ORIGINAL ARTICLE

Phylogenetic Group of *Escherichia coli* Isolated from Inflammatory Bowel Disease in Al Najaf province

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ABSTRACT

Background: *Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract that includes Crohn's disease (CD) and ulcerative colitis. Recent studies have highlighted the role of specific bacterial species in the pathogenesis of IBD, including adherent-invasive Escherichia coli (AIEC).* ***Objectives:*** *The study was designed to determine the prevalence of Crohn's disease compared with ulcerative colitis, and determination the predominant bacterial species causing inflammatory bowel disease in Al-Najaf city, Additionally the study focused on molecular detection of the phylogenetic classification of E. coli by PCR.* ***Methodology:*** *The study collected 102 stool specimens were collected from inflammatory bowel disease patients . Escherichia coli isolates were diagnosed identified by culture and biochemical reactions. Vitek-2 compact system provides an automated final identification . the PCR approach was used for detecting the phylogenic groups by targeting three marker genes chuA and yjaA and DNA fragment TSPE4.C2.* ***Result:*** *Eighty eight isolates out of 102 (88%) were presumptively identified as Escherichia coli and 6 (5.88%) isolates identified as Pseudomonas spp and Klebsiella pneumoniae while 8 (7.84%) were negative growth. There were 90 cases diagnosed as Crohn's disease and only 12 cases diagnosed as ulcerative colitis. According to the results of PCR-based phylotyping, Phylogenetic group B2 (81.8%) was the most widely dispersed phylogenetic group of E. coli, followed by Phylogenetic group A (13.6%) and Phylogenetic group D (0.04%).* ***Conclusion:*** *Crohn's disease has a higher prevalence than ulcerative colitis. Phylogenetic group B2 Escherichia coli plays a significant role in inflammatory bowel disease pathogenesis by colonizing the gut and causing inflammation.*

INTRODUCTION

Inflammatory bowel disease (IBD) is a progressive, lifelong disease that is persistent and recurrent inflammation of the digestive tract[1]

Although the exact cause of IBD is unknown, it is thought to be caused by disturbances in the immune system and microbiota resulting from a complex interplay between genetic risk factors and environmental exposures[2].

IBD has historically been classified as ulcerative colitis (UC) which affects the colon and Crohn's disease (CD) which can involve the whole gastrointestinal tract but is most common in the terminal ileum and colon and the most recurrent symptoms including abdominal pain, persistent diarrhea, blood per rectum, and mucus in feces are among the most common symptoms of IBD[3].

Due to the fact that IBD is an inflammatory gastrointestinal disease, luminal variables may play a role and as a result, gut bacteria are commonly thought to be the root of IBD relapses[4].

Numerous microbes have been proposed as having a part in the pathophysiology of IBD including *Escherchia coli*, *Klebsiella* spp, *Pseudomonas* spp, *Mycoplasma* spp, *Mycobacterium* spp, *Salmonella* spp, *Clostridium difficile*, *Listeria monocytogenes* and certain viruses have all been associated with IBD and are thought to be responsible for relapses in the illness[5].

In patients with IBD, *Escherchia coli* has been suggested as a potential cause of illness onset particularly when focused on IBD patients during disease relapses[6], several investigateons have discovered higher numbers of *E. coli* strains with virulence characteristics isolated from IBD patients compared to those from healthy controls[7].

The six well-known intestinal pathogenic *E. coli* species including Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), Enteroaggregative *E. coli*

(EAEC), Enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC)[8].

While the additional intestinal pathotypes are more closely related to the B2 and D groups, commensal *E. coli* strains are more likely to belong to the A and B1 phylogenetic groups. The phylogenetic grouping of D, E, and C strains in relation to antibiotic resistance, however, has not received much attention[9].

The aim of this study detect phylogenetic classification of *Escherichia coli* associated with inflammatory bowel disease (IBD). Adherent-invasive *E. coli* strains were found to be highly associated with ileal mucosa in inflammatory bowel disease especially CD patients[10] .

METHODOLOGY

Collection of Specimens:

The present study was carried out at Al-Najaf City extending from December 2022 to May 2023, 102 clinical specimens were collected the stool sample from patients having Inflammatory Bowel Syndrome IBS (cases) while the remaining specimens from healthy human were considered as control.

These specimens were chosen according to the diagnosis of gastroenterologists doctors which were based on clinical symptoms (e.g., diarrhea, anemia, abdominal pain, blood in stool, bowel abstraction, etc) and endoscopy. These were collected by swabs and transported in sterilized transport medium containers from four hospitals in Najaf and Specialized Hospital for Gastrointestinal and Liver Disease and Surgery.

Diagnosis of isolates:

The collected stool samples were cultured on the MacConkey agar and Eosin Methylene Blue Agar and incubated at 37°C for 18-24 hours. . Identification of bacterial isolates were done by biochemical tests including Oxidase, Catalase, and IMViC tests and finally confirmed by Vitek-2 system to make the final diagnosis of the isolates.

Genomic DNA Extraction:

Using the boiling technique, genomic DNA was successfully isolated from bacterial isolates. The RNA/DNA spectrophotometer (Biodrop) instrument directly evaluated the concentration and purity of extracted DNA. Gel electrophoresis was used to confirm and analyse the extracted DNA. The extracted DNA was used as a template in all PCR reaction .An agarose gel was prepared by mixing 1 gm of agarose powder with 100 ml of TBE buffer that had previously been packed (90 ml D.W. were added to 10 ml TBE buffer l0X, final concentration was 1 X and pH 8). When the mixture was clear, it was placed in a boiling water bath, cooled to 50 C, and ethidium bromide at a concentration of 0.5 mg/ml was added. The agarose was generously poured into a previously balanced gel tray, which had two combs fixed at each end and in the middle, and two sides of the gel tray were sealed. At room temperature, agarose takes 30 min to solidify. gently removed the combs from the tray and sealed it. Wells created by comb were used to load DNA samples. Five microliters of amplified PCR product were added to the agarose gel wells, followed by a DNA marker (ladder) in first wells. The gel tray was fixed in the electrophoresis chamber, and IX TBE buffer was added to the chamber until the gel surface was coated. At 70 volts, electrical current was provided for 1.5-2 hrs. Electrophoresis of an agarose gel with ethidium bromide staining showed amplified PCR products. The gel documentation technique was used to monitor the electrophoresis results. Positive outcomes were distinguished when the sample's DNA band base pairs were equal to the target product size. Finally, the gel was photographed using the Biometra gel documentation method.

Phylogenetic Groups of *E. coli*

Phylogenetic groups of *E. coli* (A, B1, B2, and D) were determined using a triplex PCR according to the combination of three genetic markers *chuA*, *yjaA*, and DNA fragment *TspE4.C2* as shown in table (1).

Table 1: Primers used in this study

Virulence factors	Primers		Sequences	Amplicon size (bp)	Reference
Coli haem-Utilization	***ChuA***	F	'5-GACGAACCAACGGTCAGGAT-3'	279	(Clermont *et al.*, [11])
		R	'5-TGCCGCCAGTACCAAAGACA-3'		
Stress-induced protein	***YjaA***	F	'5-TGAAGTGTCAGGAGACGCTG-3'	211	
		R	'5-ATGGAGAATGCGTTCCTCAAC-3'		
Part of lipase esterase	***TSPE4.C2***	F	'5-GAGTAATGTCGGGGCATTCA-3'	152	
		R	'5-CGCGCCAACAAAGTATTACG-3'		

These primers were used under the following conditions: 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 59°C for 30 s and 72°C for 30 s. A final extension of 72°C for 7 min was performed at the end of PCR. Each and every PCR amplification was performed using a Verity Thermal Cycler (Agilent, UK). Then, 1% agarose gel electrophoresis was used to analyze all of the PCR products, and they were all stained with red ethidium bromide dye. Finally, the gel documentation system was used to identify the electrophoresis results.

RESULTS

One hundred and two patients were examined from three hospitals (Al- Hakim General Hospital ,Al-Zahra Teaching Hospital, Al-Furat Middle Teaching Hospital and from Private Outpatient Clinics in Al-Najaf city. Patients were divided to two groups (12 were with ulcerative colitis and 90 were with Crohn's diseases). As shown in table 2

Table 2: Prevalence of infected patients with Inflammatory bowel disease according to Crohn's disease and Ulcerative colitis

Inflammatory Bowel Disease	Crohn's disease	Ulcerative colitis
Male	**34 (37.7%)**	**8 (66.6%)**
Female	**56 (62%)**	**4 (33 .3%)**
Total Number	**90 (100%)**	**12 (100%)**

Twenty six apparently normal healthy controls were enrolled for the study. Patients with Crohn's disease were males represent only 37.7% while females represent 62 % while patients with ulcerative colitis were males 66.6%) and females 33 .3% (Table 2).

Isolates were patients specimens 88 (93.9%) were *Escherichia coli* and 6 (6.8%) specimens were *Pseudomonas* spp and *Klebsiella* spp while only 8 (9%) non growth as shown in Figure 1.

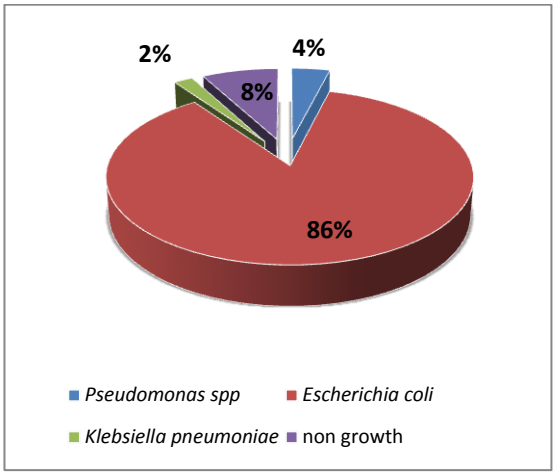

Fig 1: Bacterial isolates of Inflammatory Bowel Disease patients

The triplex PCR described by Clermont *et al* [11] was used for phylogenetic grouping analysis of *Escherichia coli* isolated from inflammatory bowel disease patients. The method enabled the detection of the four main phylogenetic groups of *E. coli*, namely (A, B1, B2, and D) targeting three marker genes *chuA* and *yjaA* and DNA fragment *TSPE4.C2*.as in shown Figure 2.

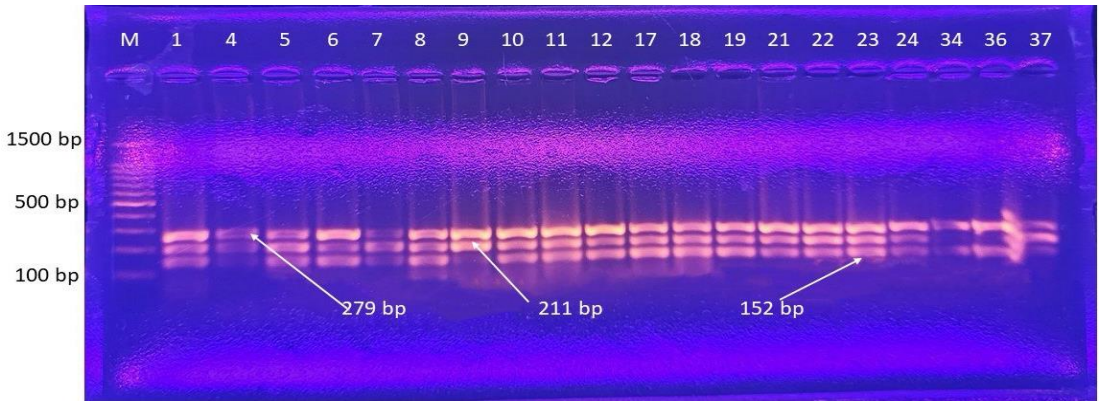

Fig. 2: Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *Escherichia coli* using primer *chu* with product 279bp and *yjaA* with product 211bp and *TSPE4.C2* with product 152bp.

The result of PCR-based phylotyping showed that the most distributed phylogenetic group of *E. coli* was "Phylogenetic group B2" which comprised 72/88 (81.8%), followed by "Phylogenetic group A" strains 12/88 (13.6%) followed by "Phylogenetic group D" that comprised 4/88 (4.5%). Strains of the "Phylogenetic group B1" were rare and were not found in their isolates as seen in Table 3.

Table 3: Percentage for *Escherichia coli* Isolates according to phylogenetic classification

Phylogenetic groups No.(%)		Genes	No. (%)
Intestinal Groups	Group A	*chuA - / yjaA +/- / TspE4.C2 –*	12(13.6%)
	Group B1	*chuA - / yjaA - /+ / TspE4.C2 +*	0(0%)
Extraintestinal Groups	Group B2	*chuA + / yjaA + / TspE4.C2 +/-*	72(81.8%)
	Group D	*chuA + / yjaA - / TspE4.C2 +*	4(4.5%)

DISCUSSION

Crohn's disease is more common in women and is associated with other autoimmune diseases/ manifestations (irises, irido-cyclitis, autoimmune thyroiditis, etc.) this is agreement with Silaghi et al [20,22].

Psychological symptoms, sleep quality, and quality of life impact CD patients differently based on sex, with females experiencing higher rates of anxiety, depression, and poorer quality of life, indicating a need for more psychological support for women with CD [13].

Overall, while there may be some variations in disease presentation and severity based on gender and smoking habits, the prevalence of Crohn's disease itself does not significantly differ between men and women [14].

E. coli represent 88 isolates this was related to the profiles of virulence factors and pathogenicity mechanisms exhibited by enteric *E. coli* pathotypes which were highly diverse and through the adaptation of critical genetic elements, the evolution of enteric *E. coli* pathotypes has led to the formation of new pathotypes that were capable of secreting toxins, aggregative colonization, multiplying in the gastrointestinal tract, and damaging various environments[15].

Other types of bacteria including *Pseudomonas aeroginosa* and *Klebsiella pneumoniae* so that intestinal microbiota and microbiome are thought to be the primary causes of a number of Gastrointestinal disorders[16].

Dysbiosis or a loss in the diversity of the intestine microbiome, has been linked to the participation of many bacterial species while the remaining were from the total number there no microbial growth [17].

Although a number of factors, including environmental and host factors like psychosocial stressors, food intolerance, antibiotics, enteric infections, altered pain perception, altered brain-gut interactions, dysbiosis (imbalance within the bacterial community), increased intestinal permeability, increased gut mucosal immune activation, and visceral hypersensitivity, are thought to be involved in the pathogenesis of IBS, though this is still unclear [18].

There is a significant association between *Escherichia coli* strains belonging to phylogroup B2 and inflammatory bowel disease (IBD), particularly Crohn's disease (CD) these Phylogenetic group B2 *Escherichia coli* plays a significant role in inflammatory bowel disease (IBD) pathogenesis by colonizing the gut and causing inflammation . Studies have shown that *E. coli* isolates from IBD patients predominantly belong to phylogroup B2, which is associated with both ulcerative colitis (UC) and Crohn's disease (CD) [10].

Murugaiyan *et al*[19] conducted that these B2 strains exhibit high levels of antimicrobial resistance, including multidrug resistance, making them challenging to treat effectively.

Furthermore, B2 strains possess specific virulence factors that enable them to reside within the human intestinal mucosa, contributing to the inflammatory process in IBD patients [20]. The presence of B2 *E. coli* strains, such as diffusely adherent *E. coli* (DAEC), has been linked to UC, while adherent invasive *E. coli* (AIEC) is associated with CD, indicating their pathogenic role in IBD flares[21]. B2 strains exhibit distinct metabolic capabilities, allowing them to efficiently utilize sugars derived from mucus glycan and potentially colonize the intestinal mucosa more effectively than other strains [22].

These findings suggest that B2 *E. coli* strains may play a role in the pathogenesis of IBD, particularly CD, by adapting to the inflammatory environment and potentially benefiting from intestinal inflammation rather than causing it[4].

Dubinsky *et al* [23] identified that B2 strains were prevalent lineage in patients with ulcerative colitis (UC) and CD by encode genotoxic molecules like colibactin .

B2 strains were closely related to adherent-invasive *E. coli* (AIEC) pathovars, which play a facilitative role in IBD flares by triggering immune cell activation and contributing to the inflammatory process in the gut tissues of IBD patients [24].

The identification of pathotypes like diffusely adherent *E. coli* (DAEC) in IBD cases highlighted the potential pathogenic role of specific *E. coli* strains in the development and progression of IBD[25,26], then stop the progression of the condition as an early disease management and it is essential to use adequate therapy in high-risk patients, then closely monitor and modify treatment in accordance with the predetermined therapeutic objectives in an effort to prevent long-term gut injury and eventual disability.

CONCLUSION

The higher prevalence of Crohn's disease (CD) compared to ulcerative colitis (UC) can be attributed to several factors, including genetic predisposition, immunological responses, and disease characteristics. B2-adherent invasive Escherichia coli (AIEC) exhibit increased virulence and pathogenicity due to several interconnected mechanisms. AIEC strains, particularly those of phylogenetic group B2, demonstrate distinct transcriptional profiles that enhance their ability to adhere to and invade intestinal epithelial cells, as well as survive within macrophages, contributing to chronic inflammation associated with conditions like Crohn's disease.

Assignment

All the participants provided informed consent for inclusion in the study and were assured that all the informations provided would be used solely for the purposes of this study and treated confidentially.

Ethical Approval Declaration

The procedures followed in this study were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki). In addition, each participant provided written consent following a concise overview of the project.

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Declarations:

Consent for publication: Not applicable

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