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Phylogenetic group determination and genetic diversity of *Escherichia coli* isolated from domestic animals' stool specimens and human clinical samples

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Abstract

Background: *Escherichia coli* consists of a wide range of strains with huge diversity in their genome, distributed in nature and the alimentary tracts of animals and humans. This study analyzed the phylogenetic group determination and genetic diversity of *E. coli* strains isolated from domestic animals and human clinical samples.

Methods: Twenty *E. coli* isolates from domestic animals were analyzed for phylogenetic grouping. Also, 100 clinical samples and 20 animal samples were evaluated by the enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCR) technique. The results and the similarity between the strains were determined based on the Dice similarity coefficient in the SAHN program of the NTSYS-pc software.

Results: The frequency of phylogroups among animal samples were A = 5%, B1 = 65%, B2 = 20%, and D = 10%. Based on the ERIC-PCR results, the clinical strains were allocated into 19 clusters. Most strains were in the E7 cluster. Fifty percent of the E. coli isolated from animal specimens belonged to the E4 group, and the lowest number of strains was in the E3 and E5 (1 strain) groups.

Conclusion: The results confirmed the efficiency and usefulness of the ERIC-PCR tool for the identification and classification of bacteria. Also, we demonstrated the most phylogroup among animal samples.

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Introduction

Escherichia coli, a gram-negative bacillus of the family Enterobacteriaceae, consists of a wide range of strains with huge diversity in their genomes, distributing in nature and the alimentary tracts of animals and humans (1-3).

Some strains of *E. coli* cause intestinal or extra-intestinal diseases in animals and humans, such as urinary tract infections (UTIs), mild diarrhea, vomiting, hemorrhagic colitis, abdominal pain, neonatal meningitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome (3-6). Pathogenic strains are derived from commensal strains following the horizontal transfer of chromosomal and extra-chromosome genes and gene deletions (7).

The structure of the *E. coli* genome, despite the high number of genes, has the appropriate potential for genetic diversity and, accordingly, pathogenicity (4). Pathogenic strains are differentiated from normal microbiota based on pathogenic factors and mechanisms (5). Today, the application of molecular genetic techniques has led to the discovery of a high percentage of microbial biodiversity and, subsequently, the assessment of the evolution of bacteria species in a proprietary habitat (8-10). Despite the occurrence of genetic recombination, these populations have a clonal structure. Many of these clones are shared by different hosts and are represented by molecular techniques and serotyping (4).

Despite the redundancy of genetic phylogroups in *E. coli*, the species of this bacterium in human and animal populations mainly fall into 4 main phylogenetic groups (A, B1, B2, and D) (3,11-13). Examination of the *E. coli* genome has proven that the distribution of isolates in different phylogenetic groups is not random. The researchers, firstly by multilocus enzyme electrophoresis and then whole-genome multilocus sequence typing, identified that strains belonging to the various phylogroups are genetic entities (13,14), and strains are not randomly divided into different groups concerning their source of isolation (15).

A comparison of the results of multilocus enzyme electrophoresis and sequence typing has shown that similar groupings are obtained; thus, electrophoresis is still a useful solution in classifying strains. The assignment of isolates to phylogroups with the development and validation of PCR evaluation to determine triplex genes has made these genes reliable markers in clinical observations (13,14). Currently, the phylogrouping by triplex PCR assay to detect the genes *chuA* and *yjaA* and a DNA fragment TspE4.C2, due to its simplicity and accuracy, being inexpensive and rapid method in obtaining valuable results, is extensively used in population genetics studies, host source relevancy and assortment of pathogenic commensal strains (4).

Enterobacterial repetitive intergenic consensus (ERIC) sequences were first characterized in *E. coli* and other members of the family Enterobacteriaceae (16,17). These sequences are a conserved and imperfect palindrome of 127 bp that repeatedly are dispersed in the whole chromosome in the bacteria intergenome (9,18-20). Since the ERIC–polymerase chain reaction (PCR) technique relies on the amplification of stochastic dispensation intergenome parts and various organisms differ in the modulation of repeated sequences, this method produces different patterns of specific primers in electrophoresis, and on this basis, bacteria can be distinguished from one another (21,22). The achievement of the ERIC-PCR technique as a facilitated typing method for an extensive and advanced extending figure of organisms makes this method appropriate for hospital-based or localized epidemiology (23).

The major purpose of the present study was to characterize the distribution of ERIC sequences among strains of *E. coli* isolated from human UTIs and domestic animals. In addition, the study aimed to compare the phylogenetic groups of *E. coli* isolates from animals with those isolated from humans and investigate the relationship between the ERIC pattern and phylogenetic groups.

Methods

Following our previous cross-sectional study on phylogroups of human isolates of *E. coli*, 100 *E. coli* strains were isolated from patients with UTI who had been referred to Sina Hospital in Hamadan, Iran, between January 2019 and January 2020. These patients were of different age groups and were examined according to standard methods of bacteriology and biochemistry. Also, the fresh fecal samples of 20 domestic animals were collected from different areas located in Hamadan Province from January to March 2021.

Phenotypic and genotypic confirmation of clinical *E. coli* isolates obtained from human specimens was done in our previous study (24). For the preliminary isolation of *E. coli* from animal fecal samples, the specimens were cultured on selective agar plates, including MacConkey and Eosin Methylene Blue agar media, and then the plates were incubated at 37 °C for 24 hours. The presence of metallic green colonies endorsed the growth of gram-negative coliform bacteria of *E. coli.* The grown colonies of *E. coli* were checked through Gram staining, IMViC test, catalase test, and urease production (25). The screened and purified strains of *E. coli* were inoculated in Nutrient Broth with the presence of 15% glycerol and then stored at -70 °C for further analysis. Genotypic confirmation of animal *E. coli* isolates was done similarly to clinical isolates. All the *E. coli* isolates were confirmed by PCR amplification of the 200 bp fragment of the 16S rRNA gene (Table 1).

Materials used in this research were bacterial genomic DNA of positive *E. coli* isolates collected from human and domestic animals. Bacterial genomic DNA for PCR amplification was extracted as previously described (26).

The animal *E. coli* strains were used for further phylogenetic characterization and statistical analysis. The phylogenetic group of each strain was determined according to Clermont and colleagues (13) by triplex PCR of *chuA* and *yjaA*

genes and the DNA fragment of TspE4.C2 (Table 1). The amplification products were electrophoresed onto 2% agarose gel containing ethidium bromide. Then, the gel was photographed under UV light, and the strains were assigned to the phylogenetic groups B2 (*chuA*+, *yjaA*+), D (*chuA*+, *yjaA*-), B1 (*chuA*-, TspE4.C2+), or A (*chuA*-, TspE4.C2-).

For performing the DNA amplification process, an ERIC sequence pair of forward and reverse primers (Metabion Company) was applied based on a report by Versalovic and colleagues (9) (Table 1). ERIC-PCRs were performed in 12.5-µL volumes containing 0.4 pmol/µL of each primer, 6.25 µL of the Taq DNA Polymerase Master Mix RED (amplicon; Denmark), 50 ng/µL of bacterial DNA (*E. coli*), and 4.75 µL distilled water. Finally, the thermocycler (BioRad T100) was programmed according to initial denaturing at 95 °C for 5 minutes, followed by 35 cycles of denaturation (95 °C for 1 minute), annealing (55 °C for 30 seconds), and extension (72 °C for 1 minute). The last step was performed at 72 °C for 5 minutes (19,27,28).

Table 1. The primers used in this study

Gene	Primer	Size of PCR product (bp)	Reference
16s rRNA-F	5'-GCGGACGGGTGAGTAATGT-3'	200	(36)
16s rRNA -R	5'-TCATCCTCTCAGACCAGCTA-3'		
ChuA-F	5'-GACGAACCAACGGTCAGGAT-3'	279	0
ChuA-R	5'-TGCCGCCAGTACCAAAGACA-3'		
YjaA-F	5'-TGAAGTGTCAGGAGACGCTG-3'	211	(37)
YjaA-R	5'-ATGGAGAATGCGTTCCTCAAC-3'		
TspE4C2-F	5'-GAGTAATGTCGGGGCATTCA-3'	152	(37)
TspE4C2-R	5'-CGCGCCAACAAAGTATTACG-3'		
ERIC-F	5'- ATGTAAGCTCCTGGGGATTCAC-3'	-	(9)
ERIC-R	5'-TCATCCTCTCAGACCAGCTA-3'		

The amplified PCR products were analyzed using 1.5% agarose gel and stained with the GelRed® nucleic acid stain. In this practice, a 100-bp DNA marker (PCRBIO Ladder IV) was used as a standard measuring means. After a 45-minute gel run, the bands were visualized under UV light and photographed via a gel documentation system.

ERIC-PCR fingerprints of amplified DNA fragments were obtained by evaluating the agarose gel electrophoresis images. The positions of the bands on each lane and each gel were recorded regarding the DNA ladder. The zero-one manual method was used to count the bands; in this regard, the presence of a given band was coded as 1, and the absence of a given band was coded as 0 in a data matrix. The data were analyzed using NTSYS-pc software version 2.10 K (Applied Biostatistics, Inc., NY, USA). The similarity between the strains was determined based on the Dice similarity. The dendrogram was constructed based on the averaged similarity of the matrix with the use of the algorithm of the unweighted pair-group method (UPGMA) in the SAHN program of the NTSYS-pc software. The nearest neighbor-joining clustering method has been used to show relations between similar groups (28).

Results

The detection of 20 animal *E. coli* isolates via the phenotypic test was by the molecular detection assay using PCR amplification of the 200-bp fragment of the 16S rRNA gene.

Following our previous study, 20 *E. coli* strains isolated from the feces of domestic animals were analyzed here. These were allocated into one of the 4 phylogenetic groups (ie, A, B1, B2, and D;). The frequency of phylogroups was as follows: A = 5%, B1 = 65%, B2 = 20%, and D = 10% (Figure 1).

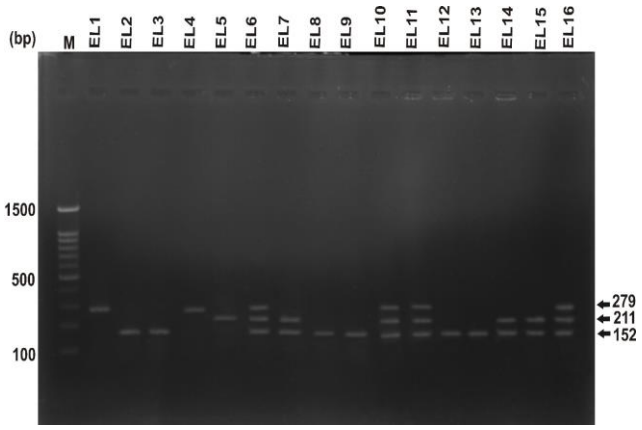

Figure 1. Triplex PCR profiles specific for animal *E. coli* isolate phylogenetic groups. Lane M: 100-bp DNA ladder; lane EL1-EL16: *E. coli* strains isolated from animal; EL1, 4: D phylogroup (*chuA*+/yjaA-); EL2, 3, 7-9, 12-15: B1 phylogroup (ChuA+/ *YjaA*+ / TspE4C2+); EL5: A phylogroup (*chuA*-/ *YjaA*+/TspE4.C2-); EL10, 11, 16: B2 phylogroup (*chuA*+/*yjaA*+/TspE4C2+).

According to our previous research (24), the combination of 3 phylogenetic markers (*chuA*, *yjaA,* and DNA fragment TspE4.C2) classified 100 *E. coli* clinical strains isolated from human urine specimens into B2 (44%), D (31%), A (21%), B1 (9.20%), and B1 (4%) phylogroup types. The clinical *E. coli* isolates have been collected from outpatients and inpatients. The patients were between 2 and 94 years old, with a mean age of 54.3 years. Previous research results indicated that most of the isolates belonged to B2 (n = 44, 44%) and D phylogroups (n = 31, 31%;).

ERIC-PCR band profiles (ERIC genotype) from *E. coli* strains isolated from human and animal fecal specimens were used to numerate bands of the gels for each sample according to their molecular weights (Figures 2 and 3). The number and diversity of bands obtained from gel electrophoresis in different samples were specified. The ERIC-PCR banding patterns in this study obtained 1 to 16 bands encompassing 120 to about 3000 bp (Figures 2 and 3).

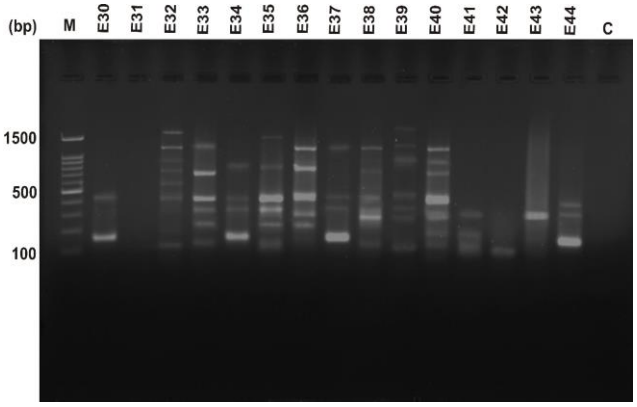

Figure 2. The DNA fingerprinting of several *E. coli* strains isolated from human specimens by ERIC-PCR. M: 100-bp DNA ladder; lane E30-E44: *E. coli* strains; C: negative control (water as the DNA template).

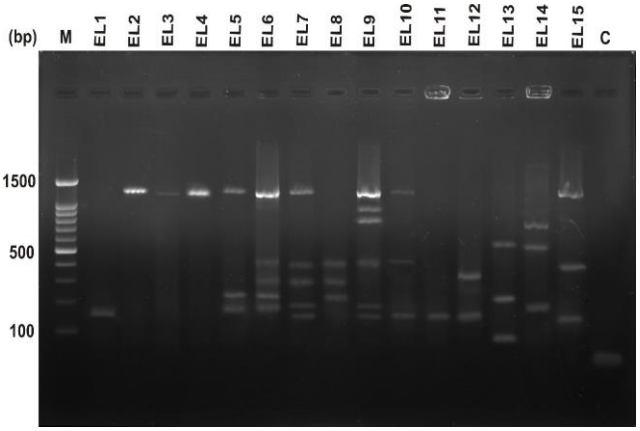

Figure 3. The DNA fingerprinting of several *E. coli* strains isolated from animal fecal specimens by ERIC-PCR. M: 100-bp DNA ladder; lane EL1-EL15: *E. coli* strains; C: negative control (water as the DNA template).

The dominant fragments in ERIC-PCR banding patterns were characterized with sizes of 200 bp (61%) and 120 bp (56%) and with sizes of 1300 bp (70%) and 200 bp (45%) in *E. coli* strains isolated from human samples and animal fecal specimens, respectively. The least frequent band was 3000 bp, which was observed in 1 clinical and domestic animal strain. However, the distinguished bands included a broad limit from 120 to 3000 bp in all samples, and higher diversity was seen among *E. coli* strains isolated from human samples than in an animal fecal specimen.

The NTSYS-pc analysis authorized the design of a phylogenetic tree for isolated strains through the attendance of a broad range of genetic heterogeneities among their populations. Two dendrograms were generated by cluster analysis. The ERIC-PCR typing of a heterogeneous population of *E. coli* isolates was genetically diverse at a 50% similarity cutoff value.

After dendrogram analysis, it was shown that ERIC-PCR differentiated the clinical *E. coli* isolates into 19 clusters, E1-E19, with 50% similarity. The results of the ERIC pattern of clinical isolates are illustrated in Table 2. Cluster analysis of clinical strains showed that there were more than 3 major groups. The highest number of strains was in the E7 group (21 strains with more than 52% similarity), and the lowest number of strains was in the E13 and E16 (1 strain) groups (Table 2). The dendrogram has grouped the 20 strains of *E. coli* strains isolated from animal fecal specimens into 7 distinct groups. Each cluster represented a particular number of strains, as well as divided inter and intra-group similarity relationships. Fifty percent of the strains with 0.53% similarity belonged to the E4 group, and the lowest number of strains was in the E3 and E5 (1 strain) groups.

Table 2. Clustering, similarity, and phylogroup of *Escherichia coli* clinical isolates in each cluster

Cluster	No. of strains in each cluster	Dendogram similarity in clusters	Phylogroup
E1	12	0.552	B2, D
E2	13	0.520	B2, D, A
E3	5	0.628	A, B1, B2
E4	7	0.528	A, B2
E5	2	0.604	B2
E6	5	0.552	A, B1, B2, D
E7	21	0.524	B2, D
E8	8	0.554	B2, D, A
E9	3	0.556	A, B2
E10	2	0.554	B1, D
E11	8	0.565	D, B1, B2
E12	2	0.750	D
E13	1	0.458	B2
E14	2	0.604	B2
E15	2	0.802	D
E16	1	0.370	A
E17	2	0.674	A, D
E18	2	0.674	A, D
E19	2	0.674	B2, D

Discussion

Escherichia coli is one of the most important opportunistic pathogens causing intestinal and urinary tract infections in animals and humans (29). This study showed that the distribution of phylogenetic groups and genetic markers among the host was not random. In a report by Zoolkiflia and colleagues, the relationship between the analyzed host and the phylogenetic group was discussed. The results of such research lead to an understanding of the communication between the phylogroup and the type of the host and disease (14).

In our studies, the triplex PCR method clustered all *E. coli* strains into 4 (A, B1, B2, and D) phylogenetic groups. A high percentage (44%) of clinical strains belonged to the B2 group, and more (65%) of the domestic animal strains were in the B1 group.

Gordon and colleagues (2008) evaluated that this method had the efficiency of separating 90% of *E. coli* strains into 4 main groups (30).

Our results demonstrated that B1 was the main phylogroup of *E. coli* isolated from domestic animals, followed by phylogroup B2. Coura and colleagues (2015) also proved that B1 was the most important phylogroup in animals, followed by phylogroup A (5). In another study, Gordon and Cowling reported the prevalence of strains of phylogroup B2 among herbivorous (31).

Some independent studies have examined the distribution of *E. coli* strains isolated from human and animal specimens between phylogroups. The results of these studies have determined the relationship between phylogenetic groups and host species. These authors concluded that domestication was the most important factor affecting the genetic structure of *E. coli* populations (7,32,33).

The genome size of the strains belonging to different phylogroups is different. Phylogenetic groups B2 and D have a larger genome than groups A and B1 and have more pathogenic factors (34). Intestinal pathogenic strains belong to the phylogroups B1 and D (31), while extraintestinal pathogenic strains belong to phylogroups B2 and D (4,15). The commensal human strains are in the A and B1 phylogenetic groups (13), while animal strains are in the B1 group (35).

Based on the comparison of ERIC-PCR results with phylogroups, the extraintestinal pathogenic strains within the major cluster (E7) with more than 50% similarity belonged to the B2 and D phylogroups (Table 2). The incidence of these human pathogenic isolates in a major cluster is genetically related to their source of prevalence and pathogenicity in the target population. Several dissimilar isolates belonging to all 4 phylogroups (A, B1, B2, D) were distributed throughout the dendrogram within E5, E10, and E12-E19 clusters (Table 2), indicating feasible horizontal gene transfer.

The results of the current research presented the genetic diversity of clinical isolates of *E. coli* correctly to detect the wide heterogeneity among *E. coli* isolates in humans and animals.

Conclusion

The maximum number (50%) of *E. coli* strains isolated from animal specimens belonged to the E4 cluster. The comparison of the results obtained from the phylogenetic analysis with the dendrogram represented that most of the isolates (70%) that fell in the E4 cluster belonged to the B1 group, followed by the B2 group. Also, the achievement of the ERIC-PCR technique as a facilitated typing method for an extensive and advanced extending figure of organisms makes this method appropriate for hospital-based or localized epidemiology.

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Ethical statement

This research is registered by ethics number IR.IAU.SRB.REC.1400.266.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

All authors have contributed substantially to the study conception and design, data collection, analysis, interpretation of results, and manuscript preparation.

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