Detection of Fimbrial Genes, Antibiotic Resistance Profile and Phylogenetic Background of Uropathogenic *E. coli* Isolated from Clinical Samples in Karaj City, Iran

Maryam Ebadi [1], Nasrin Askari [1], Maziar Jajarmi [1], Reza Ghanbarpour [2]*

[1] *Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*.

[2] *Molecular Microbiology Research Group, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*.

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ABSTRACT

***Background*:** The aim of the present study was to determine the prevalence of phylogenetic groups/subgroups, fimbrial genes, and antibiotic susceptibility of *E. coli* isolated from urinary tract infections in Karaj city, Iran.

***Methods*:** A total of 107 *E. coli* isolates were confirmed by standard bacteriological tests. The phylogenetic group, fimbrial genes and antibiotic resistance genes was determined by PCR method. Antibiotic resistance of all the isolated *E.coli* against nine antimicrobial agents was determined by disk diffusion method.

***Results*:** PCR assays showed the prevalence of fimbrial genes among the studied isolates were 31.7% and 9.3% for *papEF* and *afaBC*, respectively. Most of *papEF* genes were placed in D phylogroup (18.6%) and D1 subgroup (14.01%) and the percentage of *afaBC* (2.8%) were similar in B1, B2 and D phylogroups. The frequency of *tetA* and *tetB* genes were 22.4% and 17.7%. Isolates which contained *tetA* were distributed mainly in D group (14.01%) and those which contained *tetB* were divided in D group (7.48%). Antimicrobial susceptibility testing showed the maximum resistance rate to cephalexin (CN: 100%) and the minimum resistance level to ciprofloxacin (CP: 36.5%).

***Conclusion*:** The present study showed that phylogenetic groups A and D were predominant. Virulence factors such as *papEF* and *afaBC* belonged to D phylogenetic group. Multidrug resistance *E. coli* isolates tends to be in the non-B2 phylogenetic groups. Due to high antibiotic resistance, appropriate control should be considered in medicine to control the development of novel resistant isolates.

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*Corresponding Author: Reza Ghanbarpour, Molecular Microbiology Research Group, Shahid Bahonar University of Kerman, Kerman, Iran.

Tel: +98- 034--33202923, *E-mail:* ghanbar@uk.ac.ir

Introduction

Escherichia coli is the main cause of urinary tract infections (UTIs), including acute cystitis, pyelonephritis, and urosepsis, three important and clinically different UTI syndromes. It is mainly believed that uropathogenic *E. coli* (UPEC) originates from the distal gut microbiota (1, 2, and 3). Subsequently, UPEC tends to be distinct from the commensal *E. coli* isolates in the intestinal tract in requiring extra virulence genes, allowing their effective transition from the intestinal tract to the urinary tract. In this regard, UPEC isolates can produce diverse types of adhesins including type 1 fimbriae, P fimbriae (pyelonephritis-associated pili), S fimbriae (sfa), and Afa adhesins (afa) for afimbrial adhesins that are essential for the initiation, recognition, and adherence to receptors of the urinary tract cells (4). There are large numbers of adhesins such as pap, sfa, and afa which are effective in pathogenicity of *E. coli* isolates associated with extraintestinal infections (5).

Previous studies have found that *E. coli* isolates belong to four major phylogenetic groups (A, B1, B2, and D) and seven subgroups (A0, A1, B1, B22, B23, D1, and D2) (6, 7). ExPEC significantly belonged to B2 group and a trivial extent to D group. Isolates of the B2 and D groups harbor much virulence factors than isolates of the A and B1 groups (8, 9, 10, 11).

Now a days, unselective use of antibiotics leads to drug resistance in *E. coli* (12). It seems that there is a significant increase in the resistance of ExPEC to the first-line antimicrobial agents such as fluoroquinolones in both hospital environment and community. *E. coli* isolates with fluoroquinolone resistant also display resistance to other antibiotics such as trimethoprim/sulfamethoxazole, gentamicin, tetracycline, ampicillin, and chloramphenicol (13, 14). Trimethoprim–sulfamethoxazole (cotrimoxazole) is common antimicrobial agents recommended for UTI prophylaxis (15).

An epidemiological relation between an *E. coli* gene and UTIs recommend that the gene itself encodes a factor contributing to urovirulence or has a genetic link to such a gene. So, the genes involved in UTIs are valuable in distinguishing UPEC from non uropathogenic *E. coli* and in the improvement of strategies for management and treatment this type of disease. On the other hand, there are only a few reports about phylogenetic background of *E. coli* isolates from clinical sources in Iran. The aim of the current study was to determine the prevalence of phylogenetic groups/subgroups, fimbrial genes, and antibiotic susceptibility of *E. coli* isolated from urinary tract infections in Karaj city, Iran.

Material and method

Source of clinical samples and E. coli isolation

This study was performed on 107 *E. coli* isolates from UTIs. The isolates were belong to urine samples of patients referred to the laboratories of Karaj city, Iran. The samples were taken during Jan to Oct 2016. These isolates were identified as *E. coli* according to standard bacteriological and biochemical tests.

Two reference isolates including *E. coli* A30 (afaBC) and *E. coli* J96 (sfa/focDE, papEF) were used as positive controls for fimbrial genes. Reference isolate from the ECOR collection including ECOR62 was used as positive control for phylogrouping. Nonpathogenic *E. coli* isolate MG1655 was used as a negative control. The reference isolates were provided from Microbiology Department of Ecole Nationale Vétérinaire Toulouse, France.

PCR assay for Phylotyping

The phylogenetic groups (A, B1, B2, and D) and subgroups (A0, A1, B1, B2–2, B2–3, D1, and D2) of each isolate were determined by multiplex PCR amplification as described by Clermont et al. (2000).

PCR assay for detection of fimbrial genes and antibiotic resistance genes

DNA was extracted from *E. coli* isolates and reference isolates by lysis method with NaOH. *E. coli* isolates were tested by PCR assay for the presence of *papEF*, *afaBC*, and *sfa/focDE* genes described by Yamamoto et al. (1995). Antibiotic resistance gene including *TetA* and *TetB* were detected as described by Olowe et al. (2013). The specific primers used for detection of fimbrial genes and the antibiotic resistance genes are presented in Table 1.

Antibiotic susceptibility test

Antibiotic resistance of all the isolated E.coli against nine antimicrobial agents was determined by disk diffusion method according to Clinical Laboratory Standards Institute (CLSI 2012). Commercial antimicrobial disks were provided from Merck co. Germany. The antibiotic disks used in this study were nalidixic acid (NA; 30 µg), ciprofloxacin (CP; 5 µg), trimethoprim/ sulfamethoxazole (SXT; 25 µg), oxytetracycline (T; 30 µg), tetracycline. (TE; 30 µg), doxycycline (D; 30 µg), cephalexin (CN; 30 µg), levofloxacin (L; 5µg) and ceftriaxone (CRO; 30 µg).

Results

PCR assays showed that 107 *E.coli* isolates were belonged to four phylogenetic groups as follow: A (30.8%), B1 (7.8%), B2 (17.9%) and D (48.5%) and seven phylogenetic subgroups including A0 (29.9%), A1 (0.93%), B1 (6.54%), B2-2(0.93%), B2-3(14.01%), D1 (37.3%) and D2 (10.2%). Results showed that the most frequent phylogroup was D whereas the lowest frequency was seen in B1 group. On the other hand, the prevalence of fimberial genes among the studied isolates were 31.7% and 9.3% for *papEF* and *afa BC*, Respectively. None of isolates were not positive for *sfa/focDE* virulence gene. Most of *papEF* genes were placed in D phylogroup (18.6%) and D1 subgroup (14.01%) and the percentage of *afaBC* (2.8%) were similar in B1, B2 and D phylogroup. The frequency of *tetA* and *tetB* genes were 22.4% and 17.7%, respectively. Isolates which contained *tetA* were distributed mainly in D group (14.01%) and D1 sub phylogroup (10.28%) and also those which contained *tetB* were divided in D group (7.48%) and D1 subphylogroup (6.54%). None of the isolates were positive for *tetA* and *tetB* simultaneously.

Antimicrobial susceptibility testing showed the maximum resistance rate to cephalexin (CN: 100%) and the minimum resistance level to ciprofloxacin (CP: 36.5%). Moreover, fifty two antibiotic resistance patterns was found. The most prevalent pattern was T/TE/D/SXT/CP/NA/LOM/CRO/CN which observed in seventeen isolates (15.88%).

Discussion

The present study showed the specific association between the phylogenetic backgrounds with fimbrial genes and antibiotic resistance in uropathogenic *E. coli*. Results showed that the most frequent phylogroup was D whereas the lowest frequency was seen in B1 group. Alizadeh et al (2013) reported the most prevalent phylogenetic background were A and D phylogenetic groups. Whereas, previous studies showed that group B2 and D were the most frequent *E. coli* phylogroup in UTIs in different parts of the world (10, 11). Results showed that the highest prevalence of phylogenetic background were associated with A and D phylogenetic groups, whereas previous studies in different parts of the world found that group B2 and D were the most frequent *E. coli* biotype in UTIs (10, 11, 16) mentioned that diverse geological areas affect the distribution phylogenetic background, virulence genes, and antibiotic resistance of *E. coli* isolates. The prevalence of fimbrial genes among the studied isolates were 31.7% and 9.3% for *papEF* and *afaBC* , respectively. None of isolates were not positive for *sfa/focDE* virulence gene. Most of papEF genes were placed in D phylogroup

(18.6%) and D1 subgroup (14.01%) and the percentage of *afaBC* (2.8%) were similar in B1, B2 and D phylogroup. These results differ from reports of other studies, which showed virulence factors such as fimbriae mostly belong to phylogenetic group B2 and D groups (9, 10, 11, 17) have shown that the presence of pili P among children was associated with phylogenetic groups B2 and D. (18) showed, pyelonephritis-associated pili (papEF) and afimbrial adhesin I (afaIBC) (10.65 % both) showed highest prevalence. The prevalence of S fimbriae (sfa/focDE) among the studied strains was 6.55 %. The other studies, (19) reported that the high prevalence of *E. coli* isolates
were positive for *papEF*, *afaBC*, and *sfa/focDE* genes. Multidrug resistance in infectious disease is a global public health concern (20, 21, and 22). In the present study, the high levels of multidrug resistance of *E. coli* isolates to antimicrobial agents such as nalidixic acid, ciprofloxacin, trimethoprim/ sulfamethoxazole, oxytetracycline, tetracycline, doxycycline, cephalexin, levofloxacin, and ceftriaxone were observed. Antimicrobial susceptibility testing showed the maximum resistance rate to cephalexin (100%) and the minimum resistance level to ciprofloxacin (36.5%). Veranic et al (2016) reported the highest antimicrobial resistance of *E. coli* for ampicillin (82.79%), followed by trimethoprim-sulfamethoxazole (40.86%), nalidixic acid (19.35%), cephazolin (7.52%), nitrofurantoin (5.37%), gentamicin (2.15%) and ciprofloxacin (4.30%).

Table 1. Specific primers used for PCR amplifications of target gene.

Gene	Primer Sequence (5′-3′)	Product size (bp)	Reference
afaIBC	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	23
papEF	GCAACAGCAACGCTGGTTGCATCA AGAGAGAGCCACTCTTATACGGACA	336	23
tet(A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	577	24
tet(B)	CCTCAGCTTCTCAACGCGTG GCACCTTGCTGATGACTCTT	634	24
ChuA	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	279	9
YjaA	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	211	9
TSP	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	152	9

Conclusion

In conclusion, the present study showed that phylogenetic groups A and D were predominant. Virulence factors such as *papEF* and *afaBC* belonged to D phylogenetic group. Multidrug resistance *E. coli* isolates tends to be in the non-B2 phylogenetic groups. Further research work are needed to characterize more virulence and antibiotic resistance genes and also phylogenetic background of uropathogenic *E. coli*.

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Ethical Considerations

All ethical issues including the purpose, risk, benefits and human activities involved in this study were considered during the research.

Conflict of interest

The authors declare that they have no competing interests.

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