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Original Article

Phylogenetic Analysis and Antimicrobial Resistance Profiles of *Escherichia coli* Strains Isolated from UTI-Suspected Patients

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Abstract

Background: *Escherichia coli* as one of the most predominant pathogens is the major cause of urinary tract infections (UTI) worldwide. *E. coli* strains could be classified into distinct phylo-groups based on PCR method. Additionally, studying the antimicrobial resistance profiles of these strains is essential for finding the effective selection of treatment and evaluating the differences among resistance patterns of particular phylogenetic groups. This study aimed to determine the phylogroups of *E. coli* isolated from patients with UTI in Tehran, Iran.

Methods: The urine samples were collected from patients suspected to UTI from four hospitals in Tehran, Iran; Mofid, Vali-Asr, Bu-Ali and Tehran Heart Center (THC) Hospitals during 2014-2016. Assessing the antimicrobial resistance profile of the identified strains was accomplished using ampicillin, ceftriaxone, cefotaxime, and ceftazidime among β-lactam group; gentamicin, and streptomycin among aminoglycosides; nalidixic acid and norfloxacin from quinolones; and chloramphenicol disks. The phylogenetic characterization of 60 E. coli isolates obtained from patients with UTI was done by triplex PCR method.

Results: *E. coli* strains showed high resistance toward streptomycin (93.33%), ampicillin (86.6%) and nalidixic acid (73.33%) while resistance against chloramphenicol showed the lowest (10%). The prevalent groups were B2 (n=50/60, 83%), followed by D (n=6/60, 10%), B1 (n=3/60, 5%), and A (n=1, 1.6%).

Conclusion: The most predominant phylogenetic group was B2 with the major frequent detected with the major drug resistant (48%) compared to other Phylogenetic groups.

Keywords: *Escherichia coli*; Phylo-group; Triplex polymerase chain reaction; Urinary tract infections (UTI)

Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains are causing a great range of infections such as bacteriuria, bloodstream and urinary tract infections (1-3). Accordingly, it has been reported as the prevalent cause of bacteremia worldwide with the persistent growing rate accounting for 30% of all bacteremia (4, 5).

Urinary tract infections (UTIs) has been indicated as the most frequent bacterial infectious diseases (6) under the effect of sex and age, mostly found in females of all ages (7). The involved treatment of UTI due to the increasing prevalence of antibacterial resistance of *E. coli* strains of particular phylogenetic groups (8-11) has been considered

as a matter of importance (12). Antimicrobial susceptibility factor is very important to consider the proper drug selection for treating the patients (5, 13-18).

In recent years, various methods have been developed for determining the molecular epidemiology of microbial pathogens (19). Considering the advantages and disadvantages of the molecular typing processes, the requirements and resources of the laboratories and the aims of the investigation are the determinants for choosing the proper method. New data obtained from DNA profiling of *E. coli* reported from different hosts and habitats has advanced our analysis of ExPEC lineages (11, 20-22). Determining the phylogroups of an *E. coli* strain, Clermont et al. (23) reported a novel and easy multiplex polymerase chain reaction (PCR) method based on the presence and/or absence of chuA and yjaA genes and a DNA fragment, TspE4.C2. Recent development of genome study has determined the phylogroups of *E. coli*, represented as A, B1, B2, C, D, E and F (24-28). E and F are recognized as new groups; F designated as sister group of B2; and C designated as closely related group but distinct from B1 (29).

This study aimed to determine the phylogroups of *E. coli* isolated from patients with UTI according to the method of Clermont et al. (23); then to assess the antimicrobial resistance profile of the identified strains.

Materials and Methods

Origin of isolates and bacterial strains

To doing the study, we used the stored *E. coli* strains that have been collected previously from patients suspected to UTI from four hospitals in Tehran, Iran; Mofid, Vali-Asr, Bu-Ali and Tehran Heart Center (THC) hospitals during 2014-2016. Sterile samples were transported to the Microbiology Laboratory. Sixty *E. coli* isolates were collected and subjected to this research. All isolates were cultured on standard media, including LB agar, Muller-Hinton's agar, and LB Broth (Merck, Germany), and were incubated at 37 °C for 24 hours. Biochemical characteristics of *E. coli* strains were used for isolation and identification test. For long-term storage, isolated strains persevered at −20 °C in 20% skim milk (Merck, Germany) including 15%-20% glycerol.

Antimicrobial Susceptibility evaluation

Kirby-Bauer disk diffusion method was used for evaluation of susceptibility in culture media of Muller-Hinton's agar. Susceptibility testing was performed for 9 antimicrobial drugs including ampicillin, gentamicin, streptomycin, norfloxacin, ceftazidime, chloramphenicol, ceftriaxone, nalidixic acid, and cefotaxime. *E. coli* ATCC 25922 and ATCC 35218 were used as the quality control strains.

DNA isolation and phylogroup determination of E. coli isolates

Boiling method was used for DNA extraction of pure colonies. In summary, overnight pure cultures were heated at 95 °C for 16-24 h, centrifuged at 13,000 rpm for 2 min and kept at −20 °C. The obtained supernatant was utilized as template DNA for subsequent PCR. The integrity of extracted DNA was evaluated by electrophoresis on 2% agarose gel.

Determining the distribution of isolated *E. coli* phylogroups was performed using a reaction mixture contained 10 µL of 2x buffer (supplied with *Taq DNA* polymerase and $MgCl_2$), 1 µL of DNA genome (approximately 100 ng), 10 pmol of each appropriate primer with the total volume of 20 µL (17). Table 1 shows the primer sequences used in this study for assignment of new phylogroups.

The Mastercycler gradient (Eppendorf, USA) under the following conditions were used for PCR amplifications: initial denaturation at 95 °C for 5 min and 30 cycles for each denaturation at 94 °C for 30 sec, annealing at 56 °C for 30 sec, amplification at 72 °C for 40 sec, and final extension at 72 °C for 5 minutes.

Subsequently, PCR products were analyzed by electrophoresis equipped with a 2% agarose gel and visualized using GelDoc 2000 transillumina-

tor (Bio-Rad Laboratories, Milan, Italy). A molecular weight standard (100 bp ladder, Fermentas, Lithuania) was included on each gel.

Interpreting results for phylogrouping Phylogenetic groups were designated by the presence and/ or absence of chuA and yjaA genes and TspE4.C2 in triplex PCR (23).

Table 1: Primer sequences used in triplex phylotyping method

PCR reaction	*Primer ID*	*Target*	*Primer sequence*	*PCR product (bp)*
Triplex	chuA.1b	*chuA*	5-ATGGTACCGGACGAACCAAC-3	288
	chuA.2		5-TGCCGCCAGTACCAAAGACA-3	
	yjaA.1b	*yjaA*	5-CAAACGTGAAGTGTCAGGAG-3	211
	yjaA.2b		5-AATGCGTTCCTCAACCTGTG-3	
	TspE4C2.1b	*TspE4C 2*	5-CACTATTCGTAAGGTCATCC-3	152
	TspE4C2.2b		5-AGTTTATCGCTGCGGGTCGC-3	

Results

Drug Sensitivity Results

As illustrated in Fig. 1, the isolated samples were highly resistant to streptomycin 93.33%, ampicillin 86.6% and nalidixic acid 73.33% while chloramphenicol showed the lowest resistance (10%). Based on antibiotic categories (Table 2), the antibiotic resistances were related to β-lactams (66.9%), followed by quinolones (57.4%), aminoglycosides (54.1%) and chloramphenicol (10%), respectively.

Phylogenetic Classification

Based on the obtained results from PCR, the studied strains were divided into four phylogenetic groups with the prevalence distribution of B2 (n=50/60, 83%), followed by D (n=6/60, 10%), B1 (n=3/60, 5%), and A (n=1, 1.6%) (Fig. 2).

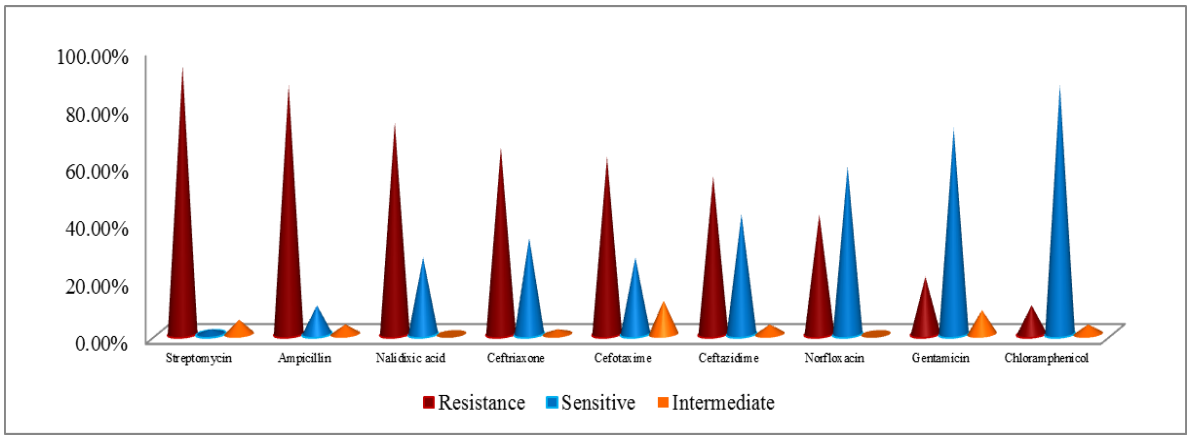

Fig. 1: Prevalence of resistance profiles

Phylotyping analysis revealed that the most prevalent multiple drug-resistant strain belonged to phylogroup B2 (48%).

We figured out the presence of a distinct difference between phylogenetic groups and resistance to all the studied antibiotics except chloramphenicol. Group B2 showed high resistance to all studied antibiotics except for chloramphenicol. Group A isolates showed only low resistance to three drugs, i.e. nalidixic acid, ampicillin, and streptomycin (Table 2).

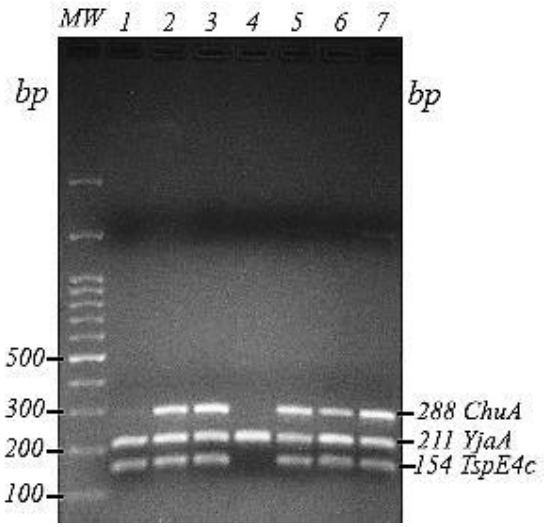

Fig. 2: Phylogenetic analysis of pathogenic *E. coli* isolates

Table 2: Prevalence of resistance among various phylogenetic groups of *E. coli* isolates

Antibiotics categories	***Antibiotic***	***B2***	***D***	***B1***	***A***	***Total***
Aminoglycosides	Gentamicin	8(13.3%)	-	1(1.6%)	-	9(15%)
	Streptomycin	46(78%)	6(10%)	3(5%)	1(1.6%)	57(93.3%)
β-lactam group	Ampicillin	44(73.3%)	5(8.3%)	2(3.3%)	1(1.6%)	52(86.6%)
	Ceftriaxone	34(56.6%)	4(6.6%)	1(1.6%)	-	39(65%)
	Cefotaxime	32(53.3%)	4(6.6%)	1(1.6%)	-	37(61.6%)
	Ceftazidime	29(48.3%)	3(5%)	1(1.6%)	-	33(55%)
Quinolones	Norfloxacin	24(40%)	1(1.6%)	-	-	25(41.6%)
	Nalidixic acid	38(63.3%)	4(6.6%)	1(1.6%)	1(1.6%)	44(73.3%)
Chloramphenicol	Chloramphenicol	4(6.6%)	2(3.3)	-	-	6(10%)

Discussion

As a worldwide health issue, the emergence, propagation, accumulation, and maintenance of antimicrobial-resistant pathogenic bacteria have been mostly considered for intensive therapeutic, prophylactic, and subtherapeutic uses of antimicrobial agents. Substantially, the increased selective pressures on both pathogenic and commensal bacteria have being employed for maintenance of antimicrobial-resistant bacteria (30).

Herein, the identification of *E. coli* through standard culture and biochemical tests from human urine samples was conducted, followed by triplex PCR to assign each isolate to a certain phylogenetic group giving in hand the recent phylogenetic studies on *E. coli* (31, 32).

Based on our finding, applying triplex PCR method divided all *E. coli* strains into four phylogroups, B2, B1, D, and A (23, 27). Our results of phylogenetic analysis showed the distribution of all pathogenic isolates into B2 (n=50/60, 83%), followed by D (n=6/60, 10%), B1 (n=3/60, 5%), and A (n=1, 1.6%).

The relation between antibiotic resistance and phylogroups showed that all *E. coli* isolates of group B1 were highly resistant to all antibiotic agents used especially penicillin and streptomycin. In contrast, only 1.6% isolates in group A were resistant to streptomycin, ampicillin and nalidixic acid.

Our findings are in line with other studies reported B2 and D as the most virulent isolates of *E. coli*. Overall, 105 *E. coli* strains were evaluated

with the phylogenic analysis showed four groups of B2 (51%) and D (20%) followed by A and D (33). B2 as the most prevalence phylogroup for patients infected by UTI in USA (34,35). Virulent extraintestinal *E. coli* strains belonged typically to group B2 and D and less often to group B1 (36,37). "A" has been reported in some researches (38, 39) as the most prevalence phylogroup which may refer to different distribution of *E. coli* strain within different social and geographic conditions. In this study, 93.33% and 86.6% of *E. coli* strains were resistant to streptomycin and ampicillin, respectively. The high frequency of ampicillin resistance among *E. coli* isolates has also been recently reported in various Asian and European countries, including Iran (14), China (40), Switzerland (41), and Italy (42), indicating inadequate treatment using these antibiotics.

Conclusion

Group B2 was the most predominant phylogenetic group and among the commonly used antibiotics for patients with UTI, isolated samples showed the highest resistance toward streptomycin. Regular monitoring of antibiotic resistance patterns will be useful to prescribe the most appropriate antibiotic and to avoid further development of antimicrobial drug resistance.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors confirm that this article content has no conflict of interest.

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