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ORIGINAL ARTICLE

Outbreak of drug resistance *Escherichia coli* phylogenetic F group associated urinary tract infection

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ABSTRACT

Background and Objectives: Urinary tract infections are one of the most commonly associated human infectious diseases caused by the bacteria *Escherichia coli. Escherichia coli* is described as having a large number of virulence genes that enable drug resistance, which is a cause for great concern. Monitoring of antimicrobial susceptibility is critical to determining the scope of the problem and selecting appropriate antimicrobial drugs. The current study aimed to identify the distribution of uropathogenic *E. coli* (UPEC) based on genetic profiles and to determine resistance patterns among isolates.

Materials and Methods: This study employed biological correlations to study the patterns of antibiotic resistance and the distribution of phylogenetic groups of 118 isolates of *E. coli* and the relationship between them, which were isolated from three hospitals in Baghdad, Iraq.

Results: The results of phylogenetic analysis showed that phylogroup F was the most common group among *E. coli* isolates (37.3%), followed by phylogroups C (20.3%), B2 (15.3%), E (14.4%), UP (4.2%), A and D (3.4%), and B1 (1.7%). The majority of antibiotic resistance patterns were related to penicillin groups (80.5%) and the least to the sulfonamide groups (67.0%). 51.7%, 42.4%, and 1.7% of isolates were Extensive Drug Resistance (XDR), Multi-Drug Resistance (MDR), and Pan Drug Resistance (PDR), respectively. Antibiotic resistance was most commonly detected in group F (35.6%).

Conclusion: Our observations revealed that the dominant phylogroup F had the highest prevalence of multi-drug resistance and extensive drug resistance among *E. coli* isolates. The newly identified phylogroups C, E, and F account for about 72.0% of the *E. coli* isolates. Such investigations should be conducted in other localities as well, in order to gain a better understanding of the pattern of antibiotic resistance patterns and the frequency of distinct phylogenetic groups.

Keywords: *Escherichia coli*; Urinary tract infection; Drug resistance; Phylogenetic F; Phylogenetic group

INTRODUCTION

Urinary tract infections are one of the most common human infectious diseases caused by *Escherichia coli*, specifically uropathogenic *E. coli* (1). In the early 1980s, Whittam and colleagues (1983) discovered a genetic substructure of *E. coli* that differs drastically within species (2), and to distinguish between certain *E. coli* species, multiple approaches have been established. Initially, Selandar and colleagues were the first to employ electrophoresis analysis to divide *E. coli* into six groups (A to F) in 1987 (3, 4). Subsequently, Clermont and colleagues developed a triplex PCR technique in 2000, which was able to distinguish four groupings: A, B_1, B_2, and D, using three marker genes: *chA*, *yjaA*, and a DNA fragment *TSPE4.C2* (4). The phenotypic classification was not conducted at random, but rather, it is based on a number of characteristics, the most important of which are virulence, resistance, serotypes, and geographic origin (5, 6). The majority of extraintestinal *E. coli* belonged to groups B_2 and D, the last

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of which was less than the first. On the other hand, groups A and B_1 were associated with commensal bacteria in the intestine (7, 8). Based on multi-locus sequence typing and whole genome data, additional *E. coli* phylogenetic groupings were discovered later (5, 6). Clermont and colleagues revised triplex PCR in 2013, by introducing a fourth set of primers to detect groups E and F, as well as new primers to distinguish cryptic clades. This method is called the Quadruplex technique (9).

An antibiotype is a type of phenotypic analysis in which isolates are classified into groups based on their resistance to antibiotics using an antibiotic susceptibility test. Antibiotypes aid in the discovery of various resistance patterns among species as well as the evaluation of epidemiological dispersion (10). Multidrug resistance and widespread drug resistance patterns in uropathogenic *E. coli* isolates are major concerns that are growing year after year (11). Antibiotic resistance leads to treatment failure and higher mortality rates (12). As a result, antimicrobial susceptibility monitoring is critical for determining the scope of the problem and selecting appropriate antimicrobial medications to treat infected patients (13).

The goal of this study was to identify the distribution of UPEC based on genetic profiles and to determine resistance patterns among isolates.

MATERIALS AND METHODS

Patients' data and specimens collection. Urine samples from 500 people suffering from urinary tract infections were obtained from three different Baghdad hospitals between December 2020 and March 2021. All study participants were evaluated for socio-demographic characteristics such as gender, age, marital status, and the site of the sample collection. A direct interview with patients was used to document clinical symptoms such as hematuria, flank pain, abdominal pain, urgency, dysuria, fever, incontinence, diarrhea, and irritability.

Laboratory diagnosis. All specimens were examined microscopically and cultivated in sentinel sites for the isolation and identification of *E. coli* isolates by selective media and standard methods, which were subsequently confirmed in microbiology laboratories at Baghdad Al-Mustansiriya University. These isolates were inoculated on blood agar, MacConkey agar, and Eosin Methylene Blue for 24 hours before being incubated at 37°C. Gram staining and other biochemical assays, such as IMViC tests, H_2S tests, carbohydrate use in TSI agar tests, motility tests, urease and oxidase tests (14), were employed to identify the bacteria.

The antimicrobial susceptibility test. *In-vitro* antimicrobial susceptibility testing of the bacterial isolates was performed by the Kirby-Bauer disc diffusion method (15). The following antimicrobial agents were used with their respective concentrations, obtained from Liofilchem Company, Italy: Amoxicillin (25 µg), amoxicillin-clavulanate (30 µg), cefoxitin (30 µg), cefixime (5 µg), cefepime (30 µg), cefriaxone (30 µg), ceftazidime (30 µg), imipenem (10 µg), gentamicin (10 µg), amikacin (30 µg), tetracycline, trimethoprim-sulfamethoxazole (25 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), nitrofurantoin (30 µg), tetracycline (30 µg), and chloramphenicol (30 µg). CLSI standard was used to compare the results (16).

Non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories was defined as multi-drug resistance (MDR) isolates, while non-susceptibility to at least one agent in all but two or fewer antimicrobial categories was defined as pan-drug resistance (PDR) isolates (17).

DNA extraction. DNA for amplification was extracted from whole cells by the boiling method as follows. Full loop colonies of overnight cultures were suspended in 1 mL of sterile distilled water and boiled for 10 minutes in a water bath. They were then centrifuged for 5 minutes at 12,000 rpm, and the supernatant was collected and stored at -20°C (18).

Phylogenic grouping by 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*) (9). Multiplex PCR was carried out in a 25 µL reaction mixture, including 12.5 µL of the master mix, 1 µL of each primer (forward and reverse), and 4.5 µL of template DNA, and completed with distilled water to reach its 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 min at 72°C; followed by 5 min at 72°C (9). Subsequently, the amplification products were separated on a 1% agarose gel and stained with ethidium bromide

stained under UV treanslumiator. The sequences of primers used are clarified in Table 1.

S**tatistical methods.** SPSS version 16.0 was used to analyze the data. Previously, a P value of less than 0.05 was considered significant. Pastversion 4.3 software was used in generating dendograms. The relative similarity of the profiles was calculated using the unweighted pair group technique with an arithmetic mean (UPGMA) and the dice correlation coefficient method.

Ethical review and approval. The study was approved by the Research Ethics Committee of the Department of Biology Sciences, Mustansiriya University. The isolates were initially isolated and diagnosed in microbiology laboratories in Medical City Teaching Hospital, Ibn-Balady, and Al-Qadisiyah Hospitals, after permission was obtained from the Ministry of Health and Environment to collect samples for the current study. Authorization was obtained from study participants to collect socio-demographic information and perform a study on the samples collected while respecting patients' privacy.

RESULTS

Socio-demographic information of patients. Of the 500 people suspected of UTIs, there were documented 118 patients with *E. coli* isolates, who were the focus of the current study, were 93 (78.8%) females and 25 (21.2%) males, where the male-to-female infection ratio is estimated to be 4:1. The majority of UTI cases were in the second age group, 37 (31.4%), which includes the age groups of 1–10 years of the study population. The social information and symptoms are summarized in Table 2.

Laboratory diagnosis. The laboratory investigation showed the presence of pus cells, red blood cells, epithelial cells, salts, and crystals, as well as mucus threads in different ratios. Most of the study samples were found to have an RBC count of <9 in their general urine examination. Similarly, with the exception of one case, all cases of suspected UTI had positive pus cells in all samples examined within > 10 and<9 of pus cell count, as listed in Table 3.

Antimicrobial susceptibility testing. In terms of the sensitivity test, we found out that the majority of the isolates exhibited multiple antibiotic resistance to the antibiotics tested, with 51.7%, 42.4%, and 1.7% displaying XDR, MDR, and PDR, respectively. It is simplified in the graphic below (Fig. 1).

Antibiogram typing. The dendrogram tree was constructed between isolates based on the inhibitory zone for all antibiotics tested and compared with the UPGUMA algorithm and Dice similarity index. All isolates that fall below the cut-off line are similar in dice distance. As shown in Fig. 2, the dendrogram typing revealed the presence of two clusters (I and II) and three isolates considered outside of the group

Table 1. The sequences of the primers used in the quadruplex PCR technique (9).

Primers ID	Gene	Primer sequence (5'-3')	Size, bp.
chuA.1b	*chuA*	5-ATGGTACCGGACGAACCAAC-3 F	288 bp
chuA.2		5-TGCCGCCAGTACCAAAGACA-3 R	
yjaA.1b	*yjaA*	5-CAAACGTGAAGTGTCAGGAG-3 F	211 bp
yjaA.2b		5-AATGCGTTCCTCAACCTGTG-3 R	
TspE4C2.1b	*TspE4C2*	5-CACTATTCGTAAGGTCATCC-3 F	152 bp
TspE4C2.2b		5-AGTTTATCGCTGCGGGTCGC-3 R	
AceK.f	*arpA*	5-AACGCTATTCGCCAGCTTGC-3 F	400 bp
ArpA1.r		5-TCTCCCCATACCGTACGCTA-3 R	
ArpAgpE.f	*arpA*	5-GATTCCATCTTGTCAAAATATGCC-3 F	301 bp
ArpAgpE.r		5-GAAAAGAAAAAGAATTCCCAAGAG-3 R	
trpAgpC.1	*trpA*	5-AGTTTTATGCCCAGTGCGAG-3 F	219 bp
trpAgpC.2		5-TCTGCGCCGGTCACGCCC-3 R	
trpBA.f	*trpA*	5-CGGCGATAAAGACATCTTCAC-3 F	489 bp
trpBA.r		5-GCAACGCGGCCTGGCGGAAG-3 R	

Table 2. Socio-demographic characteristics and symptoms of patient samples.

Characteristics	Description	Number (%)
Age (years)	Mean ± SD	15.65895 ± 14.47658
	Range (min-max)	(1 month -72 years)
Age Groups	<1	15 (12.7)
	1-10	37 (31.4)
	11-20	27 (22.9)
	21-30	19 (16.1)
	31-40	9 (7.6)
	41-50	5 (4.2)
	51-60	4 (3.4)
	61-70	1 (0.9)
	>70	1 (0.9)
Gender	Female	93 (78.8)
	Male	25 (21.2)
Marital Status	Unmarried	82 (69.5)
	Married	36 (30.5)
Attendance	Outpatient	96 (81.4)
	Ward	22 (18.6)
UTI Recurrence	New infection	34 (28.8)
	Recurrent infection	84 (71.2)
Clinical	Symptomatic	115 (97.5)
Presentation	Asymptomatic	3 (2.5)
Cause of	Unknown	39 (33.0)
Infection	Kidney infection	17 (14.4)
	Diabetic infection	17 (14.4)
	Bladder infection	16 (13.6)
	Operation	18 (15.3)
	Birth defect	9 (7.6)
	Multiple causes	2 (1.7)
Hospitals	Medical City	55 (46.6)
	Teaching Hospital	
	Ibn-Balady Childrenand	38 (32.2)
	Maternity Hospital	
	Al-Qadisiyah Hospital	25 (21.2)

Table 3. Micro-scoping examination of patient samples.

Micro-scoping Examination	Finding results under the microscope		
	Non	< 9	> 10
	n (%)	n (%)	n (%)
Red Blood Cells	11 (9.3)	80 (67.8)	27 (22.9)
Puscells	1 (0.8)	15 (12.7)	102 (86.4)
Epithelialcells	61 (51.7)	4 (3.4)	53 (44.9)
Amorphous	65 (55.1)	25 (21.2)	28 (23.7)
Crystals	103 (87.2)	4 (3.4)	11 (9.3)
Mucusthreads	106 (92.4)	0 (0.0)	12 (10.2)

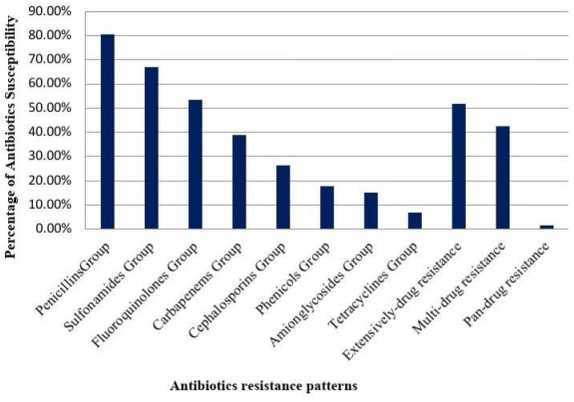

Fig. 1. Antibiotic susceptibility patterns of *Escherichia coli* isolates.

known as unique clones. The isolates within-cluster I and II have great resistance to antibiotics, ranging from MDR, XDR, and PDR patterns. Out-group isolates named 95E, 55F, and 30C are not MDR, which means they are resistant to one or two antibiotics.

Phylogenic grouping of uropathogenic *E. coli*. Phylogenetic analysis revealed that the major and predominant *E. coli* isolates were phylogroup F 44 (37.3%), followed by C 24 (20.3%), B_2 18 (15.3%), E 17 (14.4%), UP 5 (4.2%), A and D 4 (3.4%), and B_1 2 (1.7%).

Association between phylogeny and antibiotic susceptibility tests among isolates. Regarding the associations between phylogeny and Penicillin's group susceptibility of *E. coli* isolates, only types C and F showed the highest resistance compared to the others (16.1% and 30.5%, respectively). However, these distinctions were not statistically significant ($P > 0.05$).

On the other hand, phylogeny groups of isolates were more resistant to carbapenems, sulfonamides, and fluoroquinolone groups, while showing intermediate susceptibility to the cephalosporin group and high sensitivity to aminoglycosides, tetracyclines, and phenicol groups in terms of sensitivity. Only genotype F showed higher resistance than the other types. Finally, there were no significant differences ($P > 0.05$), as shown in Table 4.

Association between phylogeny and antibiotic resistance patterns among isolates. Our findings revealed that antibiotic resistance patterns were most

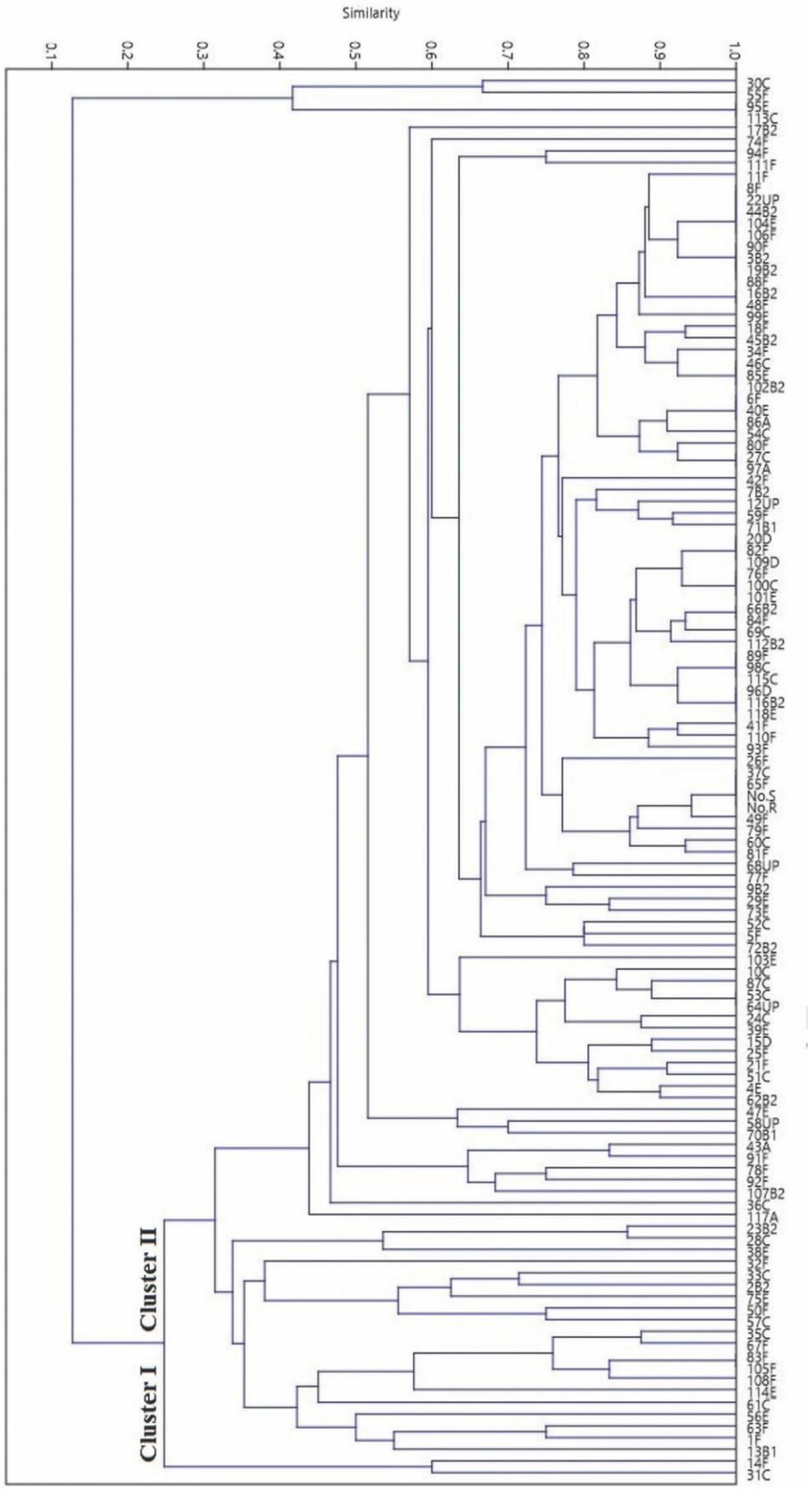

Fig. 2. UPGMA dendrograms with genetic Dice similarity coefficients analysis based on antibiotic susceptibility test results.

Table 4. Comparisons of antibiotics group susceptibility among phylogeny of *E. coli* (n=118).

Antibiotic Groups		Genotype Groups							
		A	B_1	B_2	C	D	E	F	UP
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Penicillins	S	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.7)	0 (0.0)	1 (0.9)	2 (1.7)	0 (0.0)
	R	1 (0.9)	2 (1.7)	17 (14.4)	19 (16.1)	3 (2.5)	13 (11.0)	36 (30.5)	4 (3.4)
	I	2 (1.7)	0 (0.0)	1 (0.9)	3 (2.5)	1 (0.9)	3 (2.54)	6 (5.9)	1 (0.9)
Carbapenems	S	2 (1.7)	1 (0.9)	7 (5.9)	9 (7.6)	1 (0.9)	7 (5.9)	13 (11.0)	1 (0.9)
	R	0 (0.0)	1 (0.9)	6 (5.1)	9 (7.6)	2 (1.7)	6 (5.1)	20 (17.0)	2 (1.7)
	I	1 (0.9)	0 (0.0)	5 (4.2)	6 (5.1)	1 (0.9)	4 (3.4)	11 (9.3)	2 (1.7)
Sulfonamides	S	2 (1.7)	1 (0.9)	4 (3.4)	5 (4.2)	0 (0.0)	5 (4.2)	12 (10.1)	1 (0.9)
	R	0 (0.0)	1 (0.9)	12 (10.1)	19 (16.1)	4 (3.4)	11 (9.3)	29 (24.6)	3 (2.5)
	I	1 (0.9)	0 (0.0)	2 (1.7)	0 (0.0)	0 (0.0)	1 (0.9)	3 (2.5)	1 (0.9)
Fluoroquinolones	S	1 (0.9)	1 (0.9)	1 (0.9)	9 (7.6)	1 (0.9)	4 (3.4)	12 (10.2)	1 (0.9)
	R	1 (0.9)	0 (0.0)	13 (11.0)	12 (10.1)	1 (0.9)	8 (6.8)	24 (20.3)	2 (1.7)
	I	1 (0.9)	1 (0.9)	4 (3.4)	3 (2.5)	2 (1.7)	5 (4.2)	8 (6.8)	2 (1.7)
Cephalosporin	S	0 (0.0)	0 (0.0)	1 (0.9)	4 (3.4)	0 (0.0)	4 (3.4)	5 (4.2)	0 (0.0)
	R	0 (0.0)	0 (0.0)	6 (5.2)	7 (6.0)	3 (2.5)	2 (1.7)	12 (10.2)	1 (0.9)
	I	3 (2.5)	2 (1.7)	11 (9.3)	13 (11.0)	1 (0.9)	11 (9.3)	27 (22.9)	4 (3.4)
Aminoglycosides	S	2 (1.7)	1 (0.9)	6 (5.1)	17 (14.4)	3 (2.5)	8 (6.8)	23 (19.5)	2 (1.7)
	R	0 (0.0)	0 (0.0)	3 (2.5)	4 (3.4)	0 (0.0)	3 (2.5)	8 (6.8)	0 (0.0)
	I	1 (0.9)	1 (0.9)	9 (7.6)	3 (2.5)	1 (0.9)	6 (5.1)	13 (11.0)	3 (2.5)
Tetracyclines	S	1 (0.9)	2 (1.7)	2 (1.7)	4 (3.4)	0 (0.0)	4 (3.4)	8 (6.8)	2 (1.7)
	R	0 (0.0)	0 (0.0)	2 (1.7)	2 (1.7)	0 (0.0)	1 (0.9)	3 (2.5)	0 (0.0)
	I	2 (1.7)	0 (0.0)	14 (11.9)	18 (15.3)	4 (3.4)	12 (10.2)	33 (28.0)	3 (2.5)
Phenicols S		3 (2.5)	2 (1.7)	12 (10.2)	18 (15.3)	1 (0.9)	14 (11.9)	33 (28.0)	5 (4.2)
	R	0 (0.0)	0 (0.0)	5 (4.2)	5 (4.2)	1 (0.9)	2 (1.7)	8 (7.0)	0 (0.0)
	I	0 (0.0)	0 (0.0)	1 (0.9)	1 (0.9)	2 (1.7)	1 (0.9)	3 (2.5)	0 (0.0)

prevalent in phylogenetic group F (35.6%), followed by phylogenetic groups C, B_2, and E (17.8%, 16.1%, and 13.6%), respectively. Other groups of least resistance patterns included phylogenetic groups UP (4.2%), A and D (3.4% for each), and B_1 (2.5%), as illustrated in Table 5.

DISCUSSION

The results of our study showed females had a higher rate of UTIs (78.8%) than males (21.2%). These observations are consistent with the findings of a significant number of studies (19-23), which indicate that females are more susceptible to infection than males. The increased proportion of urinary tract infections amongst female participants may be related to urogenital organ anatomical differences between the sexes (1), pregnancy, and the absence of prostate fluids. Other psychological factors, like the mechanical entry of microbes into the urethra following sexual activity, may also play a role in the increased incidence of UTIs in females (1, 24).

At least two of the following urinary complaints were diagnosed in our study, including urinary urgency, fever, flank pain, incontinence, urgency, vomiting, abdominal pain, irritability, hematuria, and diarrhea, which is equivalent to and supports the findings of previous investigators (1, 24). The presence of these clinical symptoms is an indication of urinary tract infection.

On the other hand, the current study has documented that operations are one of the most pathological factors that lead to UTIs, and this is consistent with the study documented by Gebremariam et al. (2019), which indicated an increase in urinary tract infection using catheters. This is owing to the microbes' virulence factors, including the creation of biofilm on catheters, which plays a role in pathogenesis (1, 24).

The second most prevalent conditions causing UTIs

Table 5. Prevalence antibiotics resistance patterns between phylogeny of *E. coli* (n=118).

Phenotype resistance patterns	Genotype Groups							
	A	B_1	B_2	C	D	E	F	UP
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Multi-drug Resistance	1 (0.9)	2 (2.5)	8 (6.8)	9 (7.6)	1 (0.9)	8 (6.9)	18 (15.3)	3 (2.5)
Extensively-drug resistance	3 (2.5)	0 (0.0)	11 (9.3)	11 (9.3)	3 (2.5)	8 (6.9)	23 (19.5)	2 (1.7)
Pan-drug Resistance	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)
Total (%)	4 (3.4)	2 (2.5)	19 (16.1)	21 (17.8)	4 (3.4)	16 (13.6)	42 (35.6)	5 (4.2)

are diabetes and kidney injury. A possible explanation for the elevated UTI in diabetes individuals is nerve damage induced by hyperglycemia, which affects the tissue bladder's capacity to perceive the presence of urine, allowing urine to remain in the stream for longer periods of time, which encourages bacteria to proliferate and propagate, raising infection (25). Other studies have corroborated similar conclusions to our study (21, 22, 26), reporting that UTIs are particularly common in diabetes patients. While kidney disease is one of the risk factors for UTIs, the patient's immune system is weakened, making them more susceptible to UTIs (21, 22, 27). This result is similar to the results of recent research (21, 22, 27, 28), which identified kidney injury as a risk factor for UTIs.

Microscopic examination revealed a significant association between pus cells and red blood cells as well as epithelial cells in the urine and urinary tract infections. These findings are in line with previous research (29), which suggests that the presence of pus cells, red blood cells, and epithelial cells in the urine is a reliable sign of the presence of bacteria in the urine and thus indicates a UTI. The main reason that makes these three cells a key sign of a urinary tract infection is that, due to their own role in immunity, they rise when infections occur (22, 27).

The sensitivity survey indicated that the highest development of resistance was associated with the group of β-lactam antibiotics among the isolates. The results of our study back up some of the results of studies (1, 19, 24, 30), which suggested that β-lactam antibiotics seemed to have the highest rates of resistance among isolates. This could be because these antimicrobials are frequently recommended in the initial treatment of UTIs.

Furthermore, *E. coli* has a clone locus encoding β-lactamase "chromosomal or plasmid" (2). This enables the potential for resistance acquisition across gastrointestinal related isolates (2, 31, 32). β-lactamase typically breakdown the β-lactam rings, enhance the proximity of the antibody to the specified location (PBPs), or modify the membrane structure (23, 24, 31, 32). These results suggested that these antibiotics were no longer useful as first-line therapy for UTIs with these bacteria, or that they could be retrained as useful medicine when combined with another group, resulting in a broader spectrum of activity against *E. coli*.

Sulfonamides and the fluoroquinolone achieved a high resistance rate among the isolates. These results were similar to the results of another study in Iraq, with different percentages (1, 23, 30). On the other hand, the phenicol, aminoglycoside, and tetracycline groups recorded the lowest rates of resistance. These results are consistent with the results of others (1, 23, 30), which documented low resistance ratios while respecting the difference in ratios. Personal and repeated use of medicines without consulting a doctor or random use of antibiotics, low-cost and lack of health awareness may be a reason for increased antimicrobial resistance to antibiotics.

In our present study, phylogroup F was found to be widespread across isolates. These findings contradict earlier research in Iran (19) as well as globally (1, 30, 32), which refers to B_2 as the most typical phylogroup.

These results lead us to two conclusions. The first is that the genomic backgrounds of *E. coli* in humans and food-producing animals are very similar. Phylogroup F, which includes the most common and virulent isolates that infect food-producing animals, and its spread mostly to humans, is attributed to animal-to-human transmission through the food chain (2, 31). Thus, transmitted isolates are formed through the transfer of genes between isolates by horizontal gene transfer. The second is that phylogroup F isolates inhabit a large number of virulence factors that enable *E. coli* to have a greater ability to colonize and induce UTIs (2, 31, 32).

Throughout our studies, phylogroup B_1 seemed to have the lowest rate of frequency in this study. This finding is consistent with findings from prior studies (1, 19), which indicated that group B_1 is the least prevalent group compared to other phylogenetic groups. However, previous studies suggested the reverse—that is, the majority of isolates related to Phylogroup B_2 among isolates that caused UTIs (30). Likewise, the frequency of the A and D phylogroups was dramatically lower than in other nations (1, 19, 30).

About 72.0% of the *E. coli* isolates in the current study participated in the C, E, and F phylogroups. This opposes a previous study, which found that roughly 25% of *E. coli* isolates participated in the C, E, F, and clade I phylogenies (30). Clermont et al. (2013) revealed that 1% of isolates still cannot be allocated to one phylogroup of the eight major groups (9, 30). Nevertheless, in the current investigation, 4% of isolates from UTI patients were untypeable. These findings are much lower than those from the results of Iranpour (30) which indicated that 27% of the isolates were categorized as unclassified. Untypeable isolates result from phylogroup recombination or extremely rare phylogroups (9).

Physical host health, nutritional and host genomic determinants, ecological, sociological, and topographical factors, or changes in collecting site and techniques, virulence factors, antibiotic resistance, growth rate, carbohydrate fermentation, and genome size may all reflect the varying frequency of the phylogenetic groupings documented in various studies (2, 9, 19, 21). These parameters influence the installation of isolates and, as a result, the various ranges of phylogenetic spread.

Our study clarified the phylogenetic groups F and C, which were shown to be associated with the highest incidence of MDR *E. coli* isolates. In comparison, a similar study (33), which found that non-B_2 strains had a large MDR range, and another study in Iran (30), which found that the majority of MDR UPEC belonged to phylogenetic group B_2. This could be due to the fact that phylogroup F contains a higher content of virulence genes associated with pathogenicity islands compared to other phylo-groups (2, 31, 32).

Clermont et al. (2013) discovered that phylogenic group F is a clonal isolates of group D, as well as a sister group to phylogenic group B_2 and may have been a branch of B_2 (4, 5, 6, 31). Phylogenic group E is also a clonal isolates of group D. These groups represent the most pathogenic isolates with elevated amounts of propagation, resistance, replicon, and pathogenicity island-associated genes (2, 31, 32). Thus, for this reason, phylogenic groups F and D are "converted" or "transmitted" isolates are considered highly pathogenic because they contain higher levels of adhesion genes and resistance patterns, explaining the prevalence of MDR patterns among group F and E UTI pathogens (3, 6, 9, 30, 32).

Likewise, phylogenetic group C isolates had high rates of antibiotic resistance. In comparison with our results, Tewawong et al. (19) reported that phylogenetic group C showed the highest rates of antibiotic resistance. Phylogenic group C isolates mainly consist of clonal isolates from group A and are similar to B_1 but unique. Groups A and B_1 are also daughter groups, and group B_2 is thought to represent the *E. coli* bacteria's ancestral lineage (4, 5, 9, 31, 32).

The current results promote the idea that both ExPEC isolates and commensal *E. coli* can cause UTIs (31). This is because *E. coli* is genotypically highly heterogeneous due to their highly varied and extensive genetic backgrounds arising from insertion and deletion of virulence genes via horizontal transfer (31), which makes *E. coli* have a very diversified genome and adaptive capabilities and are more aggressive (32). Furthermore, these findings highlight the fact that many UPEC isolates come from commensal isolates with low virulence genes (31, 32). These commensal isolates seem to have a higher natural tendency to acquire or maintain resistance to antibiotics since they are under more evolutionary pressure from antibiotics (2, 31, 32). Antimicrobials will be less effective as a result of *E. coli*'s creation of quiet intracellular reservoirs (QIRs), which may provide a reservoir for various resistance genes not recognized by the human defense system (2, 31, 32). As a result, these loci were less allergenic and less antibiotic-effective (2, 31, 32). By allowing germs to survive in the host and become increasingly resistant to regular antibiotics, this aids in the spread of MDR patterns and serves as a reservoir of recurring UTIs.

CONCLUSION

The results of our research revealed that certain groups of F were the most frequent, aggressive, and insensitive to antibiotics routinely recommended for individuals with urinary tract infections. For better knowledge about the prevalence and geographic dis-

tribution of *E. coli* phylogenetic groupings, analogous research in other regions is required.

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