

Molecular typing of uropathogenic *E. coli* strains by the ERIC-PCR methodMaryam Afkhami Ardakani¹, Reza Ranjbar²

¹M.Sc. of Microbiology, Department of Microbiology, Faculty of Advanced Science & Technology, pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran

²Ph.D. of Medical Bacteriology, Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Type of article: Original**Abstract**

Introduction: *Escherichia coli* (*E. coli*) is the most common cause of urinary infections in hospitals. The aim of this study was to evaluate the ERIC-PCR method for molecular typing of uropathogenic *E. coli* strains isolated from hospitalized patients.

Methods: In a cross sectional study, 98 *E. coli* samples were collected from urine samples taken from patients admitted to Baqiyatallah Hospital from June 2014 to January 2015. The disk agar diffusion method was used to determine antibiotic sensitivity. DNA proliferation based on repetitive intergenic consensus was used to classify the *E. coli* strains. The products of proliferation were electrophoresed on 1.5% agarose gel, and their dendrograms were drawn. The data were analyzed by online Insillico software.

Results: The method used in this research proliferated numerous bands (4-17 bands), ranging from 100 to 3000 base pairs. The detected strains were classified into six clusters (E1-E6) with 70% similarity between them.

Conclusion: In this study, uropathogenic *E. coli* strains belonged to different genotypic clusters. It was found that ERIC-PCR had good differentiation power for molecular typing of uropathogenic *E. coli* strains isolated from the patients in the study.

Keywords: uropathogenic *E. coli*, urinary tract infection, ERIC-PCR, molecular typing

1. Introduction

E. coli, an abbreviation for *Escherichia coli*, is a gram negative bacillus of the Enterobacteriaceae family, and it is commonly found in the intestines of warm-blooded animals. Most strains of *E. coli* are harmless, but some strains cause food poisoning, diarrhea, and urinary tract infections (1). Many bacteria can cause infection in the urinary tract. In fact, 80 to 90% of urinary tract infections in the world are caused by *E. coli*. Extraintestinal pathogenic strains of *E. coli* that cause urinary tract infections in humans are known as uropathogenic *E. coli* (2-7). Knowledge of the epidemiology of microbial populations is important in the field of medical microbiology. It is possible to identify infection reservoirs, examine the prevalence of hospitalization infections, and identify the type of microbial pathogenic agents via molecular typing (8). Enterobacterial Repetitive Intergenic Consensus (ERIC) has conserved sequences and repeated sequences that can be seen in bacteria or in some fungi. These sequences are generally found in the whole chromosome in the inter-genome (9-14). In the ERIC-PCR technique, which is based on amplification of random distribution of inter-genome parts, various organisms can be distinguished from one another. This means that bacteria differ in the frequency of repeated sequences, and, therefore, this method produces different patterns of specific primers in electrophoresis. In this technique, primers are designed to be able to attach to repeated sequences and to amplify the distances among those attached primers to repeated sequences (9-14). The population type of patients referred to Baqiyatallah Hospital is different from other hospitals in Tehran, so we aimed to do this study to examine the ERIC-PCR molecular technique in order to classify strains of *E. coli* isolated from clinical cases in Baqiyatallah Hospital.

Corresponding author:

Dr. Reza Ranjbar, Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Tel: +98.2188039883, Fax: +98.2188039883, Email: ranjbarre@gmail.com

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2. Material and Methods

2.1. Study population and bacterial isolation

In a cross-sectional study over a period of eight months, from June 2014 to January 2015, all 98 *E. coli* strains isolated from patients who suffered from urinary tract infection in Baqiyatallah Hospital were subjected. These patients were of different age groups and were examined according to standard methods of bacteriology and biochemistry.

2.2. Antibiogram Test

A disc agar-diffusion method, as recommended by Clinical and Laboratory Standards Institute (CLSI) (2013), was used to determine antibiotic sensitivity (15). The antibiogram test was performed using the antibiotics Ampicillin (AM) - Amikacin (AN) - Ciprofloxacin (CP) - Ceftazidime (CAZ) - Ceftriaxone (CRO) - Cotrimoxazole (SXT) - Norfloxacin (NOR) - Nalidixic acid (NA) - Gentamicin (GM) - Nitrofurantoin (FM), and the ATCC 25922 strain of *E. coli* was used as the quality control.

2.3. DNA preparation

After bacterial isolation, *E. coli* isolates were cultured in Luria Bertani (LB) broth. After 16-18 hours of incubation at 37 °C, DNA extraction was performed using the alkaline lysis method. The DNA concentration was determined by nanodrop.

2.4. Primers

The oligonucleotide primers used in this study were: 5'-ATGTAAGCTCCTGGGGATTACAC-3' ERIC 1 and 5'-AAGTAAGTGACTGGGGTGAGCG-3' ERIC 2 (13).

2.5. ERIC-PCR

ERIC-PCR reactions were performed in 20 µl volumes containing 1 µl of each primer, 10 µl of the master mix, 1.5 µl of MgCl₂, 2 µl of template DNA, and 3.5 µl of deionized water. The ERIC-PCR reaction was as follows: initial denaturation at 94 °C for 1 minute, with the next 30 cycles consisting of a denaturation step at 94 °C for 30 seconds, annealing at 52 °C for 35 seconds, extension at 72 °C for 4 minutes, and a final extension for 5 minutes at 72 °C. PCR products were electrophoresed using a 1.5% agarose (Merck) gel electrophoresis at 80 V for 180 minutes then visualized under UV in a gel documentation system. The zero-one manual method was used to count the bands, and, then, the data were entered on the following site: http://insilico.ehu.es/dice_upgma/, and the dendrograms were drawn.

3. Results

Of all 98 samples examined, 18 were from men and 80 were from women. The age and gender distribution of the patients with urinary tract infection were as follows: six girls under one year of age, five girls between the ages of 1-10, three girls between the ages of 10-20, 14 women between the ages of 20-30, 12 women between the ages of 30-40, 12 women and two men between the ages 40-50, 10 women and four men between the ages 50-60, six women and four men between the ages 60-70, 10 women and five men between the ages 70-80, and two women and three men between the ages of 80-90. The results of the study on antibiotic resistance indicated the maximum resistance was shown to Ampicillin and the minimum to Nitrofurantoin. ERIC-PCR patterns were used to calculate the visibility and placement of the gels according to their molecular weights and molecular markers. Bands for each sample were counted (Figure 1). After dendrogram analysis, it was shown that ERIC-PCR differentiated the isolates into six clusters, E1 - E6, with 70% similarity (Figure 2). In the strains that were studied, the maximum number, 62, belonged to the E2 cluster, and the minimum, 1, belonged to the E6 cluster. Twenty-one of the studied strains were in E1, 3 strains in E3, 2 strains in E4, 5 strains in E5, and 1 strain in the E6 cluster. Three of the 98 strains did not have bands and, therefore, could not be genotyped. The most frequent band was 1350 bp, which was found in 70 strains, and the least frequent was 170 bp, which was observed in two strains. Study and comparison of the results obtained from the antibiogram test with dendrogram yielded noteworthy results, i.e., samples in the E1 cluster were sensitive to Gentamicin and Nitrofurantoin, those in the E5 cluster were female and resistant to Ampicillin and sensitive to Nitrofurantoin, and those in E3 cluster had completely similar antibiotic patterns and were in the 70-90 age range.

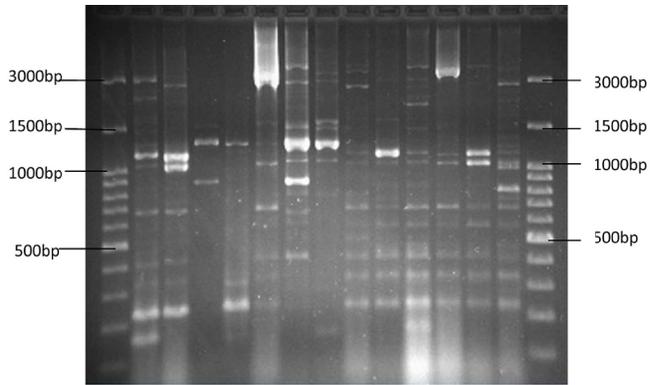


Figure 1. Results of ERIC PCR on some of the *E. coli* strains, with the first column in both sides showing Ladder 100 bp

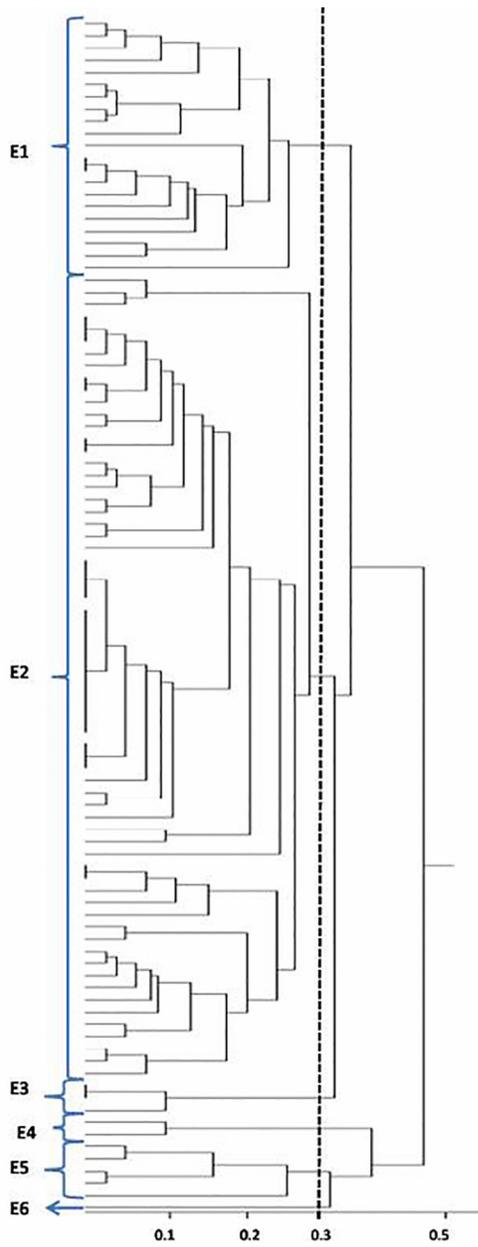


Figure 2. Dendrogram drawn for the strains studied in this research

4. Discussion

This study was conducted on 98 isolates recovered from urine samples of patients with urinary infections admitted to Baqiyatallah Hospital. All of the isolates were studied genetically using the ERIC PCR. The most frequent band was 1350 bp, which was found in 70 strains, and the least frequent band was 170 bp, which was observed in two strains. The maximum number, 62, belonged to the E2 cluster, and the minimum, 1, belonged to the E6 cluster. The comparison of the results obtained from the antibiogram test with dendrogram showed that samples in the E1 cluster were sensitive to Gentamicin and Nitrofurantoin, those in the E5 cluster were female and resistant to Ampicillin and sensitive to Nitrofurantoin, and those in the E3 cluster had completely similar antibiotic patterns and were in the 70-90 age range. Versalvic et al. concluded that fingerprinting techniques were very powerful tools for identification and classification of bacteria and in molecular epidemiology of human and plant pathogens (13).

In 2002, Wanderley Dias da Silveira et al. isolated 49 *E. coli* strains in chickens with septicemia, swollen head syndrome, omphalitis, and in some without any clinical symptoms (16). The ERIC PCR technique determined their types. These isolates were classified into four main groups, A to D, and the survey results also showed that the ERIC PCR method can be a good alternative to molecular techniques, such as RAPD PCR and RFLP (16). Meacham et al., at the University of Michigan evaluated genotyping of a large number of *E. coli* samples and concluded that more than one ERIC-PCR method should be used in determining genetic similarities through genotyping (11). In 2006, Lindsay et al. studied ERIC sequence distribution in the whole genomes of intestinal bacteria, including *E. coli* and *Shigella*. They concluded that ERIC sequence in regions close to genes with high expression levels was more inclined to be copied, and that the ERIC-PCR was widely used for genotyping because the ERIC sequence was small (12). VivekPrabhu et al. used the ERIC-PCR method for genotyping 40 samples of *E. coli* isolated from cows infected with mastitis, and 37 of their samples could be genotyped. Seven isolates were in the phylogenetic group A, 17 isolates were in the phylogenetic group B, and 13 isolates were not in any groups because no similarity with other groups. They reached the conclusion that ERIC-PCR was a quick, reliable, and suitable method for differentiating *E. coli* strains (17). In 2011, Ramazanzadeh et al. used ERIC-PCR to evaluate the genetic diversity of *E. coli* isolated from hospital samples. They studied 230 clinical samples and found that 205 of them could be genotyped in 20 clusters, with most of them being in the phylogenetic group D. The high genetic diversity in the isolated *E. coli* indicated the low rate of infection at the hospital (18). Xiu-Yan Lang et al. used ERIC-PCR for genotyping enterotoxigenic *E. coli* isolated from cows and found most isolates belonged to the phylogenetic group D. They concluded that the ERIC-PCR is a fast, efficient, and repeatable method for genotyping (19). The findings indicated that the ERIC-PCR method is a fast and excellent way to describe the diversity of phylogenetic classification and evolution racial structure. Using this method, most of the isolates were classified and separated into different categories. Another result is that *E. coli* were obtained from a variety of distinct clones that have been studied from a variety of bacteria in clinical samples.

The choice of a sub typing technique is depend on the skill level and resources of the laboratory and the purpose of the study (20). In our previous studies, we evaluated ERIC-PCR for subtyping of sporadic and epidemic strains of different foodborne bacterial pathogens in Iran (20-26) and found this technique had a good subtyping power. As one of the most important and prevalent uropathogen organism in Iran (27-30), *E. coli* was subjected here to ERIC-PCR. As indicated in previous studies, our finding showed here an acceptable discriminatory power of this methods for subtyping of uropathogenic *E. coli*.

5. Conclusions

Our results showed that the ERIC PCR technique was a rapid method for describing then genetic diversity of *E. coli* strains. The strains of *E. coli* were composed of various distinct colonies that show the variety of these bacteria in clinical samples. For future studies, we suggest the use of more samples from different hospitals and the comparison of the discrimination of the ERIC PCR using other methods, such as PFGE. We hope the results of our study will be useful for molecular epidemiology of uropathogenic *E. coli* and upgrading the public health in our country.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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