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ORIGINAL ARTICLE

Antibiotic resistance pattern and phylogenetic groups of the uropathogenic *Escherichia coli* isolates from urinary tract infections in Hamedan, west of Iran

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ABSTRACT

Background and Objectives: *Escherichia coli* is the most common causative agent of urinary tract infections (UTIs) in 90-80% of patients in all age groups. Phylogenetic groups of these bacteria are variable and the most known groups are A, B1, B2 and D. The present study aimed to evaluate the phylogenetic groups of *E. coli* samples obtained from UTIs and their relation with antibiotic resistance patterns of isolates.

Materials and Methods: In this study 113 *E. coli* isolates were isolated from distinct patients with UTIs referred to Hamadan hospitals. After biochemical and molecular identification of the isolates, typing and phylogenetic grouping of *E. coli* strains were performed using multiplex PCR targeting *chu, yjaA* and *TSPE4.C2* genes. The anti-microbial susceptibility of the isolates to amikacin, ampicillin, trimethoprim-sulfamethoxazole, amoxicillin/clavulanic acid, ciprofloxacin, cefotaxime, imipenem, aztreonam, gentamicin, meropenem, nitrofurantoin, nalidixic acid and cefazolin was determined using disk diffusion method.

Results: Of 113 isolates, 50 (44.2%), 35 (31%), 23 (20.4%) and 5 (4.4%) of samples belonged to group B2, group D, group A and group B1 phylogenetic groups respectively. All isolates were susceptible to meropenem, imipenem (100%), followed by amikacin (99.1%). The highest resistance rates were observed against ampicillin (74.3%) and nalidixic acid (70.8%). Correlation between phylogenetic groups and antibiotic susceptibilities was significant only with co-amoxiclav (P = 0.006), which had the highest resistance in phylogenetic group A.

Conclusion: Prevalence of different phylogroup and resistance associated with them in *E. coli* samples could be variable in each region. Therefore, investigating of these items in *E. coli* infections, could be more helpful in selecting the appropriate antibiotic treatment and epidemiological studies.

Keywords: *Escherichia coli*; Antibiotic resistance; Phylogenetic group; Urinary tract infections; Multiplex polymerase chain reaction

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INTRODUCTION

Urinary tract infections (UTIs) are the second most common infection after respiratory tract infection. Many bacteria are causing infection in the urinary tract system; but, *Escherichia coli* is the

most common cause of UTIs among them (1, 2). *E. coli* is responsible in 50 and 80% UTIs in outpatients and hospitalized patients in Iran, respectively (3, 4). Misdiagnosis and inappropriate treatment of UTIs can lead to severe complications such as urinary tract disorders, residual scarring in the renal parenchyma, hypertension, urethritis, cystitis and uremia (5). These bacterial infections are considered as a serious threat to the health of society (5). Consequently, UTIs along with its related problems are leading to complications that are the causative agent of about 150 million deaths annually worldwide (6, 7). According to World Health Organization (WHO) reports, the annual costs for treatment of nosocomial infections is 17-29 billion dollars with 39% of them is the expense of treatment for UTIs (8, 9).

Phylogenetic variation in bacteria could be a result of differences in geographical conditions, lifestyle, antibiotic usage pattern, antibiotic resistance, growth rate and pathogenicity. Also, different phylogenetic groups have variable genome sizes. The most known phylogenetic groups of *E. coli* are groups A, B1, B2. Groups A and B1 have smaller genomes than groups B2 and D (10-12). *E. coli* is also classified pathologically and each group is called a pathotype. In this classification, the pathotypes that to cause extra-intestinal infections cause diseases such as UTIs, neonatal meningitis, and bloodstream infections, and the pathotypes associated with intestinal infections cause diseases such as severe diarrhea in adults and children (13). External intestinal pathogens of *E. coli* usually belong to B2 and D groups, whereas the commensal strains belong to groups A and B1 and the intestinal pathogens strains belong to groups B1, A and D (3, 4, 14, 15). By examining the gene library of *E. coli* strains of different phylogenetic groups and characterization of specific gene fragments, definite genes are used as a marker in the phylogenetic grouping (16, 17). These markers are as follows: *Chu A* (required for translocation of heme in *E. coli* O157: H7). *Yja A* (gene coding for a protein of unknown function) and the *TSPE4.C2* (the gene coding for a putative DNA fragment) (18-20). The aim of the present study was to evaluate the phylogenetic groups of *E. coli* isolates obtained from UTIs and antibiotic resistance patterns of isolates to reach information about the relationship between phylogenic group and their pattern of antibiotic resistance to control UTIs.

MATERIALS AND METHODS

Sample collection. A total of 112 *E. coli* isolates from urinary tract infections of patients referred to Sina Hospital and 1 *E. coli* isolate (from 12 urine samples taken from patients' bladder) during cystoscopy surgery (for direct examination of urine before passing lower urinary tract) gathered from Shahid Beheshti Hospital in Hamadan, Iran.

Confirmation of *E. coli* isolates. The bacterial isolates were cultured on MacConkey and Eosin Methylene Blue agar (EMB) media and the plates were incubated at 37°C for 24 hours and identified by conventional microbiological methods like Gram staining, IMVIC test, catalase test and urease production (21). The bacterial isolates were confirmed by the standard biochemical tests of bacteriology. Also for molecular confirmation, DNA of 113 clinical *E. coli* isolates was extracted using the boiling method (3). The purity of the DNA was estimated by calculating the absorbance ratio in ($A_{260/280}$) nm wavelengths with a nanodrop spectrophotometer. All *E. coli* samples confirmed by PCR amplification of the 200 bp fragment of the *16S rRNA* gene. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were considered as positive and negative controls respectively (Table 1).

Antibiotic susceptibility test. All *E. coli* isolates were investigated for their resistance to 13 antibiotics based on the Clinical and Laboratory Standards Institute (CLSI 2018) guidelines. The antibiotic susceptibility was tested by disc diffusion method using the amikacin (AK-30 µg), ampicillin (AP-10 µg), trimethoprim-sulfamethoxazole (TS-25 µg), amoxicillin/clavulanic acid (AUG-30 µg), ciprofloxacin (CIP-5 µg), cefotaxime (CTX-30 µg), imipenem (IMI-10 µg), aztreonam (ATM-30 µg), gentamicin (GM-10 µg), meropenem (MEM- 10 µg), nitrofurantoin (NI-300 µg), nalidixic acid (NA- 30 µg) and cefazolin (CZ- 30 µg) (All from Mast, UK) and the isolates were defined as susceptible, resistant, intermediate.

PCR reaction to determine the phylogenetic grouping (*ChuA, YjaA* and *TspE4C2*). The phylogenetic grouping of the uropathogenic *E. coli* (UPEC) isolates was carried out using a triplex PCR for *chuA, yjaA* and *TspE4.C2* genes which allows determination of all 4 different groups (Table 1). The

Table 1. The primers used in this study.

Gene	Primers	Size of PCR products (bp)	Reference
16SrRNAF	5-GCGGACGGGTGAGTAATGT-3	200	(27)
16SrRNAR	5-TCATCCTCTCAGACCAGCTA-3		
ChuAF	5-GACGAACCAACGGTCAGGAT-3	279	(28)
ChuAR	5-TGCCGCCAGTACCAAAGACA-3		
YjaAF	5-TGAAGTGTCAGGAGACGCTG-3	211	(28)
YjaAR	5-ATGGAGAATGCGTTCCTCAAC-3		
TspE4C2F	5-GAGTAATGTCGGGGCATTCA-3	152	(28)
TspE4C2R	5-CGCGCCAACAAAGTATTACG-3		

reaction mixture of PCR was 12.5 µl in a total volume containing 6.25 µl of master mix, 0.5 µl of (10 pmol) primers (0.25 µl forward and 0.25 µl reverse), 1 µl of genomic DNA (100 ng) and 2.25 µl of distilled water (dH_2O). The multiplex PCR performed with an initial denaturation at 95°C for 3 min followed for 30 cycles of denaturation at 95°C for 30 sec, annealing step at 59°C for 10 sec, and elongation step at 72°C for 1 min. The final extension was at 72°C for 5 min. PCR products were detected by electrophoresis on 1.5% agarose gel.

RESULTS

Of the 113 *E. coli* isolates collected in this study, 70 (61.9%) were collected from outpatient and 43 (38.1%) were from inpatients. The age of the patients was between 2 and 94 years with a mean age of 54.3 years (SD: 40.8). Of the total samples, 74.3% were originated from women and 25.7% from men.

Results of *16S rRNA* gene amplification for detection of *E. coli* samples. Identification of All 113 *E. coli* isolates by the phenotypic test was compatible with molecular detection assay using PCR amplification of the 200 bp fragment of the *16S rRNA* gene. (Fig. 1).

Results of the phylogenetic analyses. According to the results of gel electrophoresis of related genes to the phylogenetic grouping of *E. coli* and observation of relevant bands, grouping was performed. Phylogroup A has *chuA-/TspE4.C2-*, phylogroup B2 has *chuA+/yjaA+/ TspE4C2+*, phylogroup D has *chuA+/ yjaA-*, phylogroup A has *chuA-/TspE4.C2-/YjaA+*. The phylogenetic analyses demonstrated that all the *E. coli* isolates belonged either to B2 (n=50, 44.2%) or D phylogroup (n=35, 31%) (Table 2) (Fig. 2).

The results of the antibiogram. Rate of antibiotic resistance among the *E. coli* isolates was approximately high; as we saw 74.3% resistance to ampicillin, 53.1% resistance to trimethoprim sulfamethoxazole, and 70.8% resistance to Nalidixic acid. However, 100% of the isolates were sensitive to imipenem and meropenem. In the case of sensitivity, most of the isolates were sensitive to amikacin and nitrofurantoin (99.1% and 96.5% respectively). The comparison of the distribution of the antibiotic resistance in four phylogenetic groups demonstrated that the prevalence of all the detected resistance was more in the A phylogroup than D and B phylogroup. Also, the Correlation between studied phylogenetic groups and antibiotic susceptibility was significant only in the case of amoxicillin/clavulanic acid ($P = 0.006$) antibiotic, which we saw the highest resistance of it in A phylogroup. We saw a different and variable pattern of antibiotic resistance among different phylogenetic groups. The final results of antibiotic resistance in different phylogenetic groups are shown in detail in Fig. 3 and Table 3.

DISCUSSION

UTIs are the most common causes of outpatient referrals to medical centers, which may sometimes require hospitalization. So far, 8 phylogenetic groups (I, F, E, D, C, B2, B1, A) have been identified in this bacterium which the most important phylogenetic groups are A, B1, B2, D. Most of *E. coli* strains that are capable of tolerating external environment belong to the group B1 (10, 11). Extraintestinal

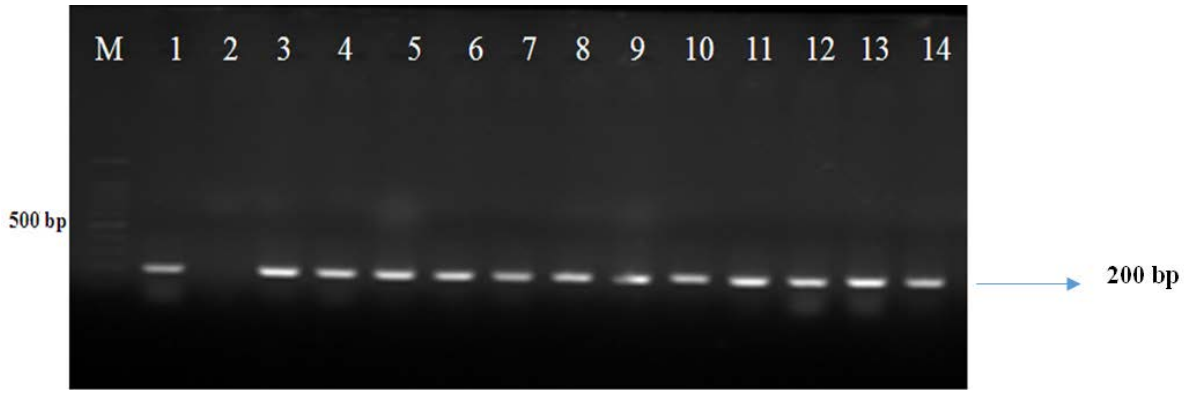

Fig. 1. Agarose gel electrophoresis of the *16S rRNA* gene. M: 100 bp marker. Line 1: *E. coli* ATCC 25922, line 2: Negative control (*Pseudomonas aeruginosa* ATCC 27853), line 3-14: *E. coli* isolates.

Table 2. Determination of phylogenetic groups of *E. coli* isolates.

Phylogenetic group	Number of isolates N (%)	Gender		In-/out-patient	
		Female N (%)	Male N (%)	In-patient N (%)	Out-patient N (%)
A	23 (20.4)	21 (91.30)	2 (8.7)	9 (39.13)	14 (60.87)
B1	5 (4.4)	4 (80)	1 (20)	1 (20)	4 (80)
B2	50 (44.2)	33 (66)	17 (34)	18 (36)	32 (64)
D	35 (31)	26 (74.28)	9 (25.72)	13 (37.14)	22 (62.85)
Total	113 (100)	84 (74.33)	29 (25.67)	41 (36.28)	72 (63.72)

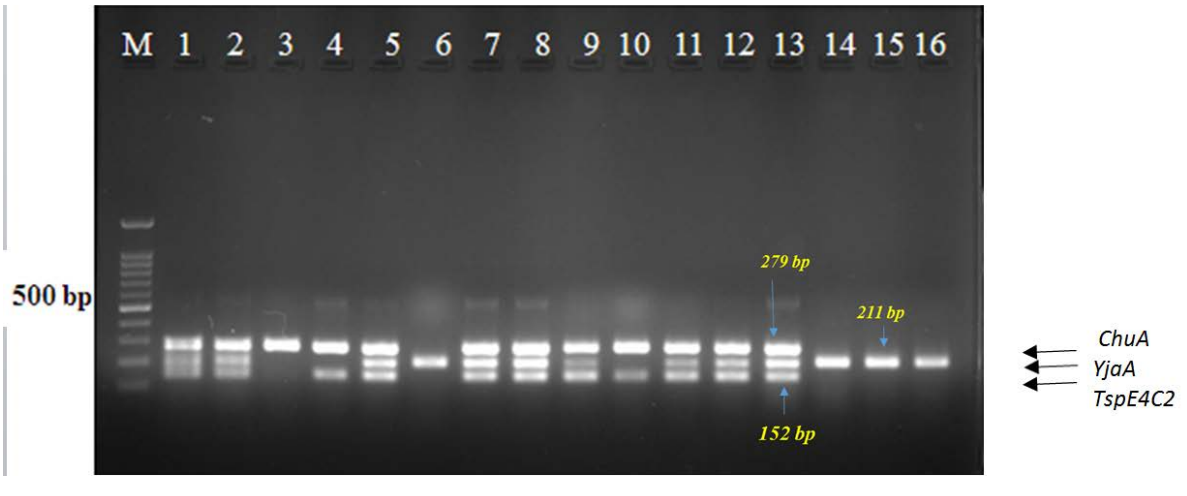

Fig. 2. M: DNA marker (100 bp) Agarose gel electrophoresis of *(ChuA, YjaA, TspE4C2)* genes in *E. coli* isolates. Lane 1-2, 5, 7-9, 11-13: B2 phylogroup *(chuA+/yjaA+/TspE4C2+)*; Lane 3-4: D phylogroup *(chuA+/yjaA-)*; Lane 6, 14-16: A phylogroup *(chuA-/TspE4.C2-/YjaA+)*

pathogenic *Escherichia coli* (ExPEC) more belong to B2 and D groups, whereas the commensal strains belong to groups A and B1 and the intestinal pathogenic strains belong to groups B1, A, and D (3, 4, 14). The best method to study phylogenetic groups is PCR as it is simple and rapid test. In addition to genome size which is not similar in different phylogroups of *E. coli*, groups A and B1 have smaller genomes than groups B2 and D (22). One of the most important aspects of treatment in UTIs are the rapid choosing of the good and inexpensive antibiotics and the main problem in the treatment of UTIs due to *E.*

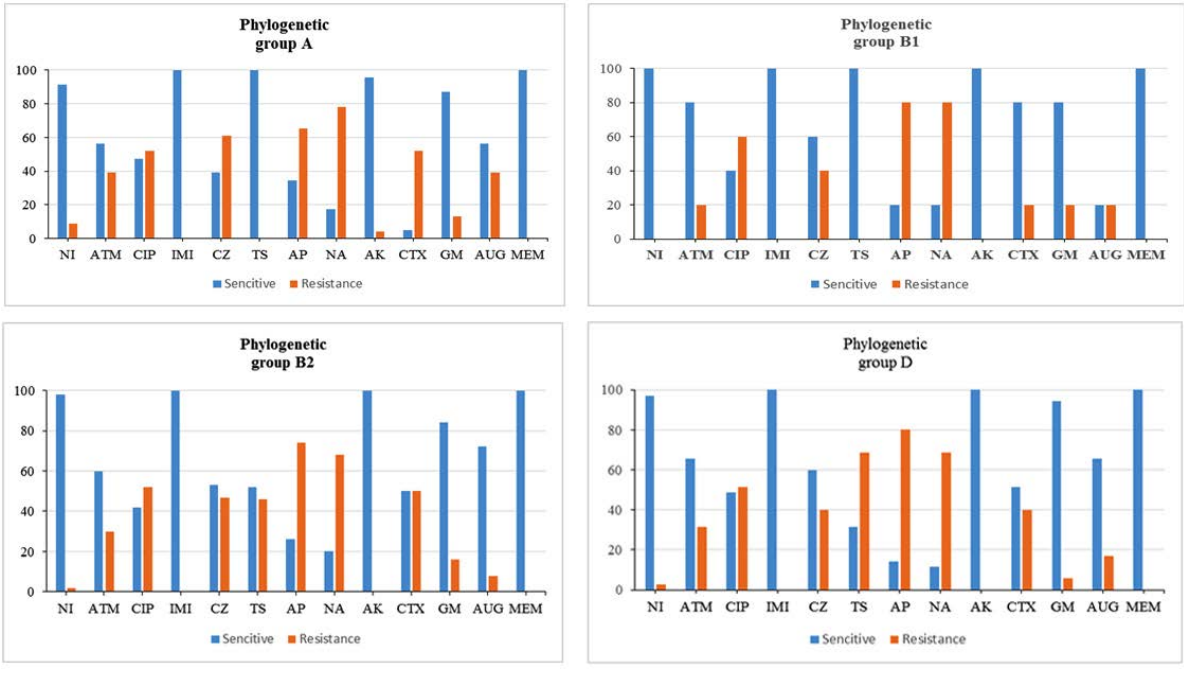

Fig. 3. Comparison of antibiotic resistance patterns between different phylogroups.
amikacin (AK), ampicillin (AP), trimethoprim-sulfamethoxazole (TS), amoxicillin/clavulanic acid (AUG), ciprofloxacin (CIP), cefotaxime (CTX), imipenem (IMI), aztreonam (ATM), gentamicin (GM), meropenem (MEM), nitrofurantoin (NI), nalidixic acid (NA), and cefazolin (CZ)

Table 3. Susceptibility pattern of the isolates against different antibiotics

Phylogenetic	NI		ATM		CIP		IMI		CZ		MEM		TS		AP		NA		AK		CTX		GM		AUG	
group	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
A	21	2	13	9	11	12	23	0	9	14	23	0	14	9	8	15	4	18	22	1	11	12	20	3	13	9
	91.3%	8.71%	56.5%	39.1%	47.8%	52.2%	100%	0	39.1%	60.9%	100%	0	60.9%	39.1%	34.5%	65.2%	17.4%	78.3%	95.7%	4.3%	4.8%	52.2%	87%	13%	56.5%	39.1%
B1	5	0	4	1	2	3	5	0	3	2	5	0	1	4	1	4	1	4	5	0	4	1	4	1	1	1
	100%	0	80%	20%	40%	60%	100%	0	60%	40%	100%	0	20%	80%	20%	80%	20%	80%	100%	0	80%	20%	80%	20%	20%	20%
B2	49	1	30	15	21	26	50	0	26	23	50	0	26	23	13	37	10	34	50	0	25	25	42	8	36	4
	98%	2%	60%	30%	42%	52%	100%	0	53.1%	46.9%	100%	0	52%	46%	26%	74%	20%	68%	100%	0	50%	50%	84%	16%	72%	8%
D	34	1	23	11	17	18	35	0	21	14	35	0	11	24	5	28	4	24	35	0	18	14	33	2	23	6
	97.1%	2.9%	65.7%	31.4%	48.6%	51.4%	100%	0	60%	40%	100%	0	31.4%	68.6%	14.3%	80%	11.4%	68.6%	100%	0	51.4%	40%	94.3%	5.7%	65.7%	17.1%
Total	109	4	70	36	51	59	113	0	59	53	113	0	52	60	27	84	19	80	112	1	58	52	99	14	73	20
	96.5%	3.5%	61.9%	31.9%	45.1%	52.2%	100%	0	52.7%	47.3%	100%	0	46%	53.1%	23.9%	74.3%	16.8%	0.8%	99.1%	0.9%	51.3%	46%	87.6%	12.4%	64.6%	17.7%

Amikacin (AK), ampicillin (AP), trimethoprim-sulfamethoxazole (TS), amoxicillin/clavulanic acid (AUG), ciprofloxacin (CIP), cefotaxime (CTX), imipenem (IMI), aztreonam (ATM), gentamicin (GM), meropenem (MEM), nitrofurantoin(NI), nalidixic acid (NA) and cefazolin (CZ).

coli strains is the bacterial resistance to many common antibiotics (16). The emergence and spread of bacterial resistant strains are often due to the genetic characteristics diversion of the bacteria and the high consumption of antibiotics (17). Here in this study, we aimed to design a survey to the phylogenetic classification of *E. coli* isolates derived from UTIs and investigate the antimicrobial resistance patterns of the isolates for further information on the regional *E. coli* phylogenetic grouping and relationship between

the phylogenetic groups and their antimicrobial resistance patterns to control UTIs. In this study, 113 *E. coli* isolates were investigated and classified into phylogenetic groups as this: 44.2% of the isolates belonged to group B2, followed by group D with 31% of isolates, group A with 20.4% of the isolates and group B1with 4.4% of isolates. Of the 113 *E. coli* isolates, 74.3% were resistant to ampicillin and 70% to nalidixic acid. Of the antibiotics tested, over 50% of the isolates were resistant to ciprofloxacin, tetracycline, ampicillin and nalidixic acid. All bacterial isolates were susceptible to meropenem and imipenem and after these antibiotics followed by amikacin (99.1%) and nitrofurantoin (96.5%). In our study, we saw the highest sensitivity to ampicillin and trimethoprim-sulfamethoxazole in phylogenetic group A. In phylogenetic group B1, the most susceptibility was seen against nitrofurantoin, cefazolin, aztreonam, nalidixic acid and cefotaxime. Isolates belonging to phylogenetic group B2 were highly susceptible to nalidixic acid, amikacin, and co-amoxiclav. Phylogenetic group D showed the highest sensitivity to ciprofloxacin, cefazolin and gentamicin. Morcatti et al., investigated 391 *E. coli* samples isolated from poultry and classified them into different phylogenetic groups of B1 and A according to highest number, whereas in the present study two phylogenetic group B2 and D had the largest number, which suggests that the host type may be influenced by the type of common phylogeny (23). In Iranpour et al. study, 140 *E. coli* samples isolated from UTIs were categorized into different phylogenetic groups, with B2 having the highest number and the most resistant was seen to amoxicillin (82.2%) and the least resistance was related to meropenem (0.7%), which our study is consistent with their results (24). Asadi et al., investigated the *E. coli* isolates from the urine culture of patients referred to Jahrom Hospital. In their study, the most common phylogenetic groups identified were group D (70%), group A (23.3%) and group B1 (6.7%). But none of the isolates belonged to the B2 group (25). Our results were unlike them, as in our study the B2 phylogroup had the highest prevalence. This difference may be due to the different geographical regions. Sohrabi et al. studied 137 isolates of UroPathogenic *Escherichia coli* (UPEC) isolated from patients with UTIs symptoms from Zanjan hospitals. They showed that the highest frequency was related to B2 group (67.15%), then group D (21.17%) and finally the group A (11.68%) and the phylogenetic group B1 were not observed in the (UPEC) isolates. According to their results of antibiotic resistance test, the highest resistance was observed against ampicillin (74.5%), azetronam (59.1%) and trimethoprim-sulfamethoxazole (55.5%), respectively and the least resistance were against imipenem (1.5%), amikacin (10.9%) and cefoxitin (11.7%). Rate of resistance in phylogenetic group B2 was more to cefotaxime, co-amoxiclav, and ciprofloxacin antibiotics; and tetracycline and trimethoprim-sulfamethoxazole resistance were higher in group D. Comparisons of resistance between phylogroup A and D showed that group A were significantly more resistant to cefoxitin, co-amoxiclav, and ciprofloxacin (26). Compared to our study, the frequency of different groups was consistent with the mentioned study, but resistance to ampicillin in group D and ciprofloxacin resistance in group B1 and co-amoxiclav resistance in group B2 were higher.

The present study and its comparison with other studies show that due to different items like geographical area, lifestyle and patterns of antibiotic in this region, we encounter the variable and different phylogenetic groups with different resistance patterns in *E. coli* isolates derived from patients with UTIs symptoms. Also, we assume that other factors such as mutations or different pathogenicity genes could be effective in the prevalence of different phylogenetic groups which must be considered in future studies. Consequently, determining the prevalence of each phylogroup in each region and examining the resistance in those phylogroups, could be more helpful to appropriate antibiotic treatment and better understanding the epidemiology of infective pathogens.

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