Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) Genotyping of *Escherichia coli* Strains Isolated from Different Animal Stool Specimens

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KEYWORDS	ABSTRACT

ABSTRACT

Background: *Escherichia coli* is a commensal-pathogenic organism, which includes a wide range of strains. Despite several advanced molecular-genomic technologies for detecting and identifying different strains of *E. coli*, Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) technique is a quick, sharp and cost effective fingerprint method. The major purpose of the present study was to determine the distribution of ERICs within *E. coli* strains isolated from different healthy animal stool specimens including hens, sheep, and cows, as an appropriate and quick molecular-genomic tool.

Methods: The animal stool samples were obtained during 1 year (October 2012 to October 2013), from animal husbandries around Tehran and Alborz provinces, Iran. After screening processes, the *E. coli* bacteria were isolated and cultured via standard microbiological methods. The DNA molecules of *E. coli* bacteria were harvested and Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was applied for bacterial molecular genotyping. The ERIC-PCR products were run on 1% gel electrophoresis. The final images regarding gel electrophoresis banding patterns were used for dendrogram generation via the GelClust software.

Results: Of 120 isolated samples, 115 different strains were recognized as *E. coli*. The fingerprint patterns involved 380 to 3280 bp bands. The predominant bands included 2900 bp, 1200 bp, and 1200 bp in stool samples of hens, sheep, and cows, respectively. The highest frequencies and diversities were seen among *E. coli* strains isolated from hens and sheep stool samples.

Conclusion: The DNA profiles were clearly detectable via specific fingerprint patterns. The ERIC-PCR seemed to be a good approach for molecular typing of *E. coli* strains isolated from different animal sources.

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Introduction

Escherichia coli includes a wide range of strains distributed in different ecosystems; from alimentary tracts in animals and humans to nature (1), however, it is usually found in the normal microflora of the human gastrointestinal tract and is intricately involved in the lives of humans (2-3). Thus, *E. coli* consists of a large range of strains

with huge diversity in their genomes (4). Some strains cause serious diseases, such as Urinary Tract Infections (UTI) (5). Uropathogenic *Escherichia coli* (UPEC) is the most frequent agent causing UTI in adults and children (6-7). There are different strains divided to commensalisms and pathogens (8-11). Nonetheless, *Escherichia coli* is recognized as an appropriate indicator for water fecal contamination by animal or human stool (12).

Large genetic variations are not distinguished throughout traditional microbiological and biochemical tests. Instead of traditional diagnostics, the application of modern and advanced technologies, such as molecular diagnostic tools and molecular fingerprints may be an appropriate choice in molecular epidemiologic investigations (5, 13, 14).

Polymerase Chain Reaction-based techniques are accurate, rapid, reproducible, sensitive, specific and reliable diagnostics, which are used for determining different DNA fingerprints (15). Among several PCR-based tools, the Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR is a simple, sharp and cost effective genotyping technology for discriminating different types of strains. Indeed, ERICs are recognized as mobile DNA particles in association with Miniature Inverted Transposable Elements (MITEs) (5, 13, 14, 16-22).

The ERIC sequences are recognized in a huge number of bacterial genomes, including *Enterobacteriaceae* family members including *E. coli*. The incomplete palindrome sequences are generally detected within transcribed areas in association with intergenic consensus. Moreover, there are different numbers of ERIC sequence copies among bacterial species. Interestingly, there is significant diversity of copy numbers among different strains of *E. coli*. This diversity evokes the evolution processes among bacterial strains within a particular species like *E. coli* (23-25).

The clonal variability in different bacterial species such as *E. coli* is performed by homolog primers to ERIC sequences, in which the appeared patterns are valuable for evaluating the phylogenetic closeness by the help of different types of software, such as GelClust (26).

For this reason, the major purpose of present study was to determine the distribution of ERICs within isolated strains of *E. coli* as an appropriate, simple and cheap molecular-genomic tool. An important point to consider is that there are different gold standards for determining *E. coli* strains. Therefore, the authors decided to use ERIC-PCR as an optional methodology in this investigation (27).

Materials and Methods

Sample collection

The animal stool samples were provided from healthy hens (50 cases), sheep (50 cases), and cows (20 cases) during 1 year (October 2012 to October 2013). This project is excluded from ethical limitations, because the animals were not touched directly by the authors.

Phenotypic and biochemical properties

The collected stool specimens were inoculated into Presence-Absence broth (PA broth) and incubated at 35±0.5°C for 24 to 48 hours. Then, the PA broth and lactose positive bacteria were inoculated into Eosin Methylene Blue agar (EMB agar) and incubated at 35 ± 0.5 °C for 24 to 48 hours.

The presence of metallic green colonies endorsed the growth of gram-negative coliform bacteria of *E. coli*. The isolated strains of *E. coli* were then inoculated in lauryl sulfate broth for screening *E. coli*. Finally, the isolated strains of *E. coli* were inoculated in EMB agar and then, the grown colonies of *E. coli* were checked throughout an IMViC test. The screened and purified strains of *E. coli* were inoculated in Brain Heart Infusion broth (BHI broth) with the presence of glycerol 15% and then stored at -70°C.

DNA extraction

The DNA extraction process was achieved via AccuPrep genomic DNA extraction kit (Bioneer, South Korea). In brief, the bacterial suspension was centrifuged at 1200 rpm in 15' and the pellet was recovered. The pellet was washed by Phosphate Buffered Saline (PBS) and then centrifuged. The supernatant was rinsed out and then, 20 µL of proteinase K was added to the gained pellet. After

performing the steps, the TE buffer was added to DNA and the final solution was directly used as a PCR template.

Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction and DNA amplification

For achieving DNA amplification process via ERIC-PCR, a pair of forward and reverse primers was needed. The primers of 5'-ATG TAA GCT CCT GGG GAT TCA C-3' (F) and 5'-AAG TAA GTG ACT GGG GTG AGC G-3' (R) were applied (28, 29). The process was performed in a volume of 25 μL, including 1 μL of bacterial DNA (*E. coli*), 12.5 μL of mastermix, 3 μL of forward and reverse primers (1.5 μl/pm per each), and the left volume was filled by 8.5 μL of PCR grade water. Finally, the thermocycler was programmed. Simultaneously, negative (PCR grade water) and positive (bacterial DNA of *E. coli*) controls were used to achieve an accurate observation of the results (18, 28, 30, 31).

Polymerase Chain Reaction Products and Gel Electrophoresis

The gained products from ERIC-PCR together with loading buffer were run on 1% gel electrophoresis loaded by SYBR Green; in this practice, a 100-base pair (bp)-DNA marker (manufactured by Fermentas) was used as a standard measuring means. After a 45 minute-gel run, the green bands were observed by UV light and photographed via gel documentation. This stage was repeated twice for checking the accuracy of the results.

Dendrogram and phylogenetic relationships

The pattern of bands in gel electrophoresis regarding ERIC-PCR products was used as the principle structure for dendrogram calculation. The GelClust is an appropriate and user-friendly software, which can be used for phylogenetic trees and dendrograms via gel electrophoresis photos (32). For constructing computerized dendrogram, the presence and absence of bands were presumed as 1 and 0, respectively. The dendrogram was designed to unweight pair group method with arithmetic mean (UPGMA), which is categorized in clustering methodologies, and is based on clustering analysis (29, 31, 33).

Results

Overall, 115 different strains of *E. coli* were isolated from 120 stool samples (Table 1). The quality and purity of harvested DNA molecules wa recognized via spectrometry. A range of 1.7 to 2.0 resulted from A_{260}/A_{280} ratio is acceptable. The A_{260}/A_{280} ratio was calculated as 1.8 (34).

The diversity and number of bands gained from gel electrophoresis regarding harvested DNA molecules from isolated *E. coli* strains in different stool samples in association with hens, sheep, and cows was determined.

Table 1. Identification of Profiles, Bands and Clusters in Association with *Escherichia coli* Strains

Methodology	Unrepeated profiles	Repeated profiles	Bands (Minimum)	Bands (Maximum)	Diversity of bands	Number of clusters
ERIC-PCR	115	5	0	6	24	10

The ERIC-PCR banding patterns have indicated 0 to 46 bands encompassing 380 bp to 3280 bp.

The highest frequencies and diversities were seen among *E. coli* strains isolated from hens and sheep stool samples. Moreover, the greatest number of similarities among DNA molecules band patterns were seen in the strains isolated from sheep stool samples. The predominant fragments in DNA fingerprints were determined with sizes of 2900 bp, 1200 bp, and 1200 bp in *E. coli* strains isolated from stool samples of hens, sheep and cows, respectively; however, the observed bands involved a wide range from 380 to 3280 bp.

The GelClust let us design a phylogenetic tree for isolated strains via the presence of a wide range of genetic heterogeneities among their populations. The cluster analysis and related dendrogram are shown in Table 2 and Figure 1, respectively.

Table 2. Cluster Analysis and Genetic Diversity of the Strains

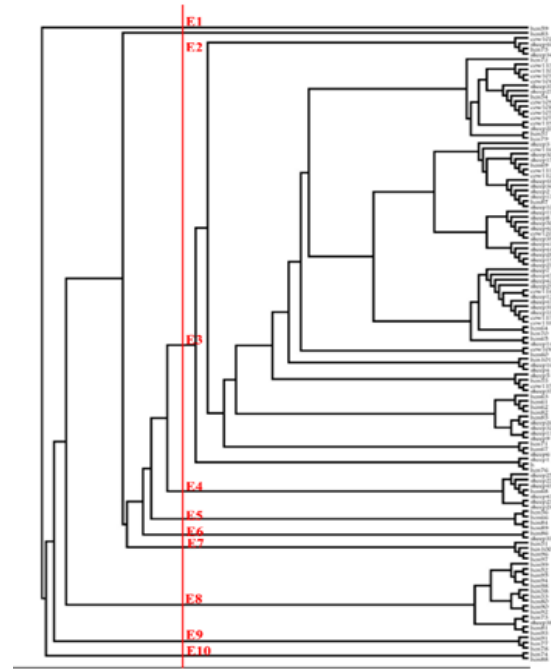| Cluster	Frequency of strains	Percentage of strains	Source
E1	1	<1%	Hens
E2	1	<1%	Hens
E3	82	69%	Hens, Sheep, Cows
E4	7	5.8%	Hens, Sheep
E5	4	3.33%	Hens
E6	2	1.6%	Hens, Sheep
E7	4	3.33%	Hens
E8	14	11.6%	Hens, Sheep
E9	3	2.5%	Hens
E10	2	1.66%	Hens

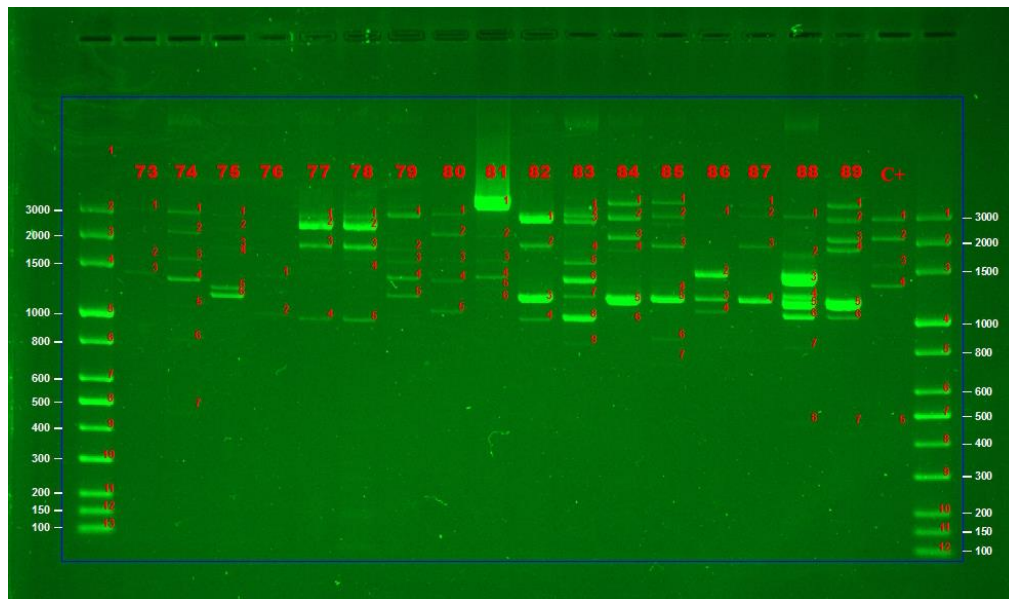**Fig 1.** Dendrogram Relating to Isolated Strains of *Escherichia coli*

Fig 2. Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction Patterns of Some *Escherichia coli* Strains

Discussion

There is a wide range of molecular biology techniques currently available, such as Amplified Fragment Length Polymorphism (AFLP), Amplified Ribosome DNA Restriction Analysis (ARDRA) different types of PCR, microarray, Pulsed Field Gel Electrophoresis (PFGE) Random Amplified Polymorphism Deoxyribonucleic Acid (RAPD), ribotyping, Restriction Fragment Length Polymorphism (RFLP). Each method and technique has its advantages and disadvantages. Of course, the applied technique in anystudy is in accordance with the study's economic facilities. Some methodologies are sharp with high accuracy

and discriminatory but they are too expensive. Among the aforementioned techniques, ERIC PCR is rapid, easy to use, and cheap with an acceptable outcome. However, its reproducibility is low (14, 15, 35-39).

In this study, 120 stool samples were obtained from 3 groups of healthy animals, including hens,

sheep, and cows during 1 year. One hundred and fifteen different strains of *E. coli* were recognized and included for fingerprinting analysis. The ERIC-PCR technique was applied to produce special amplicons for providing specific DNA fingerprint patterns by gel electrophoresis.

The predominant sizes recognized in DNA binding patterns were 2900 bp (hen), 1200 bp (sheep), and 1200 bp (cow). Furthermore, the molecular weight belonging to appeared bands ranged from 380 to 3280 bp. The binding patterns resulted in ERIC-PCR and led us to design a dendrogram by the help of the GelClust software, shown in Figure 1.

The binding patterns from gel electrophoresis were used as the basic data for designing a dendrogram using GelClust (32). In the present study, the methodology of UPGMA was applied for designing the dendrogram. Although there are different methods for designing a dendrogram, UPGMA is an easy and appropriate method to calculate, and provides us phenotypic characteristics and a rooted phylogenetic tree (38, 40, 41).

There are several investigations, which have used ERIC-PCR for detecting and determining different strains of *Enterobacteriaceae* family members such as *E. coli* (25, 42).

Casarez et al. collected 650 water samples from natural sources for detecting and genotyping *E. coli* isolates and used the BioNumerics software for ERIC-PCR fingerprints. They recognized 175 different genotypes from 555 water samples contaminated with human and animal feces (43). Interestingly, they had performed another study in 2007, which was confirmed by their recent investigation (44).

Lipman et al. applied ERIC-PCR for genotyping *E. coli* strains isolated from cows. They have successfully obtained different genotypes of *E. coli* and explained that there was some particular strains, which were the pathogenic agents of mastitis in studied cows (45).

Soltani et al. performed their study on avian hosts for recognizing and genotyping *E. coli* strains. They recognized 232 to 2690 bp bands on gel electrophoresis step and typed 65 different avian *E. coli* strains from 95 samples. They confirmed that ERIC-PCR might be a good technique for bacterial genotyping among cheap and simple molecular tools. Otherwise, there are several expensive molecular techniques as mentioned above with high discriminatory power (26).

A wide range of clinical samples like stool, urine and etc. may be used for ERIC-PCR. This method is also helpful for determining multi-drug resistant bacteria (42). The performed investigations confirm the rapidity and simplicity of ERIC-PCR as a cheap advanced molecular technology for different strains of a bacterial species (17-19, 31).

Conclusion

The DNA profiles were clearly detectable via specific fingerprint patterns. The ERIC-PCR seemed to a good approach for molecular typing of *E. coli* strains isolated from different animal sources. The ERIC-PCR is recommended for determining different strains pertaining to a bacterial species as a cheap and simple tool. The results of ERIC-PCR can be processed by different types of software, such as GelClust for generating useful dendrograms as invaluable methodology regarding classification of a diversity of bacterial strains, such as *E. coli*. However, there are also expensive genotyping methods such as AFLP with high accuracy and discriminatory power.

Acknowledgment

Conflict of Interest

The authors declare that there was no conflict of interests.

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