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 phylgroups 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 MgCl2), 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-ATGGGTACCGGACGAACCAAC-3 | 288             |
|              | chuA.2    |        | 5-TGCGGCCAGTACAAAGACA-3  |                 |
|              | yjaA.1b   | yjaA   | 5-CAAAACGTGAAGTGTCAGGAG-3 | 211             |
|              | yjaA.2b   |        | 5-AATGCCTTCCACAATCGTG-3  |                 |
|              | TspE4C2.1b| TspE4C | 5-CACATATTCCGTAAGGTACGC-3 | 152             |
|              | TspE4C2.2b| 2      | 5-AGTTATCGCTCGGTCG-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).

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. 1: Prevalence of resistance profiles

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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 been 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 phylogenetic 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.

**References**

1. Foxman B, Brown P (2003). Epidemiology of urinary tract infections: transmission and risk factors, incidence, and costs. *Infect Dis Clin North Am*, 17(2):227-41.
2. Johnson JR, Russo TA (2002). Extraintestinal pathogenic Escherichia coli: “the other bad E coli”. *J Lab Clin Med*, 139(3):155-62.
3. Russo TA, Johnson JR (2000). Proposal for a new inclusive designation for extraintestinal pathogenic isolates of Escherichia coli: ExPEC. *J Infect Dis*, 181(5):1753-4.
4. Martin GS, Mannino DM, Eaton S, Moss M (2003). The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med*, 348(16):1546-54.
5. Anvarinejad M, Farshad S, Ranjbar R, et al (2012). Genotypic analysis of E. coli strains isolated from patients with cystitis and pyelonephritis. *Iran Red Crescent Med J*, 14(7):408-16.
6. Basu S, Mukherjee SK, Hazra A, Mukherjee M (2013). Molecular characterization of uropathogenic Escherichia coli: nalidixic acid and ciprofloxacin resistance, virulent factors and phylogenetic background. *J Clin Diagn Res*, 7(12):2727-31.
7. Ejrnæs K (2011). Bacterial characteristics of importance for recurrent urinary tract infections caused by Escherichia coli. *Dan Med Bull*, 58(4):B4187.
8. Santo E, Salvador MM, Marin JM (2007). Multidrug-resistant urinary tract isolates of Escherichia coli from Ribeirão Preto, São Paulo, Brazil. *Braz J Infect Dis*, 11(6):575-8.
9. Hassan SA, Jamal SA, Kamal M (2011). Occurrence of multidrug resistant and ESBL producing E. coli causing urinary tract infections. *Aust J Basic Appl Sci*, 7(1):39-43.
10. Ranjbar R, Sami M (2017). Genetic Investigation of Beta-Lactam Associated Antibiotic Resistance Among Escherichia Coli Strains.
Isolated from Water Sources. Open Microbiol J, 11:203-210.
11. Momtaz H, Karimian A, Madani M, et al (2013). Uropathogenic Escherichia coli in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Ann Clin Microbiol Antimicrob, 128.
12. Køljalg S, Truusalu K, Stsepetova J, et al (2014). The Escherichia coli phylogenetic group B2 with integrons prevails in childhood recurrent urinary tract infections. APMHA, 122(5):452-8.
13. Tajbakhsh E, Khamesipour F, Ranjbar R, Ugwu IC (2015). Prevalence of class 1 and 2 integrons in multi-drug resistant Escherichia coli isolated from aquaculture water in Chaharmahal Va Bakhtiari province, Iran. Ann Clin Microbiol Antimicrob, 14:37.
14. Farshad S, Ranjbar R, Japoni A, et al (2012). Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic Escherichia coli strains isolated from children in Jahrom, Iran. Arch Irn Med, 15(5):312-6.
15. Jahandeh N, Ranjbar R, Behzadi P, Behzadi E (2015). Uropathogenic Escherichia coli virulence genes: invaluable approaches for designing DNA microarray probes. Cent Eur J Urin, 68(4):452-458.
16. Ranjbar R, Hagh-Ashtiani M, Jafari NJ, Abedini M (2009). The prevalence and antimicrobial susceptibility of bacterial uropathogens isolated from pediatric patients. Iran J Public Health, 38(2):134-8.
17. Ranjbar R, Hosseini S, Zahraei-Salehi T, et al (2016). Investigation on prevalence of Escherichia coli strains carrying virulence genes ipaH, estA, eaeA and bfpA isolated from different water sources. Asian Pac J Trop Dis, 6(4):278-83.
18. Khademestarki NS, Ranjbar R (2016). The phylogenetic study of Escherichia coli strains isolated from clinical cases. J Pure Appl Microbiol, 10(1):351-5.
19. Ranjbar R, Karami A, Farshad S, et al (2014). Typing methods used in the molecular epidemiology of microbial pathogens: a how-to guide. New Microbiol, 37(1):1-15.
20. Ranjbar R, Farahani O (2017). The Prevalence of Plasmid-mediated Quinolone Resistance Genes in Escherichia coli Isolated from Hospital Wastewater Sources in Tehran, Iran. Iran J Public Health, 46(9):1285-1291.
21. Abd, S, Ranjbar, R, HAKEMI, V M, et al (2014). Frequency of bla TEM, bla SHV, bla CTX-M, and qnrA among Escherichia coli isolated from urinary tract infection. Arch Clin Infect Dis, 9(1):1-5.
22. Anvarinejad M, Farshad S, Alborzi A, et al (2011). Integron and genotype patterns of quinolone-resistant uropathogenic Escherichia coli. Afr J Microbiol Res, 5(22):3765-70.
23. Clermont O, Bonacorsi S, Bingen E (2000). Rapid and simple determination of the Escherichia coli phylogenetic group. Appl Environ Microbiol, 66(10):4555-8.
24. Jaureguy F, Landraud L, Passet V, et al (2008). Phylogenetic and genomic diversity of human bacteremic Escherichia coli strains. BMC Genomics, 9:560.
25. Moissenet D, Salauze B, Clermont O, et al (2010). Meningitis caused by Escherichia coli producing TEM-52 extended-spectrum β-lactamase within an extensive outbreak in a neonatal ward: epidemiological investigation and characterization of the strain. J Clin Microbiol, 48(7):2459-63.
26. Tenaillon O, Skurnik D, Picard B, Denamur E (2010). The population genetics of commensal Escherichia coli. Nat Rev Microbiol, 8(5):207-17.
27. Clermont O, Olier M, Hoede C, et al (2011). Animal and human pathogenic Escherichia coli strains share common genetic backgrounds. Infect Genet Evol, 11(3):654-62.
28. Luo C, Walk ST, Gordon DM, et al (2011). Genome sequencing of environmental Escherichia coli expands understanding of the ecology and speciation of the model bacterial species. Proc Natl Acad Sci U S A, 108(17):7200-5.
29. Clermont O, Christenson JK, Denamur E, Gordon DM (2013). The Clermont Escherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep, 5(1):58-65.
30. Alali WQ, Scott H, Harvey R, et al (2008). Longitudinal study of antimicrobial resistance among Escherichia coli isolates from
integrated multisite cohorts of humans and swine. *Appl Environ Microbiol*, 74(12):3672-81.

31. Usein C-R, Grigore LA, Georgescu RM, et al (2011). Phylogenetic background and extraintestinal virulence genotypes of *Escherichia coli* vaginal strains isolated from adult women. *Rev Romana Med Lab*, 19: 37-45.

32. Skjøt-Rasmussen I, Ejrnæs K, Lundgren B, et al (2012). Virulence factors and phylogenetic grouping of *Escherichia coli* isolates from patients with bacteremia of urinary tract origin relate to sex and hospital-vs. community-acquired origin. *Int J Med Microbiol*, 302(3):129-34.

33. Rijavec M, Müller-Premru M, Zakotnik B, Žgur-Bertok D (2008). Virulence factors and biofilm production among *Escherichia coli* strains causing bacteremia of urinary tract origin. *J Med Microbiol*, 57(11):1329-34.

34. Kanamaru S, Kurazono H, Nakano M, et al (2006). Subtyping of uropathogenic *Escherichia coli* according to the pathogenicity island encoding uropathogenic-specific protein: Comparison with phylogenetic groups. *Int J Urol*, 13(6):754-60.

35. Johnson JR, O'Sullivan MA, Owens K, et al (2003). Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephemycins among *Escherichia coli* isolates from animals and humans. *J Infect Dis*, 188(5):759-68.

36. Hancock V, Nielsen EM, Krag I, Engberg J, Klemm P (2009). Comparative analysis of antibiotic resistance and phylogenetic group patterns in human and porcine urinary tract infectious *Escherichia coli*. *APMIS*, 117(11):786-90.

37. Zhao L, Chen X, Zhu X, et al (2009). Prevalence of virulence factors and antimicrobial resistance of uropathogenic *Escherichia coli* in Jiangsu province (China). *Urology*, 74(3):702-7.

38. Johnson JR, Owens K, Gajewski A, Kuskowski MA (2005). Bacterial characteristics in relation to clinical source of *Escherichia coli* isolates from women with acute cystitis or pyelonephritis and uninfected women. *J Clin Microbiol*, 43(12):6064-72.

39. Moreno E, Prats G, Sabaté M, et al (2006). Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli*. *J Antimicrob Chemother*, 57(2):204-11.

40. Liu Y, Liu G, Liu W, et al (2014). Phylogenetic group, virulence factors and antimicrobial resistance of *Escherichia coli* associated with bovine mastitis. *Res Microbiol*, 165(4):273-7.

41. Borsari AG, Buecher B, Brazzola P, et al (2008). Susceptibility of *Escherichia coli* strains isolated from outpatient children with community-acquired urinary tract infection in southern Switzerland. *Clin Ther*, 30(11):2090-5.

42. Caracciolo A, Bettinelli A, Bonato C, et al (2011). Antimicrobial resistance among *Escherichia coli* that cause childhood community-acquired urinary tract infections in Northern Italy. *Ital J Pediatr*, 37:3.