Annals of Microbiology and Infectious Diseases

Volume 3, Issue 4, 2020, PP 01-03

ISSN 2637-5346

The Importance of PFGE and Rapd Methods in Determination of Philogenetic Relation among *E. Coli* Sustains Isolated from Extraintestinal Infection and whose Virulance Factors are Defined

Başak BEDİR[1], AymenAziz Khalid[2], Hassan T.S. SHATUB[3], Shadman Sadiq[4*], Fatih KOKSAL[1], Ousman Bajinka[5]

[1]Department of medical microbiology, Cukurova University, Adana. Turkey.

[2]Department of Medical Laboratory technology, Imam Ja'afar Al-Sadiq University,Kirkuk, Iraq.

[3]Department of Biotechnology, Cukurova University, Adana, Turkey.

[4*]Department of Industrial and Fundamental Microbiology, Ege University, Izmir 35040, Turkey.

[5]Department of Microbiology, Central South University Changsha, Hunan Provinces, China.

***Corresponding Author:** Shadman Sadiq, Department of Industrial and Fundamental Microbiology, Ege University, Izmir 35040, Turkey.*

Abstract

In this study, 155 Escherichia coli strains were isolated to investigatethe association between PFGE and RAPD. The freeze-thaw method was applied for RAPD. The results showed PGFE had a higher discrimination power than RAPD-PCR. With the both methodsjust13(30.9%) samples showed clonal relation, the relation might be a random mutation rather than epidemic since the strains were isolated from the in-patient ofdifferent clinics.The study concluded that RAPD method is more advantageous than PEGE method in terms of the phylotyping and interpretation of obtained results.

Keywords: *E.coli , Freeze-thaw, Random mutation, PFGE method, Phylotyping*

Introduction

Escherichia coli (E. coli) is the commonest member of the Gram-negative bacteria family of Enterobacteriaceae, which was identified by Theodor Escherich.[1,11] This non-spore forming and generally motile (with a peritrichous flagellated arrangement), facultative anaerobic rod-shaped bacteria were named *E. coli* by the suggestion of Castellani and Chalmers in 1919 [2, 3, 4]. There are a diversity of *E. coli* strains, which are divided into commensal types (intra-intestinal non-pathogenic strains) and pathotypes (intra-intestinal pathogenic *E. coli* (InPEC) and extra-intestinal pathogenic *E. coli* (ExPEC)). The commensal types of *E. coli* are able to be settled within the infants' alimentary canal, just in few hours after birth as beneficial normal microbialpopulation [5, 6].

ExPEC strains are gram-negative bacteria, which are isolated from urinary tract infections (UTI), newborn meningitis, sepsis, hospital-based pneumonia, osteomyelitis and the likes, are among the leading causes of mortality and morbidity [7,8].

In this study, we aimed to determine the effectiveness of RAPD and PFGE methods in order to shed lights into epidemiologic studies.The study was designed to find whether special genotypes are mutated or random mutations bring about the virulence characteristics of ExPEC strains. If this mutation can change the genes to pathogenicin gut microbiota, even though they are commensalinthe intestinal system [9,10].

Material & Methods

A hundred fifty-five *E. coli* strains obtained from the Central Laboratory of Cukurova University were used in this study. The freeze-thaw method was used to extract test strains. The strains were verified with the amplification of genes fragment coding βeta-

D-Glukorinidaz enzymes. DNAs were used for the amplification of genes zones coding CNF-1, 2, 3 and CDT-1, 2, 3, 4 toxin genes, which are the characteristic of ExPEC. In order to find the relation between the isolates, Pulsed Field Gel Electrophoresis and Randomly Amplified Polymorphic DNA method, which was taught to be an alternative with OPA-9 (10 and 11 dimers) and OPA-11 primers in terms of repeatability, cost and time were used.

Results and Discussions

PFGE analysis with Xbal Restriction Enzymes revealed that isolates were scattered in 106 big clusters.The highest of which consisted of 5 members according to 80% similarity level.They were scattered in 128 big clusters.The highest consisted of 3 members to 85% level. RAPD method showed 95 clusters primary with 11bp OPA-9 to 85 similarity level. It was also identified that 42 (27,09%) of the test similarly scattered in main clusters and subclusters and that 25 of them placed in subcluster and 17 of them placed in single-member clusters. A real relation between only 13 (30,9%) of the 42 ExPEC strains in single-member

Cluster obtained with both groups was determined. Isolated samples of these strains were from different clinics. Figure (1).

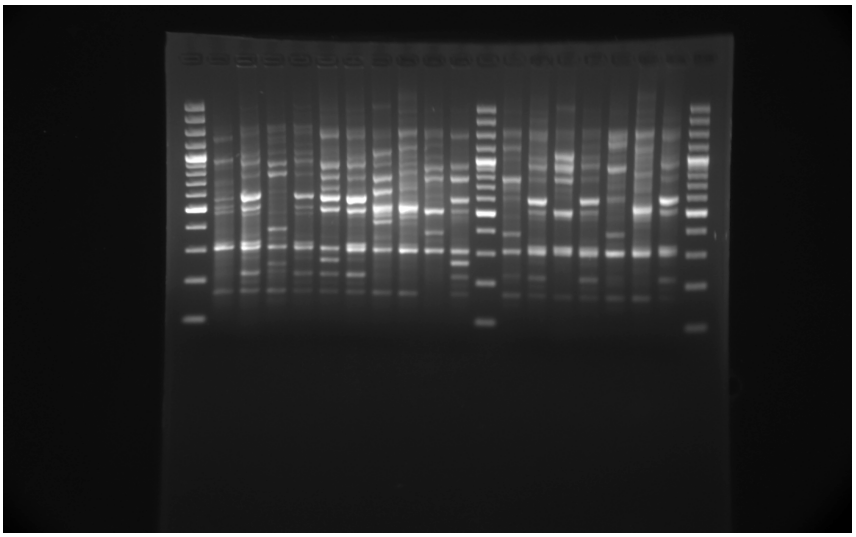

Figure1. *Band profiles of RAPD-PCR methods, In similar strains and same primer regions target linked amplification, bands are formed in the same place so there are many bands as in PFGE, dendogram is created using UPGMA*

Conclusions

PGFE showed higher discriminion power than RAPD-PCR. With both method just 13 (30,9%) samples were showing clonal relation, but the relation might be a random mutation rather than epidemic since the strains were isolated from the inpatient from different clinics. ExPEC strains come out when a delation-recombination occure among *E. coli* strains with certain zones open to mutation.

Finally, RAPD method is more advantageous than PEGE method in terms of the phylotypingand interpretation of the results.

References

[1] Lim, J. Y. et al. "A brief overview of Escherichia coli O157:H7 and its plasmid O157." *Journal of microbiology and biotechnology* 20 1 (2010): 5-14.

[2] Jahandeh N, Ranjbar R, Behzadi P, Behzadi E. Uropathogenic Escherichia coli virulence genes: Invaluable approaches for designing DNA microarray probes. *Central European Journal of Urology*. 2015;68:452-458. DOI: 10.5173/ceju.2015.625.

[3] Etymologia: Escherichia coli. *Emerging Infectious Disease Journal*. 2015;21:1310. DOI: 10.3201/eid2108.ET2108 .

[4] Schaechter M Editor. Desk Encyclopedia of Microbiology. *2nd ed. Academic press, Elsevier, UK*; 2010.

[5] Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. *Nature Reviews*. Microbiology. 2004;2:123-140. DOI: 10.1038/nrmicro818 .

[6] Donnenberg M. Escherichia coli: Pathotypes and principles of pathogenesis. *2nd ed. Academic press, Elsevier, UK*; 2013.

[7] Behzadi P, Najafi A, Behzadi E, Ranjbar R. Microarray long oligo probe designing for Escherichia coli: An in-silico DNA marker extraction. *Central European Journal of Urology*. 2016;69:105-111. DOI: 10.5173/ceju.2016.654.

[8] Jahandeh N, Ranjbar R, Behzadi P, Behzadi E. Uropathogenic Escherichia coli virulence genes: Invaluable approaches for designing DNA microarray probes. *Central European Journal of Urology*. 2015;68:452-458. DOI: 10.5173/ceju.2015.625.

[9] Robins-Browne RM, Holt KE, Ingle DJ, Hocking DM, Yang J, Tauschek M. Are Escherichia coli Pathotypes still relevant in the era of whole-genome sequencing? *Frontiers in Cellular and Infection Microbiology*. 2016;6:141. DOI: 10.3389/fcimb.2016.00141.

[10] O'brien CL, Bringer M-A, Holt KE, Gordon DM, Dubois AL, Barnich N, et al. Comparative genomics of Crohn9s disease-associated adherent-invasive Escherichia coli. *Gut* 2016;0: 1-8. DOI: 10.1136/gutjnl-2015-311059.

[11] Stanford T. Shulman, Herbert C. Friedmann, Ronald H. Sims, Theodor Escherich: The First Pediatric Infectious Diseases Physician?, *Clinical Infectious Diseases*, Volume 45, Issue 8, 15 October 2007, Pages 1025–1029, https://doi.org/10.1086/521946.

Citation: *Başak BEDİR, AymenAziz Khalid, et al. The Importance of PFGE and Rapd Methods in Determination of Philygenetic Relation among E. Coli Sustains Isolated from Extraintestinal Infection and whose Virulance Factors are Defined. Annals of Microbiology and Infectious Diseases. 2020; 3(4): 01-03.*