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Original Article

Molecular Typing of Uropathogenic *Escherichia coli* Strains Isolated from Patients by Random Amplified Polymorphic DNA-PCR (RAPD-PCR)

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Abstract

Background and Aim: Urinary tract infections (UTIs) are one of the most common pathological diseases in communities and hospitals, often caused by uropathogenic *Escherichia coli* (UPEC). The use of microbial strains typing is an integral part of epidemiological surveys of infectious diseases to identify epidemics, detect the infection source, track and recognize pathogenic strains. In this study, uropathogenic *Escherichia coli* (UPEC) strains isolated from patients living in Gorgan were typed using RAPD-PCR.

Methods: In total, 187 *Escherichia coli* strains isolated from urine samples of inpatients and outpatients of Gorgan city from 2010 to 2016 were analyzed by the RAPD-PCR method using two primers. Using GelClust and FigTree software, the respective dendrograms were plotted by unweighted pair group method with arithmetic mean (UPGMA).

Results: In our research, 614 bands were detected using two primers. The highest frequency of bands was obtained in 400 bp and 500 bp with 65 repeats and the lowest number of bands was in 2500 bp and 3000 bp with one repeat and 32 clusters. The largest number of isolates (i.e.14) was placed in cluster 16. Most bands were polymorphic, indicating high genetic diversity in isolates.

Conclusion: Analysis of 32 clusters of our study by the RAPD-PCR method showed that the studied clusters do not have a specific and unique feature and the scattering of isolates properties are equal among the clusters. Because each cluster had its characteristics, E. coli strains in the region have great genetic diversity.

Keywords: Uropathogenic *Escherichia coli*; RAPD; GelClust; Typing, UTI.

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Introduction

Escherichia coli is the most prevalent gram-negative, facultative anaerobic bacterium in human fecal flora that is commonly present as a commensal microorganism in the colon (1). There are several pathogenic types of E. coli, including enterohemorrhagic E. coli (EHEC), enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC), as well as diffuse adhering E. coli (DAEC) and uropathogenic E. coli (UPEC) (2). UPEC is a major factor of urinary tract infections (UTIs) such as cystitis, pyelonephritis, and infectious complications, leading to acute renal failure in kidney transplant patients (3), as well as UTI in over 80% of cases (4). Previous WHO reports show that urinary tract infection is a common cause of fever in 3–8% of girls and 1% of boys (5). Every year, approximately 7000000 medical visits have been attributed to UTI (6) and the annual cost of treating urinary tract infections in the United States is estimated at $ 2.47 billion (7). For effective

epidemiological surveillance and monitoring, the accurate detection, differentiation, and subtyping of strains are needed to identify potential infection sources (8). Genotyping of E. coli strains can help to reveal individual pathogens in a given host and serve as a guideline for determining the source of infection in epidemiological studies (9). Several classical typing approaches such as plasmid typing, serotyping, antibiogram, and phage typing are not only costly and time-consuming but also lack the ability to differentiate between closely related strains; therefore, we witness an increasing focus on the development and application of molecular techniques (8, 10). Molecular methods of typing include MLST, PFGE, RFLP, REP-PCR, RAPD-PCR, and ERIC-PCR, RAPD-PCR, first described by WILLIAM et al. in 1990 as well as WELSH in the same year (11), is a suitable method for overall comparison of genomes in the shortest time with proper differentiation ability. In this method, short and random oligonucleotide primers are used, a profile of DNA fragments is obtained after PCR, and a comparison of profiles from different samples indicates genetic differences between them (12, 13). The aim of the present study was to the investigation of genetic diversity of strains of UPEC isolated from patients and perform an epidemiologic study of strains in Gorgan province.

Methods

Identification of isolates and antibiotic susceptibility testing:

In this descriptive study, 187 E. coli strains isolated from mid-stream urine samples were collected from hospitalized and non-hospitalized patients with UTI who were referred to Gorgan medical centers from 2010 to 2016.

The Initial identification of bacteria isolated from UTI was performed by a culture in the blood agar and MacConkey agar. Then all the samples were identified by biochemical tests for Enterobacteriaceae, including TSI, lysine, urea, and IMVIC (2). The susceptibility of bacteria to antibiotics (i.e. cefotaxime, ceftriaxone, ciprofloxacin, gentamicin, nitrofurantoin, imipenem) was tested by disc diffusion method (Kirby-Bauer) on Mueller-Hinton agar medium based on Clinical Laboratory Standard Institute (CLSI) guidelines (14), and boiling method was used to extract genomic DNA (15).

RAPD-PCR

RAPD-PCR reaction was optimized by two random primers A (5'-CCGCAGCCAA-3') and B (5'-AAGAGCCCGT-3') made by Metabion Company (16). The PCR reaction was conducted in a final volume of 25µl containing 2.5µl of 10x PCR buffer, 2µl of 50mM Mgcl2, 1µl of 10mM dNTP, 0.5µl of Taq DNA polymerase (5 unit), 1.5 µl (10 pmol) of each A and B primer, 2 µl of the test sample with 15.5 µl of sterile distilled water and PCR amplification was performed in a Thermal Cycler (ASTEC- Pc 816) programmed as follows: one cycle at 94 °C for 5 min followed by 45 cycles of 30 s at 92 °C, 1 min at 35 °C, and 1 min at 72 °C, for DNA, denaturing, primer annealing, and primer extension, respectively (17). Reproducibility of RAPD-PCR for each primer was confirmed by performing at least two experiments per sample and a tube lacking DNA was considered as a negative PCR control. PCR products were electrophoresed on 1.5% agarose gel mixed with 0.4 µl SYBR Green dye and photographed by UV irradiation.

Data analysis

To analyze the results, the presence and absence of a band were designated with 1 and 0 in Microsoft Excel, respectively. Afterward, the similarity matrix between strains was calculated by Matching simple and Dice similarity indices using GelClust and Fig Tree software. The corresponding dendrograms were plotted using UPGMA and finally clustered with a 70% matching coefficient.

Results

From a total of 187 E. coli strains isolated in our study, 82 (44%) were taken from outpatients and 105 (56%) from hospitalized patients. Also, 120 (64%) and 67 (36%) isolates were from women and men with UTI, respectively. 50 isolates (27%) were resistant to one of the antibiotics (cefotaxime, ceftriaxone, ciprofloxacin, gentamicin, nitrofurantoin, imipenem, piperacillin) and 137 isolates (73%) had susceptibility to antibiotics listed, none of the strains showed multidrug resistance. Out of 187 isolates examined by RAPD-PCR with the

mentioned two primers, there were no bands in 23 isolates (12.3%), which were excluded from our study, and 164 isolates (87.7%) could be typed with a 70% matching coefficient and were stratified into 32 clusters. From 164 isolates, 148 showed acceptable bands with both primers but 16 isolates formed a band with only one primer. In the obtained profile, the bands were coded in the 200-3000 bp range, and the total number of bands amplified by the two primers was estimated to be 614. The highest frequency of bands was in 400 and 500 bp size with 65 repeats and the lowest number of bands was in 2500 and 3000 bp with one repeat (Figure 1). In the results of the antibiotic susceptibility, the number of resistant isolates to imipenem and piperacillin was very low in all clusters. Also, isolates with resistance to ciprofloxacin, ceftriaxone, cefotaxime were seen in all clusters. Strains in clusters 14, 15, and 29 were not resistant to any of the antibiotics tested. The largest number of isolates (i.e.14) was placed in cluster 16, which 83.3% isolates were susceptible to nitrofurantoin and in this cluster, half of the isolates were resistant to ciprofloxacin, ceftriaxone, and cefotaxime. The next largest number of isolates (i.e. 11) was placed in cluster 11, most isolates were sensitive to nitrofurantoin (90%) and imipenem (100%) and also, half of the isolates in this cluster were resistant to ciprofloxacin, ceftriaxone, and cefotaxime (table1), Most bands were polymorphic, indicating a high genetic diversity in the isolates. The analysis of 32 clusters of our research generally showed that these clusters did not have a unique feature and that the distribution has of the mentioned characteristics (such as an inpatient and an outpatient, sex, and the antibiotic resistance or susceptibility of the isolate) was the same among the clusters. Each cluster has had its characteristics, which been shown a high genetic diversity among E. coli clusters of this area. The addition to isolating the existing isolates, comparisons were made in our study between 2010 and 2016 samples, indicating that the samples have of the two years had the same distribution in different clusters and that no changes were made in E. coli clusters over six years (Figure 2).

Discussion

The RAPD-PCR molecular method has become popular due to its advantages such as high speed, low cost, high sensitivity, and no need for high technical skills (18, 19). In the present study, the molecular typing of 187 strains UPEC isolated from patients of Gorgan in 2010 and 2016 was done by simultaneous use of two primers by RAPD-PCR technique. In 2018, Farivar and colleagues in Iran performed a study to reviews the frequency of armA and KPC genes by PCR and typed 81 Klebsiella pneumoniae isolate by the RAPD-PCR method. Analysis of their results revealed 30 patterns from D1 to D30 and the isolates were classified into five clusters; however, no association was found between the presence of armA and KPC genes having a respective frequency of 66.7% and 7.4% with those derived from RAPD (20).

In our study, 32 different clusters were obtained using 2 primers on 164 isolates of UPEC, the studied clusters did not have a unique feature and the dispersion of the mentioned characteristics such as (hospitalization and outpatient, sex and antibiotic resistance or susceptibility) of the isolates were the same among the clusters because each cluster had its characteristics, which shown that the E. coli region had great genetic diversity. In a 2016 Iranian study by Afshari using RAPD PCR on 110 isolates of *E. coli* from urinary tract infection human and diarrhea and septicemia of calves in which the results were analyzed at 25% similarity, five clusters were identified, and human (UTI) and non-human (calf diarrhea and septicemia) samples were placed in separate clusters (21).

In another Indian study by Kumar and colleagues using the RAPD-PCR method on 31 E. coli isolates, E. coli strains were classified into six different groups with unique patterns. Therefore, the results of their research showed that RAPD-PCR is a powerful method for molecular differentiation in E. coli (22).

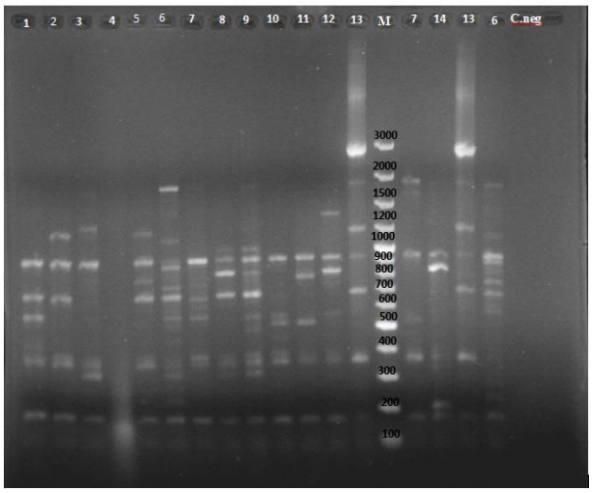

Figure 1. Results of RAPD-PCR for 14 uropathogenic *Escherichia coli* isolates and repetition of test for three isolates (6, 7, 13) together with M marker (3kb DNA ladder) and C. neg (negative control).

Table 1. Antibiotic resistance profile of 32 clusters of uropathogenic *Escherichia coli* isolates*

clusters	N	piperacillin	cefotaxime	ceftriaxone	ciprofloxacin	gentamicin	nitrofurantoin
1	9	0	5 (62%)	4 (50%)	4 (50%)	1 (11/11%)	0
2	4	0	1 (50%)	1 (50%)	0	0	0
3	1	0	1 (100%)	1 (100%)	0	0	0
4	5	0	1 (20%)	1 (20%)	1 (20%)	1 (20%)	0
5	1	0	1 (100%)	1 (100%)	1 (100%)	0	0
6	6	0	3 (60%)	3 (60%)	2 (40%)	0	0
7	4	1 (33.3%)	3 (75%)	3 (75%)	3 (75%)	2 (50%)	1 (25%)
8	4	0	1 (33.3%)	1 (25%)	1 (25%)	1 (25%)	0
9	3	0	2 (66.7%)	2 (66.7%)	3 (100%)	0	0
10	4	0	1 (33.3%)	1 (33.3%)	1 (33.3%)	0	0
11	11	1 (10%)	6 (54.5%)	6 (54.5%)	6 (54.5%)	2 (18.2%)	1 (9.1%)
12	4	0	1 (25%)	1 (25%)	2 (50%)	2 (50%)	1 (25%)
13	7	1 (14.3%)	1 (14.3%)	1 (14.3%)	1 (14.3%)	0	0
16	14	2 (15.4%)	7 (58.3%)	7 (53.8%)	8 (61.5%)	5 (38.5%)	2 (16.7%)
17	3	0	1 (33.3%)	1 (33.3%)	2 (66.7%)	2 (66.7%)	1 (33.3%)
18	2	0	1 (50%)	1 (50%)	1 (50%)	0	1 (50%)
19	3	0	2 (66.7%)	2 (66.7%)	2 (66.7%)	0	0
20	1	0	0	1 (50%)	0	0	0
21	4	0	0	1 (50%)	0	1 (50%)	0
22	7	0	1 (14.3%)	1 (14.3%)	2 (28.6%)	0	0
23	3	0	0	0	1 (33.3%)	1 (33.3%)	0
24	2	0	0	0	1 (50%)	0	0
25	7	0	4 (66.7%)	4 (57.1%)	3 (42.9%)	0	1 (14.3%)
26	3	0	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	0
27	1	0	0	0	0	0	1 (100%)
28	4	1 (50%)	1 (33.3%)	1 (33.3%)	0	0	0
30	1	0	0	1 (100%)	1 (100%)	0	0
31	2	0	1 (100%)	1 (100%)	1 (100%)	0	0
32	2	0	1 (50%)	1 (50%)	1 (50%)	0	0

*There was only one imipenem resistant strain in cluster 16 and all strains in 14, 15, and 29 clusters were not resistant to any of the tested antibiotics.

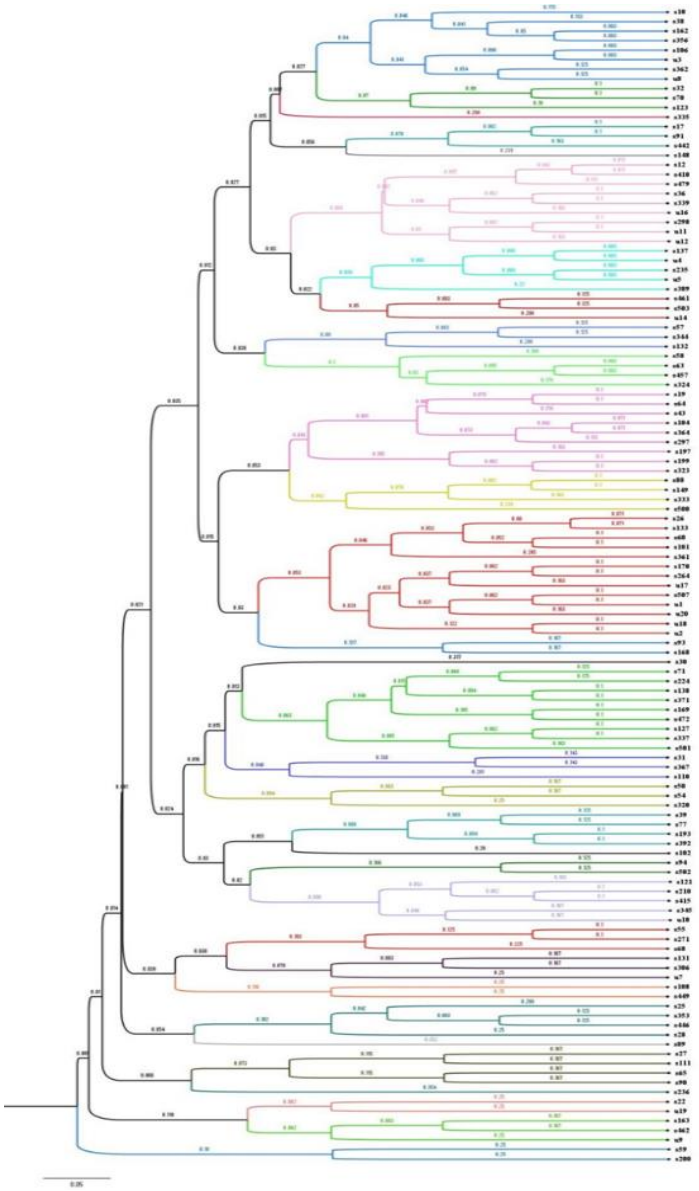

Figure 2. Dendrogram of uropathogenic *Escherichia coli* isolates' cluster analysis in RAPD-PCR by GelClust software using UPGMA.

In our study, 32 clusters of 164 isolates of UPEC were obtained. Most bands were polymorphic, indicating high genetic diversity in the strains and this case was in agreement with the studies. In 2013, Suardana et al. worked on 20 human and pig E. coli O:157 H7 samples in Indonesia using RAP-PCR with 10 different primers. Depending on the type of primer, the number of bands varied from 7 to 24 and it was stated that the pattern of isolates from the stool of human patients was similar to that of pigs (23).

In 2016, Marialouis and colleagues in India performed a study on 58 E. coli strains isolated from patients with UTI for molecular typing of drug-resistant strains by RAPD-PCR, the results showed that the prevalence of ESBL and AmpC β-lactamase producing isolates was 47% (N=27) and 12% (N=7), respectively. Also, most of ESBL– producing isolates, were sensitive to nitrofurantoin and cefoxitin (24). In 2019, Mohammadzadeh et al. had conducted a study on the genetic diversity based on pathogenicity islands marker (PAIs) and antibiotic

susceptibility on 115 strains isolated from UTI by RAPD-PCR. The results showed that according to the PAI marker, 69 strains were associated with UPEC 536 genotype and 21 isolates with UPEC J96 and 25 isolates with UPEC CFT073 strains, the highest susceptibility of the isolates to imipenem (93.9%) and nitrofurantoin (91.3%) was reported (25). In our study, approximately all clusters included resistant strains to ciprofloxacin, ceftriaxone, and cefotaxime and most clusters also contained susceptible strains to nitrofurantoin and imipenem. According to our observations, RAPD-PCR is a sensitive and cost-effective method and can be used to detect differences between isolates, which is consistent with the reported investigations. Concerning 2016 samples, our research showed that the UPEC isolates were phylogenetically similar to those of 2010 indeed, 2010 and 2016 isolates were classified in a single family according to the dendrogram.

Conclusion

Using RAPD-PCR and a sequence of two separate primers, we were able to classify the 164 isolates studied with a similarity coefficient of 70 out of 187 isolates of UPEC in 32 clusters. However, our results showed that 23 isolates in this study were uncharacteristic, indicating that the primers did not have sufficient strength and differentiation to type all strains. Nevertheless, the RAPD-PCR molecular technique can have been a suitable and at the same time simple and applicable method in most common laboratories and can been used for epidemiological studies and also in studying the genetic diversity of isolates UPEC.

Conflict of Interest

No conflict of interest is declared by the authors.

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Ethics

Written informed consent was obtained from the participants. The original study was approved by the Human Research Ethics Boards at Golestan University of Medical Sciences. Code of Ethics: 7815893040821.

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