Int J Basic Sci Med. 2018;3(4):154-158 Original Article

http://ijbsm.zbmu.ac.ir/ doi 10.15171/ijbsm.2018.27

Genetic and Phylogenetic Diversity of *Escherichia coli* Strains Collected From Fecal Specimens of Patients Admitted to Zabol Hospitals

Aliyeh Firoozkoohi[1], Zahra Rashki Ghalehnoo[2*]

[1]MSc of Molecular Genetics, Food and Drug Administration, Zabol University of Medical Sciences, Zabol, Iran
[2]Assistant Professor of Microbiology and Molecular Genetics, Department of Microbiology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran

***Correspondence to**
Zahra Rashki Ghalehnoo, Assistant Professor, Department of Microbiology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran, Tel: 0098-915 1971410, Fax: 0098-5432232191, Email: zahrarashki@yahoo.co.uk

Received June 26, 2018
Accepted September 18, 2018
Published online December 31, 2018

Abstract

Introduction: *Escherichia coli* is one of the most prevalent bacterial species which cause gastrointestinal and digestive tract infections in humans and livestock. This study examined genotypic diversity of the *E. coli* isolates taken from fecal specimens in Zabol using random amplification of polymorphic DNA (RAPD) method and phylogenetic background.

Materials and Methods: In this study, 100 isolates were collected from human samples and identified by the common biochemical tests. The isolates were categorized into four main phylogenetic groups including group A (28 isolates), group B_1 (7 isolates), group B_2 (48 isolates), and group D (17 isolates). Two primers (H1 & H2) were used to study the genetic variation of *E. coli* and the electrophoresis band pattern was analyzed by the NTSYS.

Results: The analysis of the difference in isolates using the RAPD technique showed a genetic similarity between 14% and 100%.

Conclusion: The phylogenetic groups B_2 and A were more frequent in fecal isolates. In addition, the number of isolates related to phylogenetic groups B_1 and D was significantly lower than that of the other groups.

Keywords: *Escherichia coli*, Genetic diversity, Phylogeny, RAPD-PCR

Please cite this article as follows: Firoozkoohi A, Rashki Ghalehnoo Z. Genetic and phylogenetic diversity of *Escherichia coli* strains collected from fecal specimens of patients admitted to Zabol hospitals. Int J Basic Sci Med. 2018;3(4):54-58. doi:10.15171/ijbms.2018.27.

Introduction

Escherichia coli is a type of bacteria which can colonize and survive in numerous environmental habitats and animal hosts. This bacterium and other intestinal flora bacteria often interact with their hosts in a way that the hosts provide them food, and they, in return, contribute to the regulation of immune system and host protection against the pathogens.[1] Some of the *E. coli* strains have extra-intestinal pathogenic features and are found in various organs such as blood, central nervous system, and the urinary system.[2]

Phylogenetic studies conducted with the polymerase chain reaction (PCR), multilocus enzyme electrophoresis, and ribotyping methods are of paramount importance in evaluating the genetic evolution of the *E. coli*. Identifying the phylogenetic groups of *E. coli* strains by PCR method is facilitated through determining the presence of *yjaA* and *chuA* genes and the TSPE4.C fragment. Phylogenetic association of *E. coli* standard collection of reference (ECOR) strains indicates that the *E. coli* strains belong mainly to four phylogenetic groups of A, B_1, B_2, D[3] which are among the most important groups of *E. coli* bacteria. Previous studies demonstrated that these groups are quite different in many of their characteristics such as resistance to antibiotics, growth rate, and pathogenicity. A proper understanding of these characteristics can be effective in identifying the diversity of bacteria in the environment. The phylogenetic technique has several uses. It has a biotechnological role, and it communicates between the phylogenetic and pathogenic groups. In

© 2018 The Author(s); Published by Zabol University of Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

www.SID.ir

Archive of SID

addition, this technique functions as a biotechnological screening tool for detecting the pathogenic species. Further, it is a screening means used to identify the *E. coli* K-12 species by PCR and *E. coli* strains from unknown bacterial pathogens.[4]

Furthermore, phylogenetic strains are different in terms of their genome size. The genomes of the strains in groups A and B_1 are smaller than those of the phylogenetic groups B_2 and D. Phylogenetic studies on *E. coli* strains which are responsible for digestive diseases in humans indicate that these strains mainly belong to the B_2 group, but few of them belong to group D. *E. coli* strains show vast genetic diversity depending on the severity of their underlying human diseases.[3] Moreover, random amplification of polymorphic DNA (RAPD) is one of the rapidly developing and fast PCR-based methods requires no specific DNA sequence. Additionally, its primers are designed completely randomly. However, low repeatability and inability to recognize the allelic forms are the major disadvantages of this method. Recognizing the genetic profiles of the *E. coli* isolates which are taken from diverse clinical samples can be beneficial in designing therapeutic strategies. Therefore, the present study aimed to screen the phylogenetic diversity of *E. coli* isolates from fecal specimens of the patients referring to 2 main hospitals in Zabol using the RAPD technique.

Materials and Methods

A number of 100 *E. coli* isolates were gathered from the stool specimens of patients with diarrhea who referred to 2 major hospitals, namely Imam Khomeini and Amir-Al-Momenin in Zabol from December to March 2013. Then, biochemical tests (i.e., oxidase, fermentation, movement, urease, citrate hydrolysis, nitrate reduction, the Methyl Red and Voges-Proskauer, hydrogen sulfide, and indole production) were performed to characterize the *E. coli* species. The genomic DNA of the *E. coli* isolates was extracted based on the study by Rashki et al.[5] In addition, the phylogenetic distribution of the *E. coli* isolates was determined by the Triplex-PCR, as described by Clermont et al, using primers provided in Table 1.[4] Then, 5 μL of the PCR product was loaded in 2% agarose gel, stained with ethidium bromide, and visualized using a Gel-Doc (LOURMAT, France) device (Figure 1).

Further, the RAPD-PCR reaction was performed employing H2 and H3 primers (Table 1).

The reaction condition was conducted as described by Heydari and Rashki.[6] The PCR amplicons were electrophoresed in agarose 2% and speculated under UV illumination after staining by ethidium bromide (Figure 2).

In the data matrix created in the NTSYS-pc software, version 2.02, numbers 1 and 0 denoted the presence or absence of a particular band and the phylogenetic tree was prepared for each case. The Jaccard pairwise correspondence was applied for similarities between the strains using the means of matrix correspondence and unweighted pair-group method (UPGMA).[7] The closest neighbor-joining clustering method was used to demonstrate the relationships between the similar groups.

Figure 1. An Image of the Gel Electrophoresis Produced by PCR of *chuA, yjaA* genes and TSPE4.C2 Fragment.

Table 1. Primers Used in Phylogenetic Analysis Study

Gene	Sequence of the Primer (3-5)	(bp) Size
chuA	F- GAC GAA CCA ACG GTC AGG AT R- TGC CGC CAG TAC CAA AGA CA	279
yjaA	F- TGA AGT GTC AGG AGA CGC TG R- ATG GAG AAT GCG TTC CTCAAC	211
TspE4.C2	F- GAG TAA TGT CGG GGC ATT CA R- CGC GCCAAG AAA GTA TTACG	152
H2	AAGCTTCGACTGT	Variable
H3	AAGCTTGATTGCCC	Variable

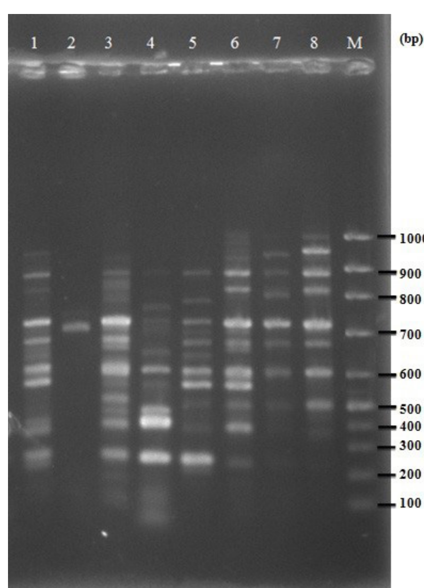

Figure 2. The RAPD-PCR Gel Electrophoresis Image.

www.SID.ir

Archive of SID

Results

Totally, 100 *E. coli* isolates collected from the stool samples were assigned into 4 groups of B_2 (48 %), B_1 (7 %), A (28 %), and D (17 %) (Table 2).

The results of the dendrogram indicated that all the isolates were in separate clusters. Furthermore, the results obtained from the RAPD-PCR demonstrated that all the isolates had a different pattern indicating a high differentiating capability of the used primers and high polymorphisms in the collected isolates (Figure 3). The genetic distance of the studied genotypes varied from 14% to 100 % using the Jaccard's coefficient in the dendrogram.

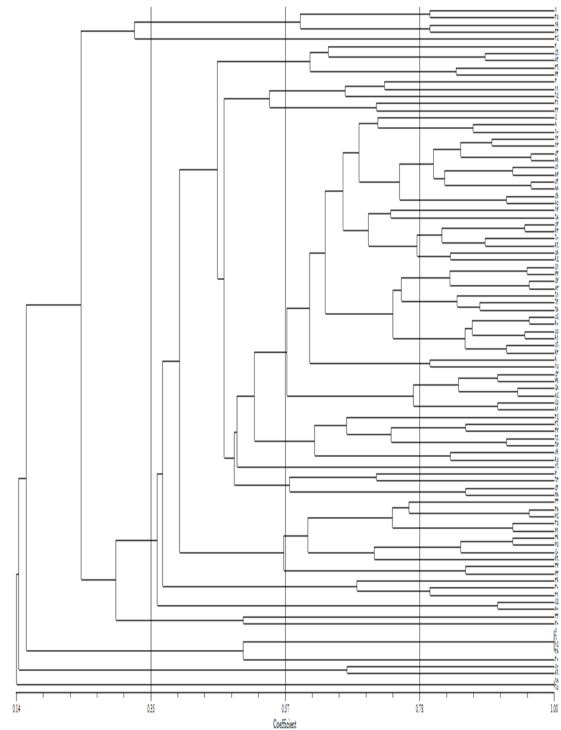

Figure 3. Dendrogram Schemed With NTSYS.

Discussion

Escherichia coli, as a common resident of the human lower intestine, is typically a part of the normal flora of the intestine. However, some strains might cause poisoning, tissue damage, and invasion. In this study, 100 isolates of *E. coli* collected from the feces were divided into four groups of B_2, B_1, A, and D with respective frequencies of 48%, 7%, 28%, and 17%, respectively. Using a Triplex-PCR method for phylogenetic analysis of the *E. coli* isolates, Derakhshandeh et al demonstrated that the isolates were related to groups A (n = 69), B_2 (n = 18), and D (n = 15), but no isolate belonged to group B_1.[8] Moreover, Lee et al indicated that phylogenetic distribution of *E. coli* strains was similar between blood and urine isolates.[9] These results regarding the phylogenetic diversity of fecal isolates in the B_2 group are quite consistent with those of the present study. Another research was conducted on 138 isolates collected from the human stool in order to assess the phylogenetic distribution of *E. coli* strains using the Triplex-PCR. Based on the results, no isolates belonged to group B_2 while the highest number of isolates belonged to group B_1.[10] In another research, the phylogenetic grouping of 168 *E. coli* isolates collected from the human stool samples was assessed in three different geographic populations including France, Croatia, and Mali. Most of the isolates were relevant to group B_2. Additionally, the remaining isolates belonged to groups A, B_1, and D with respective frequencies of 40, 34 and 15%, respectively.[11]

In their study on the phylogenetic groups of the *E. coli* strains using the Triplex-PCR, Clermont et al found that the isolates belonged to groups A (n = 43), B_1 (n = 23), D (n = 51), and B_2 (n = 113).[4] Similarly, Etebarzadeh et al used Triplex-PCR to investigate phylogenic status of the *E. coli* isolates and indicated that 65% of the strains were related to group B_2 while the other strains belonged to group D (19%) and phylogenetic group A (16%).[12] Overall, a large number of pathogenic extra-intestinal *E. coli* strains were relevant to group B_2 whereas a smaller number belonged to group D. However, the majority of the commensal strains were related to group A.[13] Lee et al in their research found that the phylogenetic group B_2 was more frequent.[9] As regards the present study, the highest frequency was related to the isolates A and B_2. Although a number of isolates were distributed into groups B_1 and D, group B_2 strains were the most common (48%) while the strains of group B_1 were the least common (7%) among the fecal specimens. This is further in conformity with the results of Escobar-Páramo et al.[3]

In the current study, molecular typing was performed on 100 *E. coli* isolates employing the RAPD method designed

Table 2. Phylogenetic Classification of the Pattern Designed Based on the Presence or Absence of the Duplication Bands

Phylogenetic Group	The Number of Isolates	Percent	*chuA*	*yjaA*	TSPE4.C2
B2	48	35	+	+	+
		13	+	+	-
D	17	6	+	-	+
		11	+	-	-
A	28	25	-	+	-
		3	-	-	-
B1	7	7	-	-	+
Total	100	100	-	-	-

www.SID.ir

Archive of SID

for detecting genetic polymorphisms in different strains of the studied isolates. In addition, it is useful for describing the distribution of the strains in the field ecology of the pathogen. Further, the RAPD, the results of which are comparable to the results of the expensive pulsed-field gel electrophoresis (PFGE) method, is a reliable technique for screening the genetic diversity of various bacterial species.[14] Based on the findings of Suardana et al, genetic maps of the *E. coli* isolates had several superiorities over the other methods using the RAPD technique. Furthermore, it is considered as time and cost-saving method with high sensitivity and easy applicability compared to methods such as amplified fragment length polymorphism (AFLP), and analysis of chromosomal DNA restriction patterns by PFGE. Therefore, such procedures can provide researchers with a wealth of information regarding the genetic diversity of various bacterial isolates.[15] Moreover, Kanungo represented that a significant genetic link was established between the *E. coli* strains, isolated from identical locations, using 2 different primer sets by exploiting the RAPD method.[16] In an analyzing the *E. coli* strains using the RAPD, Kilic et al asserted that this technique had the highest separation capacity for determining the genetic diversity of *E. coli* isolates despite a large genetic dissonance between the *E. coli* strains.[17] Additionally, Salehi et al concluded that the RAPD can be a useful method for determining the genetic differences between the *E. coli* isolates.[18] Similar to other studies, the analysis of dendrogram represented high genetic diversity in *E.coli* isolates, indicating the participation of different pollution centers in spreading the bacteria.[19,20]

Similarly, the results of the current study demonstrated that different isolates of the same *E. coli* species yielded no similar phylogenetic relationship. In addition, very negligible genetic variations may exist between the fecal *E. coli* isolates. Further studies are needed to determine the prevalence, distribution, ecology, and epidemiology of the fecal *E. coli* isolates.

Conclusion

This study was conducted to provide an efficient classification for phylogenetic isolates collected from the fecal samples of patients residing in 2 different hospitals of Zabol. Generally, the results revealed that the highest number of isolates belonged to phylogenetic group B_2, indicating a probable extra-intestinal origin.

Further, the RAPD method was employed to study the genetic difference of *E. coli* strains, which is a highly reliable and accurate technique in classifying and determining the phylogenetic diversity of bacterial strains. Analyzing the collected *E. coli* isolates from the fecal specimens across different geographic regions can provide useful information on phylogenetic epidemiology and distribution of drug-resistant strains.

Ethical Approval

The informed consent was obtained from the patients before using their samples and the data were kept confidential.

Competing Interest

There are no competing interests to declare.

Acknowledgments

The authors would like to express their gratitude to Microbiology Laboratory staff in Microbiology Department of Zabol University of Medical Sciences for their valuable assistance regarding laboratory investigation.

References

1. Suwanichkul A, Panigrahy B, Wagner RM. Antigenic relatedness and partial amino acid sequences of pili of *Escherichia coli* serotypes O1, O2, and O78 pathogenic to poultry. Avian Dis. 1987;1:809-13. doi:10.2307/1591036
2. Pitout J. Extraintestinal pathogenic *Escherichia coli*: a combination of virulence with antibiotic resistance. Front Microbiol. 2012;19:3-9. doi:10.3389/fmicb.2012.00009
3. Escobar-Páramo P, Menac'h L, Le Gall T, et al. Identification of forces shaping the commensal *Escherichia coli* genetic structure by comparing animal and human isolates. Environ Microbiol. 2006;18(11):1975-84. doi:10.1111/j.1462-2920.2006.01077.x
4. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol. 2000;166(10):4555-8. doi:10.1128/AEM.66.10.4555-4558.2000
5. Rashki A. Cervico-vaginopathogenic *Escherichia coli* in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Microb Pathog. 2014;75:29-34. doi:10.1016/j.micpath.2014.08.004
6. Heydari F, Rashki GZ. Epidemiology and drug susceptibility of *Pseudomonas aeruginosa* strains isolated from patients admitted to Zabol hospitals. J Birjand Univ Med Sci. 2016;22(4):386-391.
7. Jaccard P. The distribution of the flora in the alpine zone. New Phytol. 1912;11(2):37–50. doi:10.1111/j.1469-8137.1912.tb05611.x
8. Derakhshandeh A, Firozi R, Moatamedifa M, Motmedi A, Bahadori M, Nazari Z. Phylogenetic analysis of *Escherichia coli* strain isolated from human sample. Mol Biol Res Commun. 2013;2(4):143-149. doi:10.22099/mbrc.2013.1822
9. Lee S, Yu JK, Park K, Oh EJ, Kim SY, Park YJ. Phylogenetic groups and virulence factors in pathogenic and commensal strains of *Escherichia coli* and their association with bla_{CTX-M}. Ann Clin Lab Sci. 2010;40(4):361-367. doi:10.22099/mbrc.2013.1822
10. Unno T, Han D, Jang J, et al. Absence of *Escherichia coli* phylogenetic group B2 strains in humans and domesticated animals from Jeonam province, Korea. Appl Environ Microbiol. 2009;75(17):5659-5666. doi:10.1128/AEM.00443-09
11. Duriez P, Clermont O, Bonacorsi S, et al. Commensal

www.SID.ir

Archive of SID

Escherichia coli isolates are phylogenetically distributed among geographically distinct human populations. Microbiology. 2001;147(6):1671-1676. doi:10.1099/00221287-147-6-1671

12. Etebarzadeh Z, Oshaghi M, Mozafari A. Evaluation of phylogenetic typing of *Escherichia coli* isolated from urinary tract infection. Journal of Microbial Biotechnology. 2012;4(13):29-36.
13. Russo TA, Johnson JR. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. Int J Infect Dis. 2000;181(5):1753-4. doi:10.1086/315418
14. Escobar P, Le Menac'h A, Le Gall T, et al. Identification of forces shaping the commensal *Escherichia coli* genetic structure by comparing animal and human isolates. Environ Microbiol. 2006;8:1975-1984. doi:10.1111/j.1462-2920.2006.01077
15. Suardana IW, Artama WT, Widiasih DA, Mahardika IG. Genetic diversity of *Escherichia coli* O_{157}: H_7 strains using random amplified polymorphic DNA (RAPD). Int Res J Microbiol. 2013;4(72):e8. doi:10.3103/S1068367418020039
16. Kanungo S. A simplified analysis of different *Escherichia coli* strains by using RAPD technique. Not Bot Horti Agrobot. 2009;37(2):25-39. doi:10.15835/nbha3723235
17. Kilic A, Muz A, Ertas HB, Ozbey G. Random amplified polymorphic DNA (RAPD) analysis of *Escherichia coli* isolated from chickens. FU Sag Bil Vet Derg. 2009;23(1):1-4. doi:10.1099/jmm.0.46373-016.
18. Salehi TZ, Madani SA, Karimi V, Khazaeli FA. Molecular genetic differentiation of avian *Escherichia coli* by RAPD-PCR. Braz J Microbiol. 2008;39(3):494-7. doi:10.1590/S1517-838220080003000015
19. Asadi H, Yari R, Zargar M. Genetic variation of *Escherichia coli* bacteria isolated from Urinary Tract Infections using RAPD-PCR (Persian). J Mazandaran Univ Med Sci 2016; 26(134): 114-123.
20. Tabrizi S, Golestani Imani B, Karimi F. Genetic the effect of different doses of silver nanoparticles on *Escherichia coli* genome using randomly amplified polymorphic DNA (RAPD) Molecular Markers (Persian). Biotchnol Appl Microbiol J. 2014;3(1):37-48. doi:10.22090/jwent.2018.01.005

www.SID.ir