Original Article

Molecular Genotyping by ERIC-PCR of Uropathogenic *E. coli* Isolated from Patients with Urinary Tract Infections

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Abstract

Background: *Escherichia coli* is the most common organism found in urinary tract infections (UTIs). A cutting-edge molecular genomic-based method, the enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR), was employed to identify and detect different *E. coli* strains. **Objectives:** In this investigation, we aim to evaluate the association of *E. coli* as a main causative agent of UTIs and determine the distribution of ERIC sequences in urine samples. **Materials and Methods:** *E. coli* were isolated and cultured using standard microbiological procedures. The DNA from these *E. coli* bacteria was extracted and subjected to *16S rRNA* gene confirmation via PCR and ERIC-PCR for molecular genotyping. The resulting ERIC-PCR products were separated via 2% gel electrophoresis, and the resulting gel electrophoresis banding patterns were employed to generate dendrograms with GelJ software. **Results:** The results showed that out of 100 patients' urine samples, 31% were positive for *E. coli*. The fingerprint patterns revealed bands ranging from 100 to 5000 bp in size. The most frequent band was 1350 bp, which was found in 17 isolates, while the least frequent was 150 bp, which was observed in two isolates. Six clusters (E1–E6) were identified with 70% similarity among them. **Conclusions:** UPEC strains were genetically classified using ERIC-PCR technology, showing promising genotype differentiation capabilities. Unique colonies within *E. coli* clones highlighted diversity in the tested clinical samples, which is expected to enhance public health and contribute to the molecular epidemiology of UPEC *E. coli*.

Keywords: *E. coli*, ERIC-PCR, genotyping, molecular analysis, uropathogenic

Introduction

In the discipline of medical microbiology, understanding the epidemiology of contagious inhabitants is crucial. Molecular typing can be used to discover contagion reservoirs, investigate the occurrence of hospitalized toxicities, and determine the nature of bacteriological pathogenic representatives.[1] *Escherichia coli* encompasses a broad spectrum of strains found in various environments, ranging from the digestive systems of animals and humans to natural habitats.[2] It is primarily a part of the normal gastrointestinal microflora in humans and plays an integral role in our lives.[3] Interestingly, *E. coli* exhibits extensive genomic diversity.[4] The *E. coli* strains recovered from human origins are divided into four clusters based on their evolutionary relationships: A, B1, B2, and D. yjaA and TspE4. C2 and chuA are three frequently used genes for determining the phylogeny and pathogenicity of *E. coli*.[5] These strains are categorized into three groups based on their pathogenicity and infection site: commensal, intestinal, and extraintestinal pathogenic strains, with the latter being more prevalent among urinary tract infection (UTI) patients.[6] These opportunistic pathogens have a broad range of infection behaviors and are considered contaminants of a variety of dietary sources.[7,8] Pathogenic *E. coli* strains manufacture a wide spectrum of virulence factors, including adhesins and aerobactin, which are composed of diverse adhesion factors that allow the bacteria to

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cause a variety of illnesses due to their capacity to adhere to various tissues and organs.[9] The virulence factors that affect *E. coli* pathogenicity include capsular structure, endotoxins, invasive mechanisms, adhesion, hemolysis, cytotoxic necrotic characteristics, and effacement.[10] As a result, the bacteria are further categorized into distinct pathogenic groups based on their potential to establish virulence factors as well as the severity of infection and illness patterns: EPEC, EHEC, ETEC, EIEC, and EAEC.[11]

Uropathogenic *E. coli* (UPEC) is the predominant causative agent of UTIs in both adults and children.[12,13] Traditional microbiological and biochemical tests often fail to identify significant genetic variations among these strains. In contrast, modern molecular diagnostic tools and molecular fingerprinting techniques have proven to be valuable in epidemiological investigations.[14,15] PCR-based methods offer accuracy, speed, reproducibility, sensitivity, specificity, and reliability for distinguishing different DNA fingerprints.[16] Among these tools, the Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR is a straightforward and relatively inexpensive genotyping technology for separating among different strain types.[17] ERIC contains well-preserved repetitive sequences that are usually located in the intergenome region of bacteria. These repetitive sequences are mobile DNA elements that are often associated with miniature inverted transposable elements and are prevalent in numerous bacterial genomes, including those of the Enterobacteriaceae family, such as those of *E. coli*.[18] ERIC-PCR amplifies randomly distributed intergenic sequences.[19] The number of ERIC sequences varies among bacterial strains, indicating evolutionary processes within specific *E. coli* strains.[18] To assess the various species of *E. coli* strains, primers homologous to the corresponding ERIC sequences were used. The resulting ERIC sequences are employed for evaluating the phylogenetic relationships using GelJ software.[20]

The main objectives of this study were to 1) evaluate the association of *E. coli* as a causative agent in urine samples of patients with signs and UTI symptoms and 2) use ERIC-PCR as a suitable, rapid, and cost-effective molecular genomic tool to determine the distribution of ERIC sequences within the isolated *E. coli* strains.

Materials and Methods

Sample collection

A total of 100 urine samples were obtained between November 2022 and March 2023 from females and males aged 5–50 years. Sterile screw-cap capped containers were used for collecting midstream urine samples to confirm the diagnosis of recurrent UTIs. The UTI patients were diagnosed according to clinical criteria (signs and symptoms) and general urine laboratory examination, where the samples were analyzed microscopically for pus cells, red blood cells, epithelial cells, crystals, bacteria, and the presence of a positive urine culture of at least 10^5 CFU/mL bacteria.[21]

Biochemical and phenotypic characterization

The collected urine samples were inoculated into Blood Agar, MacConkey Agar, and Mannitol salt agar with a sterile standard loop (0.001 mL) at 37 °C for 24 h. The cell morphology of the pure bacterial colonies was investigated using Gram stain reagents. All the bacterial isolates were diagnosed by manual and automated biochemical tests. The VITEK-2 system was used for automated identification of ID-Gram-positive cocci (ID-GP cards) and ID-Gram-negative cocci (ID-GN cards) in accordance with the manufacturer's instructions. The identification of the TSVR was further confirmed via the use of the *16S rRNA* gene to detect bacterial colonies via PCR.[22]

DNA extraction

All isolates were cultured in Luria Bertani (LB) broth. Using the Presto™ Mini gDNA Bacteria Kit (Geneaid Biotech, Taiwan), the genomic DNA of the investigated UPEC isolates was extracted after a 24-h period at 35 °C. All PCR-based tests use extracted DNA as a DNA template. This assay was performed according to the manufacturer's guidelines.[23]

PCR amplification of the *16S rRNA* gene

Molecular identification of UPEC isolates was carried out according to the methods of Sabat *et al.*,[24] in which *16S rRNA*-specific primers (forward 5′ GGAAGAAGCTTGCTTCTTTGCTGAC-3′, reverse 5′ AGCCCGGGGATTTCACATCTGACTTA-3′) were used to produce a 544 bp product. PCR amplification of DNA was performed using a gradient thermal cycler (Eppendorf, Germany) with a final mixture volume of 25 µL. PCR yields were investigated through 1.5% agarose gel electrophoresis with a UV transilluminator and subsequent discoloration with ethidium bromide. The mixtures and conditions of the PCR amplification are illustrated in Table 1.

ERIC-PCR

These reactions were performed according to the methods of Movahedi *et al.*[25] with minor modifications. The process was carried out in a volume of 25 µL: 1 µL of each primer (ERIC 1: 5′-ATGTAAGCTCCTGGGGATTCAC-3′, and ERIC 2: 5′-AAGTAAGTGACTGGGGTGAGCG-3′); 12.5 µL of master mix (GoTaq® G2 Green Master Mix, Promega, USA); 3 µL of DNA template; and 7.5 µL of PCR grade water. Concurrently, positive (*E. coli* DNA) and negative (PCR grade water) controls were added. The ERIC-PCR procedure in the thermocycler involved preliminary

Table 1: Uniplex PCR mixtures and conditions for identification of the *16S rRNA* gene. Modified from *Sabat et al.*[24]

PCR mixtures		Type of cycle	PCR conditions	
Contents	Volume (μL)		Condition	Number of cycles
Master mix	12.5	Initialisation	95 °C for 5 min	1
Forward primer	2.5	Denaturation	94 °C for 1 min	30
Reverse primer	2.5	Annealing	60 °C for 1 min	
Template DNA	3	Extension	72 °C for 1 min	
Nuclease free water	4.5	Final Extension	72 °C for 10 min	1

denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 45 s, extension at 72 °C for 1 min, and a final extension for 6 min at 72 °C. The yields obtained from ERIC-PCR were then electrophoresed through a 2% gel at 75 V for 75 min. A 100 bp DNA marker (Geneaid Biotech, Taiwan) was used as a standard measuring means. The bands were visualized using a UV gel documentation system (Cleaver Scientific Ltd., UK).

Analysis of the dendrogram and clustering

The primary structure for calculating the dendrogram was the band pattern observed in the gel electrophoresis of the ERIC-PCR results. The GelJ software was used for creating dendrograms based on hierarchical clustering via gel electrophoresis images. For constructing a computerized dendrogram, the existence of bands was assumed to be 1 to zero, and the nonexistence of bands was assumed to be zero. Based on the clustering analysis, a dendrogram was created via the unweighted pair group method with arithmetic mean (UPGMA), which is classified according to clustering techniques.[15]

Statistical analysis

The statistical comparison of the two datasets was performed using the Student's *t* test.

Ethical approval

Verbal consent was obtained from all patients prior to their participation in this study. The Committee of Publication Ethics at the College of Medicine, University of Babylon, Iraq, granted approval for this study under reference number BMS/0203/016, dated June 18, 2019.

Results

Distribution of UPEC strains isolated

The biochemical and phenotypic characterization tests revealed various microorganisms in the urine samples, with *E. coli* being the most prevalent (31%) [Table 2]. The VITEK-2 reports indicated a 99% similarity among all isolates. Additionally, molecular identification using *16S rRNA* confirmed that among the 100 urine samples, 31 were identified as uropathogenic *E. coli*. The PCR products were obtained from uropathogenic *E. coli* isolates with specific primers that generated a 544 bp amplicon of the *16S rRNA* gene, as shown in Figure 1.

Table 2: The main microorganisms identified in the urine samples of 100 patients

Type of microorganisms	Number	Percentage %
Enterococcus faecalis	8	20
Enterococcus faecium	4	
Streptococcus agalctiae	3	
Staphylococcus epidermidis	2	
Staphylococcus aureus	3	
Escherichia coli	31	77
Klebsiella pneumonia	14	
Enterobacter cloacae	12	
Proteus merabilis	10	
Citrobacter	5	
Acinetobacter	5	
Candida albicans	3	3

ERIC-PCR arrangements

Owing to their molecular weightiness and molecular indicators, ERIC-PCR was used to analyze the discernibility and positioning of the DNA bands. The ERIC-I and ERIC-2 primers were used. Agarose gel electrophoresis revealed a DNA banding pattern comprising amplifiable bands extending from 1 to 13 with sizes varying from 100 to 5000 bp [Figure 2]. The most frequent band size was 1350 bp, which was found in 17 isolates, while the least frequent band was 150 bp, which was observed in two samples only. After dendrogram analysis, in the strains that were studied, 29 samples had ERIC sequences. However, of the tested samples, two (UPEC-12 and UPEC-26) did not display DNA fragments and, therefore, could not be genotyped. The genotyped isolates were classified into six different genotypic clusters (E1–E6), as shown in Figure 3 and Table 3, with 70% frequency similarity among them [Figure 4].

Discussion

Urinary tract infections are most frequently caused by *E. coli*, and many serotypes of this bacterium known as UPECs are implicated in the development of these

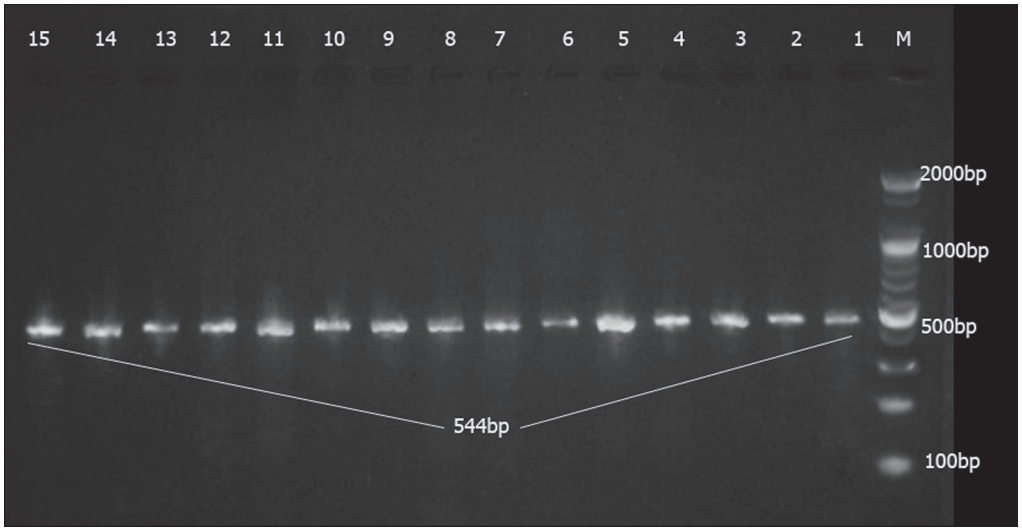

Figure 1: Agarose gel electrophoresis of PCR products obtained from uropathogenic *E. coli* strains. Specific primers used to generate a 544 bp amplicon of the *16S rRNA* gene. Lanes 1–15 represent the UPEC1-15 strains, and Lane M represents the 100 bp DNA ladder

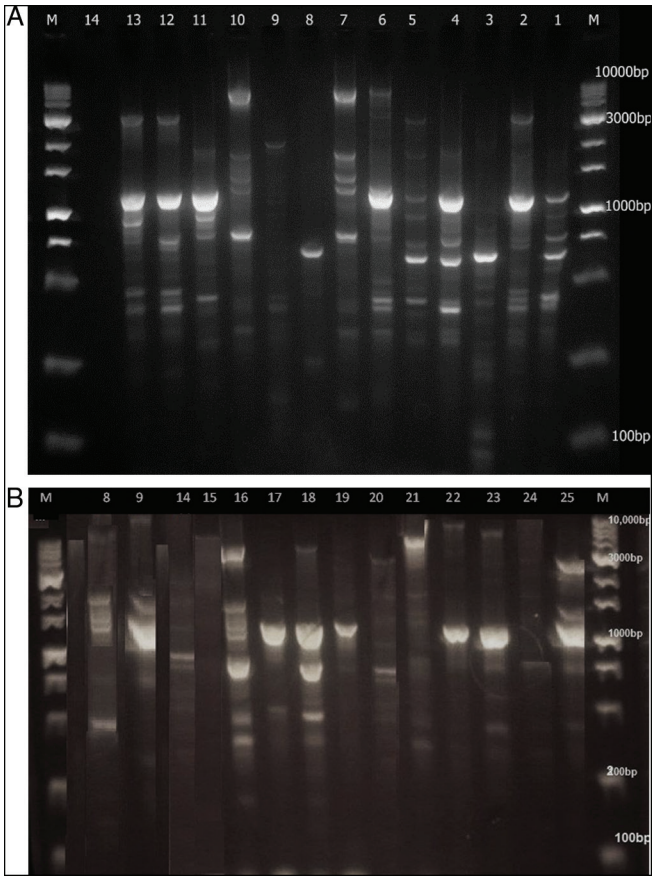

Figure 2: Genomic fingerprint-derived profile generated by the Enterobacterial repetitive intergenic consensus (ERIC) PCR tool for the tested uropathogenic *E. coli* isolates. (a) Lane M represents the 100 bp universal DNA ladder; lanes 1–14 represent UPEC 1-13 isolates. (b) Lane M represents the 100 bp universal DNA ladder; 15–25 represent UPEC 15–25 isolates. Lanes 8, 9, and 14 are repeats of samples 8, 9, and 14

infections.[26] Various pathogens have been identified [Table 2], with *E. coli* being the predominant pathogen in diagnosed UTI cases (31%). This finding is consistent with the findings of Ibrahim *et al*. and Al-Janabi *et al*.[27,28] who revealed that the most common microorganism isolated from urine samples of patients with UTIs was *E. coli*.

In the past few decades, bacterial strains have been categorized using phenotypic techniques like serotyping. Many techniques, including ribotyping, ERIC-PCR, random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR), and restriction fragment length polymorphism (RFLP), have been employed recently to identify the origin and source of microbes. These techniques have proven effective in differentiating between different bacterial strains.[15,29]

A practical approach for classifying *E. coli* strains is to use PCR-based techniques, particularly the ERIC-PCR. In order to identify *E. coli*, Casarez *et al*. used the ERIC-PCR approach in 2007 on 650 samples of water polluted with human and animal feces. From 555 samples of contaminated water, they were able to identify 175 distinct genotypes.[30]

In correspondence to our results, Ardakani and Ranjbar examined 98 *E. coli* samples from patients who had UTIs and used the ERIC-PCR method to separate them into 6 clusters (E1–E6). They demonstrated that the most common band found was 1350 bp, which was detected in 70 samples, while the least common band was 170 bp, which was detected only in 2 samples.[31] ERIC-PCR was also utilized by Movahedi *et al.* to determine the presence of *E. coli* in urine samples of patients with UTIs. After analyzing the degree of affinity among the *E. coli* isolates,

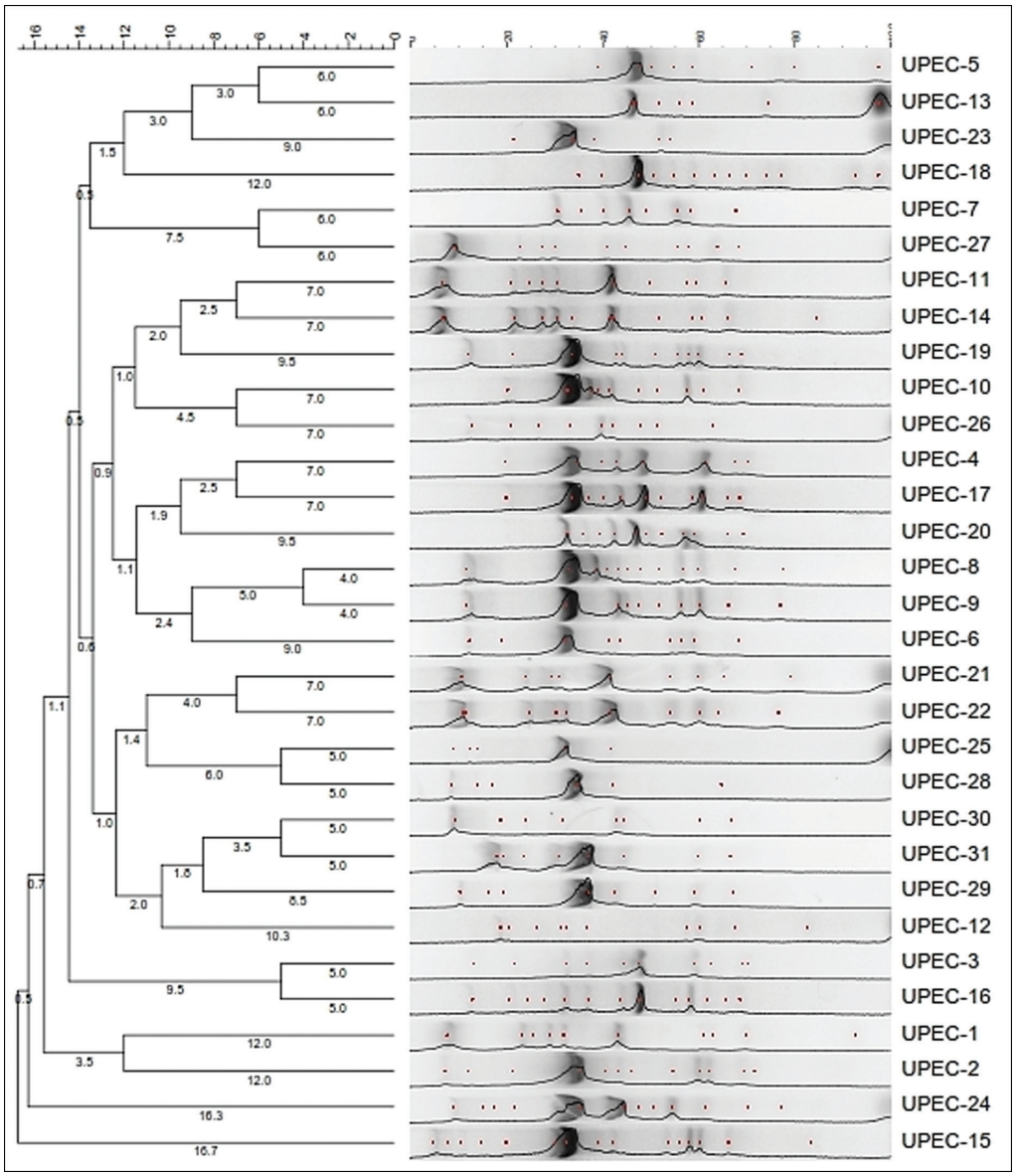

Figure 3: The ERIC-PCR-derived cladogram representing the relationships among 31 uropathogenic *E. coli* strains. The bar represents the distance values. This cladogram was generated by UPGMA

Table 3: ERIC genotype cluster of the studied uropathogenic *E. coli* samples

ERIC genotype cluster	No. of isolates	Name of isolates
ERIC1 (E1)	2 isolates	UPEC 1,2
ERIC2 (E2)	2 isolates	UPEC 3,16
ERIC3 (E3)	8 isolates	UPEC 21,22,25,28,30,31,29,12
ERIC4 (E4)	6 isolates	UPEC 4,17,20,8,9,6
ERIC5 (E5)	5 isolates	UPEC 11,14,19,10,26
ERIC6 (E6)	6 isolates	UPEC 5,13,23,18,7,27

27 ERIC patterns were found, of which 13 were unique (unique type) and 14 were similar across isolates (common type).[25]

Out of the 92 separated specimens, 115 distinct *E. coli* strains were detected, with the stool samples from chickens and sheep exhibiting the highest frequency. They concluded that ERIC-PCR approach is an effective and suitable technique for genotyping *E. coli* strains recovered from various animal sources.[15] Regarding the distribution of polymorphic bands, the gel appearance banding configurations of the pathogenic *E. coli* strains were different. The most frequent band size was 1350 bp, which was found in 17 isolates, while the least frequent band was 150 bp, which was observed in two samples only. According to Ranjbar *et al.* the distributions of the polymorphic bands of the studied *E. coli* strains were different; the most common band size was found to be 1500 bp, which was identified in 23 isolates, while the slightest common band size was 100 bp, which was shown in only one isolate.[15]

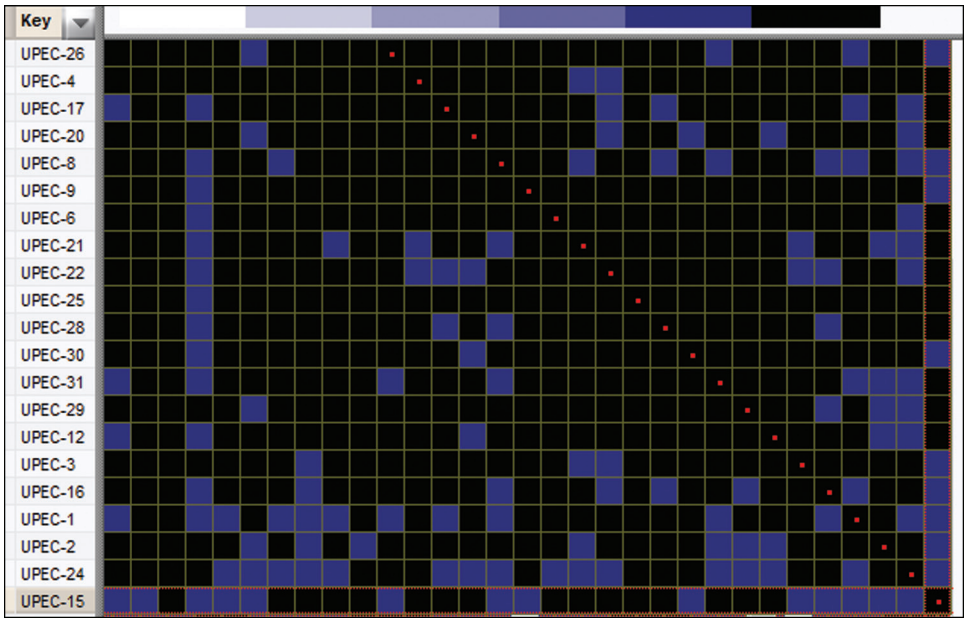

Figure 4: A heatmap representing the genetic similarity matrix among the studied uropathogenic *E. coli* strains obtained by ERIC-PCR. Any sample with less than 30% similarity was disregarded

Conclusion

In conclusion, one of the most significant and widespread microorganisms, UPEC strains, were genetically classified in the current study using the ERIC-PCR technology. The analysis of isolates in various subgroups shows that this technique has a respectable genotype differentiation capacity. The current study's findings demonstrated that the ERIC-PCR approach is an easy, quick, and affordable way to characterize the genetic diversity of various *E. coli* strains, including strains that are part of the UPEC. The different unique colonies that make up the *E. coli* clones illustrate the diversity of these colonies in clinical samples. Here, we advise the use of additional samples from various hospitals in future research and the comparison of the ERIC-PCR results with those of other techniques, such as PFGE. Several software programs, such as GelJ, can be used to interpret the ERIC-PCR findings and produce helpful dendrograms, which are invaluable for classifying a variety of bacterial species, including *E. coli*. We anticipate that our findings in this study will improve public health in our nation and aid in the molecular epidemiology of uropathogenic *E. coli*.

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Conflicts of interest

There are no conflicts of interest.

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