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GENETIC IDENTIFICATION OF *E. COLI* ISOLATES ASSOCIATED WITH GUT DISORDERS USING RAPD PCR

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ABSTRACT

Objective: To isolate and identify *E. coli* strains from samples taken from patients with gut disorders, analyze the genetic diversity among isolates using RAPD-PCR to detect genetic variation among them, To assess the genetic relationship between isolates to determine their genetic closeness or divergence, and to correlate this relationship with the clinical condition of patients. **Methods:** Samples were collected from Diwaniyah Teaching Hospital, Digestive System Unit. Twenty E. coli bacteria were isolated from 35 samples from patients suffering from intestinal disorders accompanied by watery and bloody diarrhoea, from both sexes, males and females, using agricultural biochemical methods and genetic diversity using RAPD PCR and branching tree analysis. **Results**: 20 isolates of *E. coli* bacteria were selected from patients suffering from gut disorders to determine their genetic heterogeneity using ERIC-PCR. and shows the presence of polymorphic variants within each of the six groups. The number of polymorphic variants (genetic variants) among isolates can be estimated at approximately 8. **Conclusion**: in this study, males were more likely than females to be affected by gut disorders. The age group most affected by gut disorders and the highest rate of isolation of *E. coli* bacteria was from 21-30 years. *E. coli* bacteria also had genetic diversity with six clusters and eight genetic variations.

KEYWORDS: Genetic diversity of *E.coli*, Gut disorders, RAPD PCR.

1. INTRODUCTION

Escherichia coli is one of the most common bacteria in the human gastrointestinal tract, playing a dual role: it is part of the normal flora, but some strains can be pathogenic and are associated with gut disorders.[1] To distinguish pathogenic strains from non-pathogenic strains, genetic analysis is an essential tool for determining genetic diversity among bacterial isolates.

Among the molecular techniques used, random amplified polymorphic DNA (RAPD-PCR) is a rapid and effective method for studying genetic variation without the need for prior knowledge of the genetic sequence.[2] This technique is used to detect genetic diversity among bacterial isolates based on different amplification patterns, which helps in tracing sources of infection and understanding their relationship to various diseases, including intestinal disorders.[3] This study contributes to the analysis of the genetic diversity of E. coli isolates isolated from patients with intestinal disorders, using RAPD-PCR technology, which enables the assessment of potential genetic differences between isolates and their role in virulence or causing clinical symptoms.

2. MATERIALS AND METHODS

2.1. Sample Collection

Samples were collected from hospitals in Diwaniyah City, specifically from the Internal Medicine Clinic and the Gastroenterology Clinic, between December 2024 and March 2025. The study included the collection of 35 clinical samples from patients with gastrointestinal disorders. Stool samples were collected using sterile containers and transported directly to the laboratory within 30 minutes.

2.2. Sample Culture

Stool samples were transported directly to the laboratory within 20-30 minutes and cultured on blood agar, MacConkey agar, and EMB medium. They were then incubated at 37°C for 24 hours. Any medium that did not show growth within 24 hours was incubated for an additional 24 hours before the results turned negative.

2.3. Randomly Amplified Polymorphic Polymerase Chain Reaction (RAPD PCR)

This technique was performed to study the genetic diversity of Escherichia coli using ERIC PCR (as follows):

1. Extract bacterial genomic DNA using a Genomic DNA Extraction Kit (Presto™ Mini DNA Bacteria Kit) and instructions provided by American Generaid.[4]
2. Screen the DNA using a Nanodrop device to detect and quantify nucleic acid concentrations. This is detected by determining the DNA concentration (ng/μL) and measuring its purity by reading the absorbance at a wavelength of 260-280 nm.
3. Prepare the PCR master mix using the AccuPower® PCR PreMix kit and processing instructions from Bioneer (Korea).[5]
4. The thermocycler was programmed to amplify the DNA according to the method of Townsend et al. (19) for each primer. Thermocyclers were repeated for 30 cycles for each primer.
5. Prepare agar gel according to the method of Sambrook et al.[6]
6. Electrophoresis of agar gel (1.5%) at 100 V and 80 mA for 60 min to detect extracted DNA bands and amplified DNA representing amplified sizes or PCR products.

Table 2-1: Primers sequences and product size used in PCR amplification and Eric PCR.

Gene name		Oligo sequence (5'-3') (primer)	Product Size(bp)
16sRNA	F	TCAGCAAGAGGATTTCTCA	**625**
	R	GGCAGCACTATTACTCCCA	
ERIC 1	ATGTAAGCTCCTGGGGATTCAC		**500-2000**
ERIC 2	AAGTAAGTGACTGGGGTGAGCG		

2.4. Statistical Analyse

All the results of the present study were statistically analyzed, and the Statistical Package for Social Science (SPSS) was used for this purpose. Version 23, where the statistical test, square xi (chi-square) was applied for the purpose of comparing the percentages of all study variables. 95% and the probability level is less than 0.05 ($P < 0.05$).

3. RESULT AND DISSUASION

Gut disorder are a group of conditions that affect gastrointestinal function and include a wide spectrum of symptoms, such as functional diarrhea and abdominal bloating. These disorders are often associated with alterations in the microbial balance (dysbiosis) within the gut, leading to abnormal immune responses and chronic mucosal inflammation. Intestinal bacteria, such as Escherichia coli (E. coli), play a pivotal role in some of these disorders. Certain pathogenic strains of this bacteria have been shown to be more abundant in patients with intestinal disorders compared to healthy individuals Numerous studies have shown that microbial imbalance can contribute to the development of symptoms, such as abdominal pain, bowel irregularities, and inflammation, by affecting the immune system, intestinal barrier function.

Table 3.1: Prevalence of Escherichia coli isolates obtained from patients with gut disorders.

Type and No. of specimens	Total samples	Positive sample No.(%)	
		Positive Culture for *E.coli*	16srRNA Molecular
Gut disorder	35	20 (57 %)	20/20 (100%)

X2=15, p.value <0.001: There is a strong statistically significant correlation between the results of the Positive Culture and 16srRNA Molecular tests. This indicates that the 16srRNA Molecular test agrees well with the Positive Culture for *E. coli* test.

Out of 35 stool samples collected from patients with gut disorders, 20 (48%) were positive for Escherichia coli based on conventional culture methods. All 20 culture-positive isolates were confirmed as *E. coli* using 16S rRNA gene amplification by PCR, yielding a 100% molecular confirmation rate. This result highlights the reliability and specificity of molecular techniques such as 16S rRNA sequencing for bacterial identification, especially when compared to traditional culture-based methods, which may fail to detect certain fastidious or subdominant strains.[7]

The 48% culture positivity rate suggests that *E. coli* may be associated with a subset of gut disorder cases, while other microbial agents or non-infectious factors may contribute to the remaining cases. The complete molecular confirmation of cultured isolates validates the presence of *E. coli*, setting a strong basis for further genetic characterization using ERIC-PCR.

These findings emphasize the value of integrating molecular tools into microbiological diagnostics to improve accuracy and detection rates. Future analysis using ERIC-PCR on these isolates will help to determine the genetic diversity and clonal relationships among E. coli strains, which may offer insights into their potential pathogenicity and association with specific gastrointestinal symptoms.[8]

Table 3.2: Prevalence of *E.coli* bacteria in patients with gut disorder according to age and gender.

Patient profile	Status	Gut disorder		No. % of *E. coli* n=20		Statically analysis
		n	%			
Age	10 - 20	7	20	3	42	**X2=21.38 p.value=0.00069** significant difference at P<0.05
	21 - 30	9	28	8	88	
	31 - 40	3	8	1	33	
	41-50	9	25	5	55	
	51-60	4	11	2	50	
	61-70	3	8	1	33	
Sex	Male	26	74	16	61	**X2=0.793 p.value=0.373** no significant difference at P<0.05
	Female	9	25	4	44	

Analysis of the demographic distribution of Escherichia coli-positive isolates revealed a higher prevalence among male patients (61%) compared to females (44%). This aligns with a male-to-female ratio of approximately 4:1, indicating that male patients with gut disorders were more likely to harbor *E. coli* isolates. Such a trend may be attributed to behavioral factors (e.g., occupational exposure, hygiene practices) or physiological and immunological differences between sexes. Although some previous studies have found no significant sex-related differences, others have reported a male predominance in enteric E. coli infections.[9]

Age-wise, the 21–30-year-old group showed the highest rate of *E. coli* isolation (88%), followed by the 31–40 group (55%). This age-related pattern suggests that young adults may be more vulnerable to gut colonization or infection by E. coli, possibly due to lifestyle, dietary habits, or environmental exposure. Similar age-based trends have been noted in earlier studies, particularly in low- and middle-income regions, where exposure to contaminated food and water is more frequent.[10] These findings highlight the need for targeted public health measures, especially among young adult males in high-risk settings.

Table 3.3: Distribution of Diarrhea Types and *E. coli* Infection among Gut Disorder Patients.

Type of Diarrhea	Age (years)	No. Infected (n=35)	*E. coli* Positive (n=20)
Watery	10–50	19 (54.3%)	12 (63.1%)
Bloody	>50	16 (45.7%)	8 (50%)
Total	—	35 (100%)	20 (57.1%)

X2=0.61, p.value=0.43, watery diarrhea appears to be more closely associated with E. coli infection than bloody diarrhea but No Statistically Significant.

The table shows the distribution of diarrhea types caused by E. coli by gender. The results of the current study showed that watery diarrhea was more common than bloody diarrhea among patients with gastrointestinal disorders, accounting for 54.3% of cases. This is consistent with[11], who reported that most diarrhea cases caused by bacterial infections, especially E. coli, are watery.[12] also indicated that pathotypes such as ETEC and EPEC of E. coli often cause this type of diarrhea. Among 20 patients positive for E. coli, the majority (63.1%) had watery diarrhea, reinforcing the relationship between infection with this bacteria and watery diarrhea. On the other hand, the incidence rate was slightly higher among males than females, which is consistent with Al-Gallas et al., who found higher incidence rates among males, possibly due to behavioral or environmental factors. However, the results of Moyo et al.[13] did not show significant gender differences, suggesting that the effect of gender may vary depending on demographic and geographic factors. The age group covered by the study (10–50 years) is also in line with Scallan et al.'s[14] finding that older, active populations are more susceptible to infection due to dietary behavior and lifestyle. These findings reflect general agreement with previous literature, with minor variations that may be due to the nature of the sample and the geographic region.

RAPD-PCR analysis for *E. coli* isolates

The RAPD-PCR dendrogram tree depicting the genetic relationships among 20 *E.coli* isolates as show in figure Based on the of RAPD-PCR electrophoresis and dendrogram tree analysis the results show as following:

1. Clusters

The dendrograms was shows that the 20 *E.coli* isolates in gut disorder can be divided into 6 distinct clusters as (A, B, C, D,E and F).

2. Polymorphic Variants

The dendrogram indicates the level of genetic similarity or diversity among isolates. The horizontal distance between branching points in the dendrogram represents the degree of genetic variation among isolates and shows the presence of polymorphic variants within each of the six groups. The number of polymorphic variants (genetic variants) among isolates can be estimated at approximately 8, as shown in the following table.

Table 3.4: The *E. coli* isolated from gut disorder patients according to RAPD PCR.

Isolate No.	Cluster No.	No. polymorphic variants
E3, E9, E14, E15, E20	A	1
E7, E8	B	2
E17	C	1
E10, E11, E12, E13	D	1
E5, E6, E16, E18	E	1
E2, E4,E19,E1	F	**2**
Total: 20	**6**	**8**

X2=3.75, P.value> 0.05: There is no statistically significant relationship between the sampling distribution and the number of polymorphic variants across clusters. This suggests that the distribution of polymorphic variants may be random among clusters.

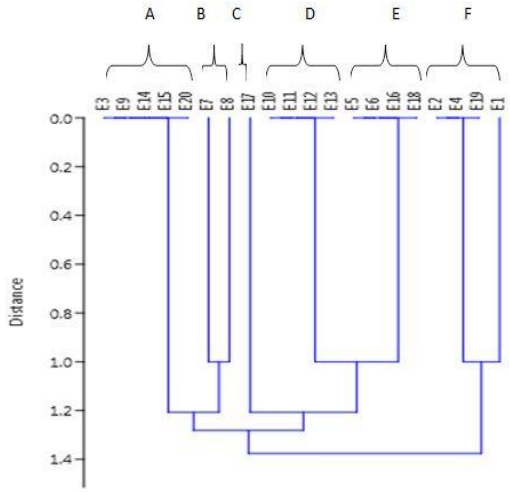

Figure 1: RAPD-PCR dendrogram tree analysis of the ERIC gene in *E. coli* isolates using (Paleontological Statistics version 4.0). The cluster analysis using (algorithm Ward's method) revealed 6 cluster variants among 8 polymorphic variants in 20 *E. coli* clinical isolate from Gut disorder.

This result is consistent with previous studies that used DNA fingerprinting techniques such as ERIC-PCR or RAPD-PCR to assess the genetic diversity of E. coli strains. For example,[15] demonstrated that ERIC-PCR was able to differentiate closely related E. coli isolates based on polymorphic banding patterns, which reflect underlying genomic variation. Similarly, a study by[16] found that ERIC-PCR analysis of E. coli isolates from clinical samples revealed polymorphic clusters, indicating high levels of genetic variation. Furthermore, Tzimtzimis et al.[17] reported that ERIC-PCR could successfully cluster E. coli isolates into distinct genetic profiles, supporting the use of this method for epidemiological surveillance and molecular classification. The identification of approximately eight polymorphic variants in the current study is consistent with the ranges observed in these previous investigations, reinforcing the conclusion that E. coli strains from patients with gastrointestinal disorders often exhibit significant genetic diversity.

CONCLUSION

In this study, males were more likely than females to be affected by gut disorders. The age group most affected by gut disorders and the highest rate of isolation of *E. coli* bacteria was from 21-30 years. *E. coli* bacteria, watery diarrhea appears to be more closely associated with *E. coli* infection than bloody diarrhea, They also had genetic diversity, with six clusters and eight genetic variations. From this, we can conclude that the greater the genetic diversity of E. coli isolates, the greater their ability to cause disease.

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