Genetic variation among *Escherichia coli* isolates from human and calves by using RAPD PCR

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Abstract:

BACKGROUND: Various strains of *Escherichia coli* (*E. coli*) are known as major causes of intestinal and extraintestinal infections in humans and various animal species. Molecular methods are important for the identification of bacterial isolates and nucleotide sequence variations, as well as information on tracking bacterial agents related to the outbreaks, the frequency of the bacterial genetic structure, and the evolution of microbial populations. **OBJECTIVES:** The purpose of the present study was to evaluate the efficiency of the RAPD method to differentiate *E. coli* strains. **METHODS:** In this study, 110 isolates of *E. coli* were analyzed by the RAPD PCR method using two 10bp oligonucleotides. These strains were isolated from humans with urinary tract infections and neonatal calves affected by diarrhea or septicemia. **RESULTS:** Data analysis showed that 87.5% of human *E. coli* isolates were correctly classified in the human host group, while 94.3% of calf *E. coli* isolates were correctly placed in calf groups. It also demonstrated that 100% and 93.3% of isolates were accurately assigned to diarrheic and septicemic calf groups, respectively. **CONCLUSIONS:** Genetic variation analysis indicated that the percentage of polymorphism among *E. coli* isolates from humans with urinary tract infections, diarrheic calves, and septicemic neonatal calves were 54.71%, 61.22%, and 62.5%, respectively.

Introduction

Different strains of *Escherichia coli* (*E. coli*) are known as important agents in intestinal and extraintestinal infections in humans and various animal species. Clinical infections in young animals may be confined to the intestines (intestinal colibacillosis and diarrhea), or may occur as septicemia (colisepticemia and general colibacillosis) or as toxemia (toxemic colibacilli) (Rostamzad et al., 2010). This organism is also a major cause of community-acquired urinary tract infections (Zalewska'Piatek, 2011).

Many methods are used for the identification of *E. coli* isolates. Bacteriological and serological methods are not sensitive enough for distinguishing bacterial isolates (Anand, 2001). Molecular methods are important for the identification of bacterial strains and nu-

cleotide sequence variations, as well as information on tracking the outbreaks, genetic structure, and evolution of microbial populations. However, these methods can help to differentiate strains that are specific to certain hosts. Moreover, they are useful guides for epidemiological studies in identifying the source of infection and the mode of disease transmission.

RAPD-PCR is one of the molecular techniques in which single oligonucleotides with arbitrary sequences are used for the synthesis of DNA. The strain-specific DNA fragments were amplified which require no prior knowledge of the nucleotide sequence of the target DNA. Since this method is PCR-based, small amounts of DNA (even at nanograms) are sufficient (Shehata, 2008). This method was successfully used for typing bacteria such as *Camphylobacte rjejuni* (Owen and Hernandez, 1993), *Listeria monocytogenes* (Mazurier and Wernars, 1992), and *Pseudomonas fragi* (Tanaka et al., 1993). Several studies were performed for typing *E. coli* strains by using RAPD PCR. In all of these studies, RAPD PCR was evaluated as an effective and important method in epidemiological surveys (Wang et al., 1993; Bando et al., 1998; Carvalho et al., 2007; Al-Darahi et al., 2008; Maityand Guru, 2008).

In the present study, the genetic diversity and clonal relationships of 110 isolates of *E. coli* from calves with septicemia and diarrhea along with humans with urinary tract infection were evaluated using the RAPD-PCR technique.

Materials and Methods

Bacteria: In this study, a total of 110 isolates of *E. coli*, including 40 human isolates from urinary tract infections, 40 isolates from diarrheic calves, and 30 isolates from septicemic calves, were used. Human isolates were collected from several medical diagnostic laboratories in Mashhad, Iran, during the summer and autumn of 2010. Septicemic and diarrheic calf isolates were collected from commercial dairy farms around Mashhad, Iran, in the summer of 2010. The isolates were confirmed as *E. coli* by using standard biochemical methods, and they were kept in nutrient broth with 15 % glycerol at -20 °C.

RAPD PCR: The random amplified polymorphic DNA fingerprinting method was used to differentiate *E. coli* isolates. Two primers with the size of 10 bp, OPAC 7 (GTGGCCGATG), and OPAC 11 (CCTGGGTCAG) were used (Gomes et al., 2005).

Extraction of DNA: DNA was extracted from the isolates using a commercial DNA extraction kit (Bioneer, South Korea).

Amplification of DNA: Amplification of bacterial DNA was performed using premix PCR kit (20 µl volumes) (Bioneer, Southern Korea). Every reaction contained 1 µl of oligonucleotide primer, 6 µl of the DNA template, and 13 µl of deionized distilled water. The PCR condition was carried out as follows: 94˚C for 3 minutes for initial denaturation, followed by 45 cycles of 94˚C for 1 minute, 40˚C for 1 minute, and 72˚C for 2 minutes, plus a final extension of 72˚C for 7 minutes.

Gel electrophoresis: The amplified products were visualized by standard gel electrophoresis on 1.5% agarose gel in TAE buffer (89 mMTris, 89 mM glacial Acetic acid, 0.5 M EDTA) containing 1 µg ml-1ethidium bromide for 45 min at 100 V.

A 100 bp DNA ladder molecular weight marker (Fermentas, UK) was included in each electrophoretic run to allow the identification of the amplified products. PCR products were visualized under UV illumination and catalogued with a gel documentation system.

Interpretation of PCR fingerprint images: Scanned images were analyzed using the Photocap software. Bands were assigned on a presence-absence basis. The software estimated band sizes for RAPD PCR data.

Statistical analysis: The data were analyzed using the SPSS software, version 16. Because the data were binary, the Jaccard distance matrix and Ward's hierarchical cluster technique were used. Isolates were clustered and displayed in the Dendrogram form. On the other hand, for differentiating isolates on the basis of host and the kind of infection, the discriminant method was applied, using the SPSS software, version16.

Results

A total of 28 bands were produced by primer OPAC7 among human isolates with sizes ranging from 350 to 3000 bp. Bands with the sizes of 900 and 650bp were repeated 16 and 15 times, respectively (Fig. 1). The electrophoresis of PCR products with primer OPAC11 among human strains revealed 26 bands with the sizes of 500 to 3000bp. Band 900bp was repeated 16 times.

The electrophoresis of PCR products with primer OPAC7 and OPAC11 for diarrheic calf isolates showed 22 and 24 bands, respectively. Band sizes ranged from 350 to 3000bp. A typical band with the size of 1000bp was repeated 19 times with primer OPAC7 (Fig. 2), and the typical bands with the sizes of 2000bp and 500bp were repeated 18 and 17 times, respectively. The electrophoresis of PCR products with primer OPAC7 for isolates from septicemic calves showed 28 bands with the sizes of 500 to 3000bp (Fig. 3). Band 900bp with 17 repeats was the typical band. A total of 20 bands by primer 2 with sizes ranging from 450 to 3000bp were produced among septicemic isolates. Typical bands with the sizes of 2300bp and 900bp were repeated 22 and 24 times, respectively. All *E. coli* isolates were grouped into 5 major groups, A, B, C, D, and E (Fig. 4). Group A included 50% of septicemic isolates from calves and 50% of isolates from urinary tract infections (UTI). Group B included 85 isolates from diarrheic calves and

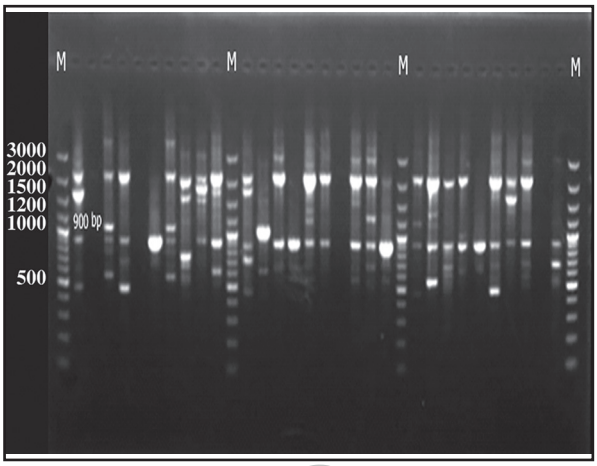

Figure 1. RAPD patterns of *E. coli* isolates from human using primer OPAC7. M: Marker 100 bp plus (Fermentas).

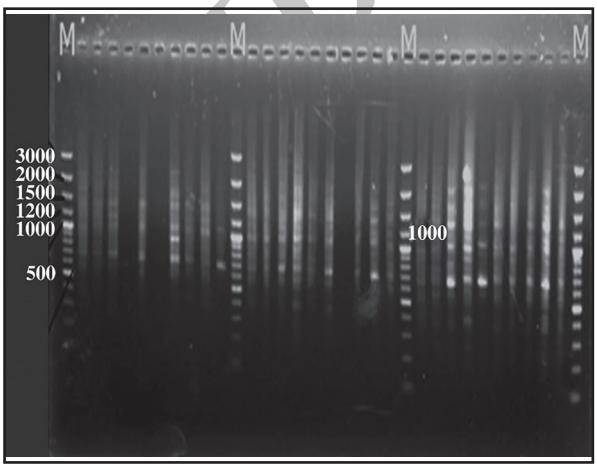

Figure 2. RAPD patterns of *E. coli* isolates from from diarrheic calves using primer OPAC7. M: Marker 100 bp plus (Fermentas).

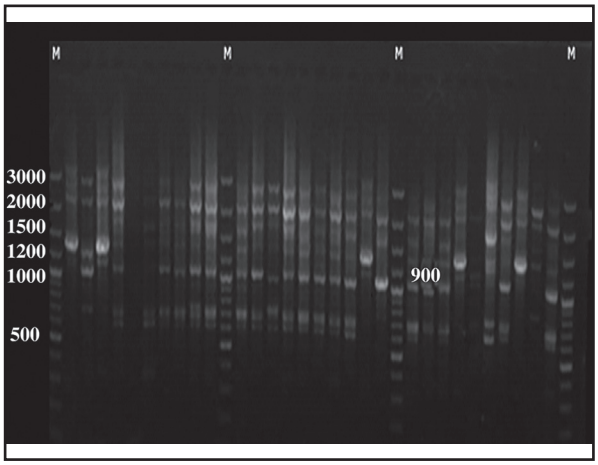

Figure 3. RAPD patterns of E. coli isolates from septicemic calves using primer OPAC11. M: Marker 100 bp plus (Fermentas).

15% of isolates from septicemic calves. Group C contained 5.5% of isolates of UTI samples and 4.44% of isolates from diarrheic calves.

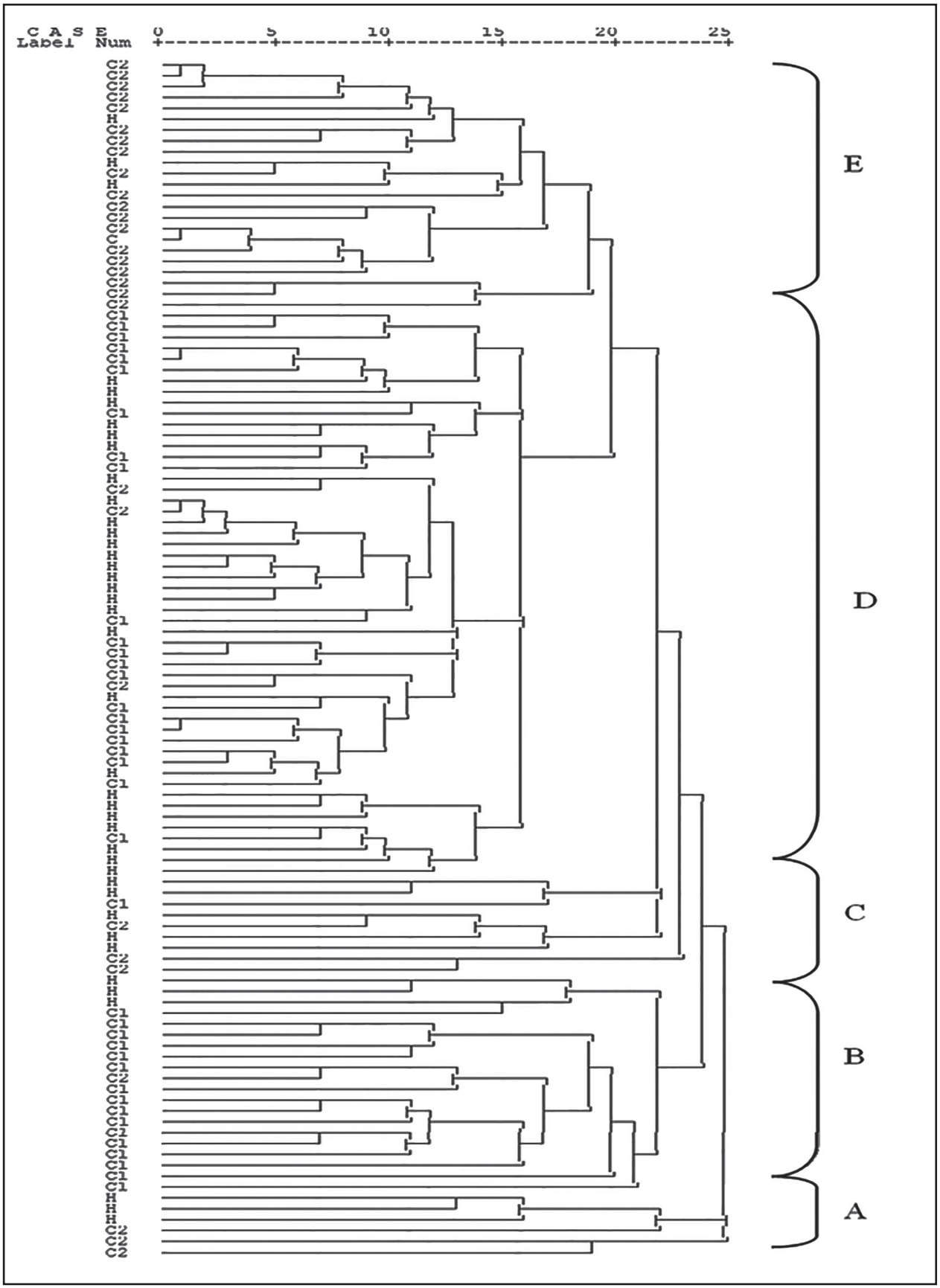

Figure 4. Dendrogram based on two RAPD primers (OPAC7 and OPAC11). H: human isolates, C1 : diarrheaic calves isolates, C2: septicaemic calves isolates.

Group D comprised of 50% of isolates from UTI, 3.42% of isolates from diarrheic calves, and 7.5% of isolates from septicemic calves. Group E contained 9.86% of isolates from septicemic calves, 6.8% of isolates from UTI, and 3.4% of isolates from diarrheic calves.

The results showed that there were clonal relationships among *E. coli* from different sources. On the other hand, genetic variation was clear among the isolates from each source.

Discriminant analysis of the results showed that *E. coli* isolates were significantly different based on the host. However, isolates from diarrheic and septicemic calves can correctly be differentiated. Isolates from diarrheic and septicemic calves were significantly placed with 100% and 93.3% accuracy in their own groups, respectively. Additionally, based on these results, human isolates were significantly classified with the accuracy of 87.5% in their own group. However, 94.3% of the isolates from calves were significantly assigned in their own group.

Discussion

E. coli is a normal flora in the digestive tract of animals. However, some pathogenic strains can cause severe disease in humans (Griffin and Tauxe, 1991). Cattle are the main reservoir of *E. coli*, with different prevalence ranges (Hancock et al., 1997). *E. coli* is an indicator organism for the fecal contamination of water. Some strains of *E. coli* known as O157: H7 can contaminate drinking water and can lead to disease outbreaks (Leung et al., 2004). Following the outbreak of the disease caused by this organism in 1982, in many countries, including America, Britain, and Japan, E coli infections were considered as a public health threat (Lih-Ching et al., 2001). Several major diseases, including urinary tract infection, septicemia, meningitis, and diarrhea were caused by E coli. Therefore, methods of microbial source tracking have drawn attention to themselves. Several methods are designed to classify the strains of E.coli. The evaluation of classification systems in epidemiological studies were based on important criteria, including the differential ability of the technique, typeability, and repeatability. Several methods based on phenotype and genotype are designed to differentiate *E. coli* strains (Tenover, 1997). Phenotypic methods like serotyping and phage typing have little discriminating power. Thus, genotypic methods with high discriminating power, low cost, and quick and easy usage had to be utilized for the classification of *E. coli* isolates (Rostamzad et al., 2010).

Recently, many methods have been applied for microbial source tracking. Methods such as ribotyping (Rodtong and Tannock, 1993), ERIC PCR (Parabhu et al., 2010), RAPD PCR (Gomes et al., 2005; Nowrouzian et al., 2001), and RFLP (Kamerbeek et al., 1997) were successfully applied for the differentiation of different bacterial strains. One of the most widely used methods, especially for the differentiation of *E. coli*, is RAPD-PCR. In this study, RAPD PCR was used to differentiate strains of *E. coli* based on the host and also the kind of infection.

A study on the differentiation of E.coli strains isolated from humans and animals was carried out using RAPD-PCR by Tseng et al. (2001). They reported the RAPD-PCR technique as a sensitive method for the fingerprinting of E.coli strains (Tseng et al., 2001). The genetic and molecular characterizations of pathogenic *E. coli* in laboratory rodent species were analyzed effectively using RAPD PCR (MaityandGuru, 2007). In the study of Carvalho et al. (2007), a clonal relationship was shown between the enteropathogenic *E. coli* isolates from human and primates using RAPD-PCR (Carvalho et al., 2007).

This study showed that this method has a relatively high power for differentiating *E. coli* strains. The isolates were divided into five groups. Most of the isolates in each group had

a common source. In many epidemiological studies, RAPD PCR was used to trace the origin. RAPD-PCR was also used for the molecular typing of other bacteria such as Shigella, *Staphylococcus aureus*, and *Pasteurella multocida* (RostamZad et al., 2010; Shehata, 2008; Ozbey et al., 2004). In our study, *E. coli* isolates were divided into 5 major groups based on 35 pleomorphic bands, using two different primers.

In a similar study, RAPD PCR was effectively used for the differentiation of *E. coli* strains from human and animal origin (Tseng et al., 2001). The results of the present study showed that *E. coli* isolates from humans and calves with the correct rates of 87.5% and 94.3% were classified in their own groups, respectively. On the other hand, our results showed that RAPD PCR was able to differentiate *E. coli* isolates from diarrheic and septicemic calves with the correct rates of 100% and 93.3%. The selection of suitable primers for RAPD PCR is an important aspect. In this study, we used two primers which were already used in other studies. However, using several primers for typing by RAPD PCR can increase the typeability of the method. The results of several other studies have proved the greater effectiveness of RAPD PCR in comparison to other molecular typing methods (Wang et al., 1993; Leung et al., 2004; ZahraeiSalehi et al., 2008; Saxena et al., 2014).

In this study, there were genetic variations among *E. coli* isolates from each source. This may be due to different origins, occurrence of mutations, and horizontal gene transmission. On the other hand, because of the difference of intestinal tract conditions among different species, the clonal relationship of *E. coli* isolates in specific hosts can be considered.

Conclusion: RAPD PCR was used to differentiate the isolates of *E. coli* based on the host and kind of infection. *E. coli* isolates from humans and calves with the correct rates of 87.5% and 94.3% were classified in their own groups, respectively. In addition, our results showed that RAPD PCR was able to differentiate *E. coli* isolates from diarrheic and septicemic calves with the correct rates of 100% and 93.3%, respectively.

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تنوع ژنتیکی جدای‌های اشریشیا کلی از انسان و گوساله‌ها به روش RAPD-PCR

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چکیده

زمینه مطالعه: سویه‌های مختلف اشریشیا کلی به عنوان عوامل مهم عفونت‌های داخل و خارج روده‌ای در انسان و گونه‌های مختلف دامی شناخته می‌شوند. روش‌های مولکولی، برای شناسایی جدایه‌های باکتریایی، تنوع سکانس نوکلئوتیدی، اطلاعاتی در زمینه ردیابی عوامل باکتریایی دخیل در همه گیری‌ها، شناسایی تکراردر ساختار ژنتیکی و نیز شناسایی تکامل تدریجی باکتری‌ها، اهمیت دارند. **هدف:** هدف از این مطالعه ارزیابی میزان کارایی روش RAPD-PCR در تفکیک جدایه‌های اشریشیا کلی می‌باشد. **روش‌کار:** در این مطالعه ۱۱۰ جدایه اشریشیا کلی از منابع مختلف به کمک دو الیگونوکلئوتید ۱۰bp با سکانس‌های اختیاری به روش RAPD-PCR مورد بررسی قرار گرفتند. این جدایه‌ها از افراد مبتلا به عفونت ادراری، گوساله‌های نوزاد مبتلا به اسهال یا سپتی سمی، جدا شده بودند. **نتایج:** تجزیه و تحلیل اطلاعات نشان داد که ۸۷/۵٪ ازجدایه‌های اشریشیا کلی جدا شده از انسان به درستی در گروه میزبان انسان طبقه‌بندی شدند، درحالی که ۹۴/۳٪ از جدایه‌های اشریشیا کلی به درستی در گروه‌های مربوط به گوساله قرار گرفتند. همچنین نشان داده شد که ۱۰۰٪ و ۹۳/۳٪ از جدایه‌ها به درستی به ترتیب درگروه‌های گوساله اسهالی وسپتی سمی قرار گرفتند. **نتیجه‌گیری‌نهایی:** آنالیز تنوع ژنتیکی نشان داد که درصد پلی مورفیسم در بین جدایه‌های اشریشیا کلی از افراد مبتلا به عفونت ادراری، گوساله‌های مبتلا به اسهال و گوساله‌های سپتی سمی به ترتیب ۵۴/۷۱٪، ۶۱/۲۲٪ و ۶۲/۵ ٪ بودند.

واژه های کلیدی: *E. coli*، تنوع ژنتیکی، RAPD-PCR

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