:29-39.

Magiorakos, A.P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18(3):268-81.

Manges, A.R.; Geum, H.M.; Guo, A.; Edens, T.J.; Fibke, C.D. and Pitout, J.D. Global Extraintestinal Pathogenic *Escherichia coli* (ExPEC) Lineages. Clin. Microbiol. Rev. 2019; 32(3): e00135-18.

Maniam, L.; Vellasamy, K.M.; Ong, T.A.; the, C.S.; Jabar, K.A.; Mariappan, V.; *et al*. Genotypic characteristics of Uropathogenic *Escherichia coli* isolated from complicated urinary tract infection (cUTI) and asymptomatic bacteriuria-a relational analysis. PeerJ. 2023; 11: e15305.

Matthews, S.J. and Lancaster, J.W. Urinary tract infections in the elderly population. Am. J. Geriatr. Pharmacother. 2011; 9:286-309.

McCann, E.; Sung, A.H.; Ye G.; Vankeepuram, L. and Tabak, Y.P. Contributing Factors to the Clinical and Economic Burden of Patients with Laboratory-Confirmed Carbapenem-Nonsusceptible Gram-Negative Urinary Tract Infections. Clin. Outcomes Res. CEOR. 2020; 12:191-200.

Medina, M. and Castillo-Pino, E. An introduction to the epidemiology and burden of urinary tract infections. Ther. Adv. Urol. 2019; 11:1756287219832172.

Nasrollahian, S., Halaji, M., Hosseini, A., Teimourian, M., Armaki, M. T., Rajabnia, M., *et al*. Genetic diversity, carbapenem resistance genes, and biofilm formation in UPEC isolated from patients with catheter-associated urinary tract infection in north of Iran. Int. J. Clin. Pract. 2022; 9520362.

Nasrollahian, S., Graham, J. P., & Halaji, M. (2024). A review of the mechanisms that confer antibiotic resistance in pathotypes of E. coli. Frontiers in Cellular and Infection Microbiology, *14*, 1387497.

Nayaju, T.; Upreti, M.K.; Ghimire, A.; Shrestha, B.; Maharjan, B.; Joshi, R.D.; *et al*. Higher Prevalence of Extended Spectrum β-Lactamase Producing Uropathogenic Escherichia coli Among Patients with Diabetes from a Tertiary Care Hospital of Kathmandu, Nepal. Am. J. Trop. Med. Hyg. 2021; 105(5):1347-55.

Niu, H.; GU, J. and Zhang, Y. Bacterial persisters: molecular mechanisms and therapeutic development. Signal Transduct. Target Ther. 2024; 9(1):174.

Painuli, S.; Semwal, P.; Sharma, R. and Akash, S. Superbugs or multidrug resistant microbes: a new threat to the society. Health Sci. Rep. 2023; 6: e1480.

Sarshar, M.; Behzadi, P.; Ambrosi, C.; Zagaglia, C.; Palamara, A. T. and Scribano, D. FimH and anti-adhesive therapeutics: A disarming strategy against uropathogens. Antibiotics, 2020: 9: 397.

Schwartz, D.J.; Kalas, V.; Pinkner, J.S.; Chen, S.L.; Spaulding, C.N.; Dodson, K.W. *et al*. Positively selected FimH residues enhance virulence during urinary tract infection by altering FimH conformation. Proc. Natl. Acad. Sci.2013; 110: 15530-7.

Simoes, E.; Silva, A.C.; Oliveira, E.A. and Mak R.H. Urinary tract infection in pediatrics: An overview. J. Pediatr. (Rio. J.) 2020; 96:65-79.

SPSS (2019). Statistical Packages of Social Sciences-SPSS/ IBM Statistics 26 step by step. 16th Edition. https://doi.org/10.4324/9780429056765.

Tanabe, R.H.; Dias, R.C.; Orsi, H.; de Lira, D.R.; Vieira, M.A.; Dos Santos, L.F.; *et al*. Characterization of Uropathogenic *Escherichia coli* Reveals Hybrid Isolates of Uropathogenic and Diarrheagenic (UPEC/DEC) *E. coli*. Microorganisms. 2022; 10(3):645.

Tandogdu, Z. and Wagenlehner, F.M. Global epidemiology of urinary tract infections. Curr. Opin. Infect. Dis. 2016; 29:73–79.

Terlizzi ME, Gribaudo G, Maffei ME. UroPathogenic *Escherichia coli* (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-antibiotic Antimicrobial Strategies. Front Microbiol. 2017; 8:1566.

Terlizzi, M.E.; Gribaudo, G. and Maffei, M.E. UroPathogenic *Escherichia coli* (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-antibiotic Antimicrobial Strategies. Front Microbiol. 2017; 8:1566.

Thanert, R.; Choi, J.; Reske, K.A.; Hink, T.; Thanert, A.; Wallace, M.A.; *et al.* Prevention Epicenters Program. Persisting uropathogenic *Escherichia coli* lineages show signatures of niche-specific within-host adaptation mediated by mobile genetic elements. Cell Host Microbe. 2022; 30(7):1034-47.

Thanert, R.; Reske, K.A.; Hink, T.; Wallace, M.A.; Wang, B.; Schwartz, D.J.; *et al.* Comparative Genomics of Antibiotic-Resistant Uropathogens Implicates Three Routes for Recurrence of Urinary Tract Infections. MBio. 2019; 10: e01977–19.

Whelan, S.; Lucey, B. and Finn, K. Uropathogenic *Escherichia coli* (UPEC)-associated urinary tract infections: the molecular basis for challenges to effective treatment. Microorganisms, 2023; 11: 2169.

Wojciuk, B.; Majewska, K.; Grygorcewicz, B.; Krukowska, Z.; Kwiatkowska, E.; Ciechanowski, K.; *et al*. The role of uropathogenic Escherichia coli adhesive molecules in inflammatory response-comparative study on immunocompetent hosts and kidney recipients. PLoS ONE. 2022; 17: e0268243.

Zhou, Y.; Zhou, Z.; Zheng, L.; Gong, Z.; Li, Y.; Jin, Y.; et al. Urinary Tract Infections Caused by Uropathogenic *Escherichia coli*: Mechanisms of Infection and Treatment Options. Int. J. Mol. Sci. 2023; 24(13):10537.

Huemer, M., Mairpady Shambat, S., Brugger, S. D., & Zinkernagel, A. S. (2020). Antibiotic resistance and persistence—Implications for human health and treatment perspectives. *EMBO reports*, *21*(12), e51034.