Original Article

The Prevalence of Microcin B17 and Colibactin Genes in *Escherichia coli* Isolated from Patients with Inflammatory Bowel Disease

Huda Najeh AL-Baroody, Mohanad Mohsin Ahmed[1], Kawkab A.H. Alsaadi

Department of Biology, College of Science, University of Karbala, [1]College of Medicine, University of Karbala, Karbala, Iraq

Abstract

Background: *Escherichia coli* (*E. coli*) is the most prevalent bacterial species in inflammatory bowel disease (IBD) patients stool, these isolates have been shown to differ from *E. coli* isolated from healthy individuals by owning many virulence factors including the production of toxins such as microcin B17 and coliba. **Objective:** The purpose of our study to demonstrate the prevalence of microcin B17 and colibactin genes in *E. coli* isolated from IBD patients stool in Karbala governorate hospitals. **Materials and Methods:** Sixty stool samples were collected from patients with IBD (30 from Crohn's patients and 30 from ulcerative colitis) aged from 5 to 70 years old, of both sexes, who visited the Center of Digestive Tract and Liver Diseases in Karbala from August 2021 to September 2022. All patients were in active IBD, which is confirmed by testing fecal calprotectin production. Besides, 30 stool samples from healthy individuals were collected as control. *E. coli* was isolated from all samples and analyzed to detect the presence of toxin microcin B17 gene (*McbA*) and colibactin gene (*clbB*) by polymerase chain reaction using amplification of these genes. **Results:** The result is high prevalence of microcin B17 gene in *E. coli* isolates in both Crohn's (27 [90%]) and ulcerative colitis (25 [83%]) isolates, compared with fecal commensal *E. coli* from control (5 [16.6%]) isolates only. While the prevalence of colibactin gene less than the micricinB17 gene with 10 isolates only from all sixty patients of IBD. Fecal calprotectin was between 50 and 200 µg/mg for most patients, which mean they had active gastrointestinal inflammation, whereas in 14 of them, their results were more than 200 µg/mg, which mean they were in acute inflammation condition. **Conclusion:** microcinB17-producing *E. coli* may have a greater role in the pathogenesis of inflammatory bowel disease due to high prevalence of its gene in IBD patients.

Keywords: Calprotectin, colibactin, *E. coli*, IBD, microcin B17

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are examples of inflammatory bowel diseases (IBDs) that relapse and remit, which are becoming more common and prevalent around the world. They are linked to significant mortality, decreased quality of life for those who suffer, and an increasing societal burden in both direct and indirect costs.[1] IBD is becoming more common around the world. IBD's pathophysiology is unknown and has yet to be discovered.[2] Interactions between genetic, environmental, immunological, and microbial factors cause IBD.[2] Many bacteriological studies in IBD patient's fecal microbiota have demonstrated that a diverse range of bacterial species, including *Escherichia coli*, is more common in the stool of these patients[3] The species *E. coli*, in addition to being an important member of the normal intestinal microflora of humans and other mammals, possesses many virulence factors that lead to a variety of diseases. *E. coli* virulence factors can influence a variety of eukaryotic cellular functions, such as cell signaling, ion secretion, protein synthesis, mitosis, cytoskeletal function, and mitochondrial function.[4] In the gut microbiota, these *E. coli* strains have become more prevalent over the past

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_251_23

Address for correspondence: Dr. Huda Najeh AL-Baroody, Department of Clinical Laboratories, College of Applied Medical Sciences, University of Karbala, Karbala, Iraq. E-mail: albaroodihn@gmail.com

Submission: 03-Mar-2023 **Accepted:** 01-May-2023 **Published:** 23-Jan-2026

How to cite this article: AL-Baroody HN, Ahmed MM, Alsaadi KAH. The prevalence of microcin B17 and colibactin genes in *Escherichia coli* isolated from patients with inflammatory bowel disease. Med J Babylon 2025;22:990-4.

30 years, incorporating the control of fitness/virulence variables into networks that react to certain environmental signals, such as siderophores, colibactin, and microcins.[5]

Microcins are bacterial-inhibitory molecules with a low molecular weight that are produced by ribosomes. They participate in competitive and mutualistic interactions with other Enterobacteriaceae in the intestine.[6] Bacteriocin determinants found frequently in *E. coli* isolates from IBD patients could indicate that IBD *E. coli* strains are more virulent. Furthermore, promoters of the SOS response and iron-dependent ferric uptake regulator tightly regulate bacteriocin expression.[7]

Microcin B17 (43 amino acid) inhibitor DNA gyrase was discovered on a seven-gene operon encoding a 69-amino acid precursor to microcin, *McbA*.[8] Microcin B17 is a class I microcin that has been posttranslationally modified, which causes cell death by destabilization of gyrase-dependent DNA cleavage.[9]

Microcin B17 was the initial compound derived from thiazole/oxazole-modified microcins and linear azole-containing peptides. These ribosomal peptides are posttranslationally modified to form oxazole and thiazole rings from serine and cysteine residues.[10] Microbial oxazoles activate aryl hydrocarbon receptors, which cause intestinal inflammation.[11]

Colibactin, another secondary metabolite encoded by the pks gene island, is identified in many Enterobacteriaceae, including the pathogenic *E. coli*, and is typically found in the mucosa of patients with IBD and colorectal cancer (CRC). In cell lines and preclinical models, *E. coli* containing this biosynthetic gene results in DNA damage and tumor progression.[12] CD and UC patients have a higher lifetime risk of developing CRC. Lakatos and Lakatos[13] suggest that colibactin-producing *E. coli* may have a greater role in the pathogenesis of colitis-associated CRC than sporadic disease in non-IBD patients because of higher levels of colibactin exposure.

Materials and Methods

Sample collection and *Escherichia coli* isolation

Sixty stool samples were obtained from patients with IBD aged 5–70 years old, of both sexes, from August 2021 to September 2022. Furthermore, information was obtained from them via a questionnaire paper, which included questions about their gender, age symptoms, and the effect of certain diet. In addition, 30 stool samples were taken from healthy individuals of various ages and sexes as controls. Fecal samples were delivered to the laboratory in sterile containers.

The specimens were first prepared by picking a small amount of stool (1 g) with a stick and diluting it with 19 mL of normal slain, then streaking the diluted stool on MacConkey agar and incubating it for 24 h at 37°C. Lactose fermenting colonies were selected and recultured on eosin-methylene blue (EMB) to look for green metallic sheen colonies. Depending on Lupindu,[14] additional molecular identification by polymerase chain reaction (PCR) method was used to confirm the identity by using the amplification of *16s rRNA* gene [Table 1].

Calprotectin detection

All patient samples were evaluated for calprotectin production using the Calprotectin (50 + 200 kit) (CerTest BIOTEC, Spain), which is divided into two parts:

1. Prepared and diluted the sample in a stool sample collection tube.
2. Calprotectin comb card test, which includes two strips (50 and 200).

Test result has been read later than 10min after adding the diluted stool sample to the strips, if calprotectin concentration was <50 μg/mg, which mean neither active gastrointestinal inflammation nor risk of relapse (CD or UC) if the result was between 50 and 200 μg/mg, which might mean active gastrointestinal inflammation and if the result was more than 200 μg/mg that indicate they had a cute inflammation.

DNA extraction

DNA was extracted from bacterial isolates by using extraction kit (Favorgen Biotech Corporation, Taiwan, China).

Polymerase chain reaction techniques

Series of PCR reactions were conducted to detect the genes of microcin B17 and colibactin by using the primers in Table 1, these primers were provided by Scientific Researcher Co, Iraq.

Table 1: Primers used in the study

Gene	Primer sequence (5′-3′)	Amplicon size (bp)	Annealing temperature °C	Ref.
16S-rRNA	F-CATGCCGCGTGTATGAAG AA	100	60	[15]
	R–CGGGTAACGTCAATGAGCAAA			
McbA	F-TCCGCCTGCGGAATTAATGA	203	55	This study
	R-TACTGATTGCCACCGTCCTG			
clbB	F-GCGCATCCTCAA GAGTAAATA	280	60	[15]
	R-GCGCTCTATGCTCATCAACC			

Table 2: Levels of calprotectin in Crohn's disease and ulcerative colitis

Type of disease	Concentrations of FC tests	
	>200 (μg/mg)	50-200 (μg/mg)
CD	7 (23.3)%	23 (76.7)%
UC	5 (16.7)%	25 (83.3)%
Total	12 (20)%	48 (80)%
P-value = 0.374		

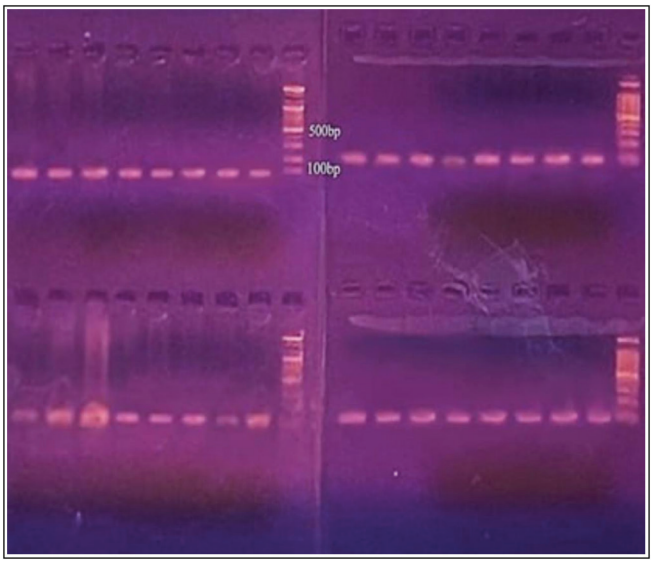

Figure 1: Electrophoresis of 1% agarose gel stained with ethidium bromide of *16S rRNA* amplified gene product (size 100 bp) using DNA template of *E. coli* isolates at 70 V for 1–2 h. DNA ladder (from 100 to 1200 bp), all lanes show positive with single band 100 bp

Ethical approval

The research was carried out in accordance with the ethical principles outlined in the Helsinki Declaration. A local Ethics Committee reviewed and approved the study protocol, according to the document with the number 3727 on the date September 28, 2021.

Results

Sample collection and *Esherichia coli* isolation

The identification of *E. coli* isolates was performed by examining bacterial culture, microscopically characteristic, and PCR technique. The suspected colonies on MacConkey agar were pink in color (Lactose fermenter), the colonies had metallic sheen appearance on EMB agar. Microscopic examination of Gram stain isolates revealed Gram negative small bacilli.

Calprotectin detection

Fecal calprotectin results in Table 2 were between 50 and 200 μg/mg for most patients, whereas 14 patients results were more than 200 μg/mg.

Polymerase chain reaction techniques

All *E. coli* isolates were confirmed by PCR technique by the amplification of *16S rRNA* gene with product 100bp [Figure 1].

On the other hand, the amplification of the toxin microcin B17 (*McbA*) gene for all *E. coli* isolates shows high prevalence in 27 (90%) isolates from Crohn's patients and 25 (83%) isolates from UC compared with fecal commensal *E. coli* in control (5 [16.6%]) only [Figure 2].

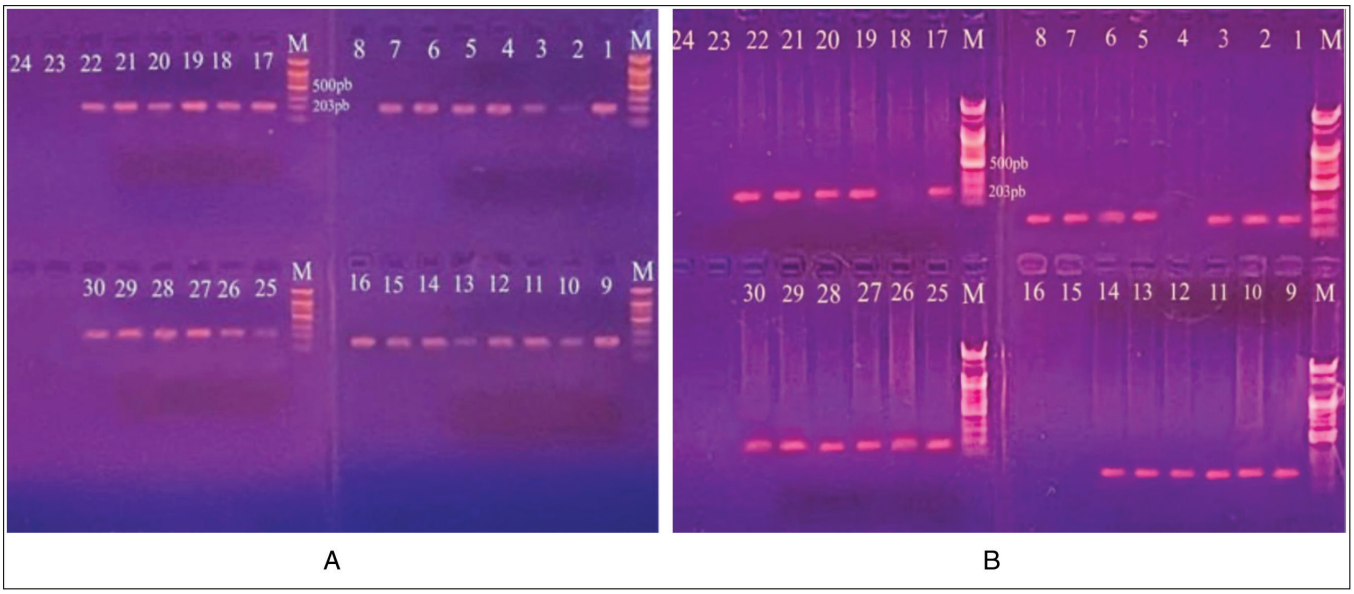

Figure 2: Electrophoresis of 1% agarose gel stained with ethidium bromide of *McbA* amplified gene product (size 203 bp) using DNA template of *E. coli* isolates, 70 V for 1–2 h, DNA ladder (from 1200 to100 bp). (A) isolates from Crohn's disease and (B) isolates from ulcerative colitis

Table 3: The toxins genes distributions in both CD and UC isolates

Toxins	Crohn's patients sample	Ulcerative colitis sample	Control sample
	n = 30	*n* = 30	*n* = 30
Microcin B17 genes	27 (90%)	25 (83.3%)	5 (16.6%)
Colibactin genes	4 (13.3%)	6 (20%)	2 (6.6%)

The results of amplification of colibactin in all *E. coli* isolates were demonstrated in Table 3.

Discussion

A comparative genomic analysis of IBD *E. coli* isolates revealed that these isolates represented a heterogeneous population that was more similar to extraintestinal pathogenic *E. coli* than to the classic diarrheagenic pathotypes.[16] We found in our study high prevalence of microcin B17 gene in *E. coli* isolates from patients with IBD compared with the control; these findings correspond with Micenková *et al.*'s[17] study when he found that the frequency of bacteriocinogenic isolates was significantly higher in IBD *E. coli* (66.9%) compared with fecal commensal *E. coli* isolates (54.2%), including a higher prevalence of the colicin B determinant.[17] Microcin genes found in high prevalence in extracellular *E. coli* with compared with diarrhea-associated *E. coli* and nonpathogenic *E. coli*.[16] Iyer *et al.*[11] have been defined the oxazole class of aromatic organic compounds as a new source of both environmental and microbial gastrointestinal inflammation inducers in 2018 that cause CD1d-dependent intestinal inflammation and are distinguished by the occurrence of a five-membered oxazole ring, which modulates natural killer T cell-dependent inflammation.[11] Microcin B17 was the first compound found in the family of thiazole/oxazole modifications microcins and linear azole-containing peptides; these ribosomal peptides are posttranslationally altered to form oxazole and thiazole rings from serine and cysteine residues.[10]

The effect of microcin B17 not limited to the intestine cell only but can enhance the harmful effect of other pathogenic bacteria such as *E. coli* O157:H7, which produces virulence factors such as the locus of enterocyte effacement, and Shiga toxins (Stx), which causes hemolytic uremic syndrome and hemolytic colitis (Stx). The activation of the bacterial SOS response, which is caused by microcin B17, is linked to the induction of the prophage and subsequent upregulation of Stx toxin.[18]

The prevalence of colibactin genes in *E. coli* isolate in this study were less than many studies, the reason for these results is attributed to two important factors, the important one is the type of specimens, in this study, patient's stool was used, whereas in a study by Arthur *et al*.,[19] intestinal mucosal biopsy samples were collected, and he has demonstrated a greater incidence of colibactin-encoding *E. coli* in IBD patients (14/35 vs. 5/24 in healthy controls). The second one is the period of infection, the longer one, the greater exposure to bacteria producing this toxin. Accumulating evidence indicates that *E. coli* producing colibactin may be affected by the inflammation environment, which is inducing the activity of genes toxin by increasing epithelial oxygenation, decreasing mucosal barrier integrity, or promoting bacterial biofilm formation.[20] Pathogenic *E. coli*, which harboring colibactin gene, is frequently abundant in mucosal tissue taken from patients with CRC and IBD.[12] Furthermore, patients with IBD, such as CD and UC, have a higher lifetime risk of developing CRC. Due to higher levels of colibactin exposure, and years of chronic inflammation, cancer onset is up to 15–20 years earlier than in sporadic cases. These CRC incidence rates are consistent with cumulative probabilities of 2% after 10 years, 8% after 20 years, and 18% after 30 years in IBD patients[21] Inflammation may promote *E. coli* carcinogenic activity (i.e., colibactin-induced DNA damage in intestinal cells) by promoting producing colibactin *E. coli* proliferation, improving their adhesion to the mucosa, or/and boosting the expression of colibactin genes.[20]

Conclusion

E. coli-producing toxins were found to be the most abundant bacteria in the stool of patients with IBD with a high prevalence of microcin B17 and low prevalence of colibactin, their role in the development of inflammation is not known yet and needs to be investigated.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Wark G, Samocha-Bonet D, Ghaly S, Danta M. The role of diet in the pathogenesis and management of inflammatory bowel disease: A review. Nutrients 2020;13:135.
2. Mohammed BI, Amin BK. Sociodemographic characteristics, smoking, and family history of patients with inflammatory bowel disease, northern part of Iraq. Med J Babylon 2022;19:615-9.
3. Pascal V, Pozuelo M, Borruel N, Casellas F, Campos D, Santiago A, *et al*. A microbial signature for Crohn's disease. Gut 2017;66:813-22.
4. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. Nat Rev Microbiol 2004;2:123-40.
5. Martin P, Marcq I, Magistro G, Penary M, Garcie C, Payros D, *et al*. Interplay between siderophores and colibactin genotoxin biosynthetic pathways in Escherichia coli. PLoS Pathog 2013;9:e1003437.
6. Baquero F, Lanza VF, Baquero M-R, Del Campo R, Bravo-Vázquez DA. Microcins in Enterobacteriaceae: Peptide antimicrobials in the eco-active intestinal chemosphere. Front Microbiol 2019;10:2261.
7. Gillor O, Vriezen Jan AC, Riley MA. The role of SOS boxes in enteric bacteriocin regulation. Microbiology (Reading) 2008;154:1783.

8. Davagnino J, Herrero M, Furlong D, Moreno F, Kolter R. The DNA replication inhibitor microcin B17 is a forty-three-amino-acid protein containing sixty percent glycine. Proteins Struct Funct Bioinf 1986;1:230-8.
9. Heddle JG, Blance SJ, Zamble DB, Hollfelder F, Miller DA, Wentzell LM, *et al*. The antibiotic microcin B17 is a DNA gyrase poison: Characterisation of the mode of inhibition. J Mol Biol 2001;307:1223-34.
10. Collin F, Maxwell A. The microbial toxin microcin B17: Prospects for the development of new antibacterial agents. J Mol Biol 2019;431:3400-26.
11. Iyer SS, Gensollen T, Gandhi A, Oh SF, Neves JF, Collin F, *et al*. Dietary and microbial oxazoles induce intestinal inflammation by modulating aryl hydrocarbon receptor responses. Cell 2018;173:1123-34.e11.
12. Dougherty MW, Jobin C. Shining a light on colibactin biology. Toxins 2021;13:346.
13. Lakatos PL, Lakatos L. Risk for colorectal cancer in ulcerative colitis: Changes, causes and management strategies. World J Gastroenterol 2008;14:3937.
14. Lupindu AM. Isolation and characterization of Escherichia coli from animals, humans, and environment. In: Escherichia Coli-Recent Advances on Physiology, Pathogenesis and Biotechnological Applications. London, UK: IntechOpen Limited 2017:187-206.
15. Lyadorai T, Mariappan V, Vellasamy KM, Wanyiri JW, Roslani AC, Lee GK, *et al*. Prevalence and association of pks+ Escherichia coli with colorectal cancer in patients at the University Malaya Medical Centre, Malaysia. PLoS One 2020;15:e0228217.
16. Micenkova L, Štaudová B, Bosák J, Mikalová L, Littnerová S, Vrba M, *et al*. Bacteriocin-encoding genes and ExPEC virulence determinants are associated in human fecal Escherichia coli strains. BMC Microbiol 2014;14:1-9.
17. Micenková L, Frankovičová L, Jaborníková I, Bosák J, Dítě P, Šmarda J, *et al*. *Escherichia coli* isolates from patients with inflammatory bowel disease: ExPEC virulence- and colicin-determinants are more frequent compared to healthy controls. Int J Med Microbiol 2018;308:498-504.
18. Mosso HM, Xiaoli L, Banerjee K, Hoffmann M, Yao K, Dudley EG. A putative microcin amplifies Shiga toxin 2a production of Escherichia coli O157: H7. J Bacteriol 2019;202:e00353-19.
19. Arthur JC, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan T-J, *et al*. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 2012;338:120-3.
20. Tang-Fichaux M, Branchu P, Nougayrède J-P, Oswald E. Tackling the threat of cancer due to pathobionts producing colibactin: Is mesalamine the magic bullet? Toxins 2021;13:897.
21. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: A meta-analysis. Gut 2001;48:526-35.