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Exploring the Association of Campylobacter jejuni with inflammatory bowel diseases: insight from a clinical study

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ABSTRACT

Aim: To demonstrate a possible association between Campylobacter jejuni and inflammatory bowel disease Iraqi patients.

Materials and methods: The study involved two groups. The inflammatory bowel disease patient group consisted of 90 participants. This group was further classified into two subgroups: Crohn's disease (n) and Ulcerative colitis (n). The control group consisted of 90 healthy participants who had no history of inflammatory bowel disease. Age and gender distributions in inflammatory bowel disease patient and control groups were computed. The association between inflammatory bowel disease and C. jejuni was determined by identifying the bacterial infection using various detection methods, including culturing, biochemical tests and real-time PCR (Polymerase chain reaction). The association was further assessed by comparing the symptoms of IBD patients and C. jejuni positive samples.

Results: Finding of the conventional identification showed 5% prevalence of *C. jejuni* among inflammatory bowel disease patients whereas the real-time Polymerase chain reaction. Results showed significantly higher prevalence. Based on real-time Polymerase chain reaction. Results, a significant association was found between *C. jejuni* infection with inflammatory bowel disease and Crohn's disease patients (P-value=<0.001).

Conclusions: No significant differences observed in detection of inflammatory bowel disease and Crohn's disease using conventional and molecular methods. The study provides valuable insights into a possible association between *C. jejuni* and inflammatory bowel disease.

KEY WORDS: *C. jejuni,* inflammatory bowel disease, culturing, biochemical test

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ABBREVIATIONS

IBD - Inflammatory Bowel Disease

CD - Crohn's Disease

UC - Ulcerative Colitis

PCR - Polymerase Chain Reaction

INTRODUCTION

Inflammatory Bowel Disease (IBD) is a recurring inflammatory disorder that affects the gastrointestinal system. It comprises two main types: Crohn's disease (CD) and ulcerative colitis (UC). IBD is most prevalent in North America and Western Europe, with a rate of 0.3%. However, in recent year, there has been an increase in its occurrence in other regions globally, making it a significant healthcare concern worldwide [1], while IBD can affect individuals of all age groups, it tends to be more commonly diagnosed in late adolescents and young adults [2]. This particular age bracket seems to be more susceptible to developing IBD, although it can still occur at any age. Furthermore,

there is a notable gender-based variation in the distribution of IBD within this demographic. Studies have shown that IBD can affect both males and females, but the prevalence and disease patterns may differ between the genders. For instance, males tend to have a higher incidence of Crohn's disease, while females have a higher incidence of ulcerative colitis. The reasons for these gender-based differences are not yet fully understood and require further research [2-6]. Though the specific cause of IBD is still unknown, it is believed to be a consequence of a patient's genetics, microbiome, immunological response, and environment, which generate an excessive and inappropriate immune reaction to commensal flora in genetically predisposed individuals [7-9]. Previous studies have indicated a connection between IBD and microbes in patients with IBD [1, 7]. C. jejuni is one of the reported causes of gastroenteritis in humans [7]. The disruptive nature of C. jejuni infections on normal gut homeostasis have been unveiled, which, in turn, is believed to contribute to the development of IBD [8]. Despite efforts to implement treatment measures, the prevalence of C. jejuni infections continues to escalate globally. In Iraq, as in numerous other nations, IBD constitutes a prevailing health concern, posing significant challenges to human well-being [9-10], however, despite its substantial impact, the research landscape concerning the relationship between IBD and the bacterium C. jejuni remains notably underexplored. Addressing this research gap in the etiology of IBD is imperative not only for expanding our scientific understanding of its etiology in a specific region but also for potentially developing improved diagnostic and therapeutic strategies that are specifically tailored to the unique healthcare landscape of the country in question. The identification and confirmation of C. jejuni in clinical samples involve a multifaceted strategy encompassing several techniques. The process usually initiates with the isolation and culture of the bacteria on selective media. Subsequently, a combination of biochemical and molecular tests is used to confirm the existence of the pathogen [11]. The clinical presentation of IBD is known to appear differently among affected person, with symptoms ranging from abdominal pain and diarrhea to more serious indication, such as weight loss and rectal bleeding [12]. Contrasting the symptom profiles of IBD patients to those individuals carrying positive C. jejuni samples can contributes to a more nuanced understanding of the potential effects of bacterial presence on symptomatology. This clinical study aimed to provide insights in to the possible association of C. jejuni with IBD through the detection of the bacteria in stool samples of local patients with IBD diseases. Strain typing of Campylobacter conscious has previously been done using a variety of typing techniques, including multi-locus sequence typing (MLST) and amplified fragment length polymorphisms (AFLP) 23 S rRNA PCR [13]. Multilocus sequence typing (MLST) is a commonly used typing technique that may identify population patterns and lineages in microorganisms [14]. Research has demonstrated that MLST has a good discriminatory power for Campylobacter jejuni, Campylobacter coli, and developing Campylobacter species. The ease with which sequence data can be shared across laboratories for use in international epidemiological research is one of the benefits of MLST [15]. This includes the identification of the bacteria within the studied groups through conventional and real time PCR techniques, also examined the clinical symptoms of IBD patients in comparison to individuals who had positive C. jejuni samples.

MATERIALS AND METHODS

SAMPLES PREPARATION

The study included a total of 180 participants, 90 diagnosed with IBD by specialist in gastroenterology comprising the patient group. The remaining 90 participants were apparently healthy individuals and formed control group. The samples for the study had been collected during a period ranging from December 2022 to the end of March 2023, from Al-Imamain

Al-Kadhimain Medical City. The study was conducted in the laboratories of Microbiology Department in the College of Medicine-Al-Nahrain University.

INCLUSION CRITERIA

Inclusion criteria were taking samples from patients with IBD over than 18 years old, while exclusion criteria were avoiding patients have or had previously any type of malignancy, or have other disease such as muscular dystrophy (MD), thyroid disorder, congenital adrenal disease, and renal disorder, any congenital or inherited metabolic disorder. Also, pregnant or lactating women and patient on antibacterial drugs with the last three days were avoided. Small quantities of diarrheal stool samples were collected from all participants by plastic spoon and transferred to pre-labeled clean dry plastic container with locked cup under sterile condition. Samples were then transported to the laboratory in cool box within three hours of collection [16].

ETHICAL APPROVAL

This study was approved by the Institutional Review Board of the College of Medicine; Al-Nahrain University under the number 239 and date 30/7/2019.

BACTERIAL IDENTIFICATION

CONVENTIONAL METHOD

Totally, 5 g of freshly collected faucal sample was pre-enriched by suspending the faeces in 45 ml buffered nutrient broth and incubating the suspension at 42°C for 18 hours in a 50-ml closed culture tube were immediately inoculated onto the chromogenic medium CASA (AES Chemunex, France) proved highly efficient for C. jejuni and incubated to 48 hours at 42°C under conditions that were microaerophilic. Gram staining, microscopy examination, and Oxidase, catalase, and indoxyl hydrolysis biochemical tests were used to confirm the identity of the suspected colony. The same colonies were tested for cytochrome oxidase enzyme production by placing a portion of the test colony onto oxidase paper impregnated with NNN'N' tetramethyl-p-phenylene-diamine dihydrochloride (Oxoid, Basingstoke, UK). Purple color change was recorded as positive reaction. Reactive colonies were processed for DNA and a portion stored in skimmed milk at -80°C for further characterization [17]. Following this, a series of biochemical assessments were performed, encompassing glucose fermentation, nitrate reduction, oxidase and catalase tests, as well as hippurate hydrolysis tests [18-20].

DNA PREPARATION

Strains isolated from our hospital were cultured in brainheart infusion (BHI) broth medium and shaken in an oscil-

Table 1. Primer sequence and PCR product

Gene	Primer	Amplified DNA fragment
CAMP-JEJUNI	F: 5'-TAAAAGCGGTGGATTTGGAC-3> R: 5'-CTTTTTCCTTTTTGCCACCA-3'	167 bp

Source: compiled by the authors of this study

Table 2. PCR reaction solution for detection of CAMP-JEJUNI

Component	Volume [μL]
GoTaq® qPCR Master Mix	10
Forward Primer	0.5
Reverse Primer	0.5
Supplemental CXR Reference Dye	0.2
DNA template	4
Nuclease-Free Water	4.8
Total volume	20

Source: compiled by the authors of this study

Table 3. Real-time PCR protocol

No.	Steps	Temperature	Time	Cycle No.
1	Initial denaturation	94°C	6 min	1
2	Denaturation	94°C	40 sec	
3	Annealing	56 °C	45 sec	35 cycles
4	Extension	72 °C	1 min	_
5	Final extra extension	72 °C	10 min	1

Source: compiled by the authors of this study

Table 4. Account of IBD patient group and control group based on sex and age

	Dama a manhia D		Study	Study groups			
	Demographic D	ata	IBD Patient	Control	- P value		
Λαο.	(Mean :	±SD) years	34.21±10.16	33.47±11.06	- 0.779		
Age -	(Mir	า-Max)	(19-57)	(18-70)	- 0.778		
	Female –	n	36	42	_		
Say	remale	[%]	45.0	52.5	- 0.215*		
sex	Sex Male —	n	44	38	- 0.215"		
		[%]	55.0	47.5			

^{*}P value less than 0.05 was considered as statistically significant

Source: compiled by the authors of this study

Table 5. Growth of *C. Jejuni* in Skirrow's medium

lator at full temperature of 37°C for 20–24 h. DNA was then extracted using a bacterial genomic DNA extraction kit to prepare for subsequent experiments. DNA extraction .The bacterial genomic DNA extraction kit used in this paper was purchased from Alpha DNA Ltd. (Canada).

REAL TIME PCR METHOD

Extraction of DNA from the entirety of stool samples was executed meticulously, adhering to the comprehensive guidelines provided by the Geneald Presto Stool DNA Ex-

traction Kit. Using a LAMBDA Bio PLUS, the concentrations and purities of the extracted DNA were calculated from the absorbance at 260 nm (A260) and the A260/A280 ratio. Spectrophotometer (Perkin-Elmer, Norwalk, CT, USA), and sterile deionized water was used to dilute the DNA to the proper quantities. Assessment of DNA concentration and subsequent purification steps were meticulously conducted through employment of the Nano-drop system. Designing of primers was performed using the robust Primer 3 plus software. These primers, specifically designed for the V4 region, underwent a meticulous validation process, inclusive

Bacteria culture	n	[%]
No growth	76	95.0
Growth	4	5.0
Total	80	100

Source: compiled by the authors of this study

Table 6. Occurrence of *C. jejuni* in IBD patients according to disease type

		IB	IBD			
C. jejuni		Crohn's disease	Ulcerative colitis	P value		
		[%]	[%]			
Do atovio aultuvo	No growth		92.9	0.347*		
Bacteria culture	Growth	2.6	7.1			

^{*}P value less than 0.05 was considered as statistically significant Source: compiled by the authors of this study

Table 7. PCR detection of *C. jejuni* in studied groups

D ₀	-4	Study	Groups	Duralina
Da	cteria -	IBD	Controls	P value
	Median	796.68	370.34	
C. jejuni [copy/m]	Percentile 05	193.22	32.5	<0.001*
	Percentile 95	5685.9	2340.02	\0.001

^{*} P value less than 0.05 was considered as statistically significant Source: compiled by the authors of this study

of scrutiny under the University Code of Student Conduct (UCSC) programs, alongside alignment with corresponding reference sequences housed within the National Center for Biotechnology Information (NCBI) database. The synthesis and lyophilization of these primers were entrusted to the expertise of Alpha DNA Ltd. (Canada), (Table 1).

A total of 40 μ L of the PCR mixture were spun down with a minicentrifuge. PCR protocol (Table 3).

COMPARING CLINICAL SYMPTOMS IN INDIVIDUALS WITH IBD AND C. JEJUNI POSITIVE SAMPLES

The symptom data from the IBD patients were collected and compare with signs exhibited in samples that indicate a positive presence of *C. jejuni* [19].

RESULTS

AVERAGE AGE AND GENDER DISTRIBUTION IN IBD PATIENTS

The average age of individuals in both studied groups was computed, the mean age among individuals diagnosed with IBD is 34.21 ± 10.16 years and among healthy individuals is 33.47 ± 11.06 . Examining the gender distribution within this cohort, males accounted for a predominant 55% representation, while females constituted 45%. Statistical analysis indicat-

ed that no statistically significant disparities, bearing a P-value of less than 0.05, emerged between the patient cohort and the control group in relation to both age and gender (Table 4).

BACTERIAL DETECTION

CONVENTIONAL IDENTIFICATION OF C. JEJUNI

The outcomes arising from the cultivation of *C. jejuni* showed that the bacterial growth rate stood at approximately 5% within the studied samples. In contrast, the control group presented an entirely negative outcome, with no occurrences of *C. jejuni* (Table 5).

Notably, the prevalence of *C. jejuni* growth exhibited distinct patterns based on the subcategories of the disease. For example, among individuals diagnosed with Crohn's disease, the presence of *C. jejuni* was observed in approximately 2.6% of cases. While the occurrence of *C. jejuni* growth within the subset of individuals afflicted by ulcerative colitis, registered at a relatively higher rate of 7.1%. However, these observed differences were not found to be statistically significant (P-value < 0.05) (Table 6).

The dark field or phase contrast preparations showed that all bacterial isolates have a distinctive darting motility. Gram stain preparations revealed Gram negative, curved or spiral rods. All isolates were positive for reduced nitrate, oxidase and catalase, indoxyl acetate and hippurate-tests, while were

Table 8. Molecular detection of *C. jejuni* in IBD types

			BD	— P Value
		Crohn's disease	Ulcerative colitis	P value
	Median	834.14	758.52	
C. jejuni (copy/mL)	Percentile 05	177.4	198.35	0.583
	Percentile 95	5478.3	5893.5	_

Source: compiled by the authors of this study

Table 9. Signs and symptoms of IBD patients in association with C. jejuni

Signs and	- · · / · ·					Bacteria c	ulture						
symptoms			No G	rowth					Grow	th			P value
	Present				Abser	nt		Present	Absei		sent		
Bloody diarrhea	n		[%]	n		%	n	[9	%]	n		[%]	0.408*
	34		97.1	42		93.3	1	2	.9	3		6.7	
		Yes			No			Yes			No		_
Smoking	n		[%]	n		[%]	n	[9	%]	n		[%]	0.647
	17		94.4	59		95.2	1	5	.6	3		4.8	
		Present			Abser	nt		Present		Al	osent		_
Abdominal pain	n		[%]	n		[%]	n	[9	%]	n		%	0.608
	25		96.2	51		94.4	1	3	.8	3		5.6	
D: 1		Yes			No		Yes			No		_	
Biology Therapy	n		%	n		%	n		%	n		%	0.728
	6		100.0	70		94.6	0	0	0.0	4		5.4	
		B1			B2		B1 B2						
Behavior	n		[%]	n		[%]	n	[9	%]	n		[%]	0.136*
	26		100.0	11		91.7	0	0	0.0	1		8.3	
				lo Growth					Grow	th			
	E1: Ulce proc			ft sided UC		xtensive UC				eft sided UC	E3: Exten- sive UC		
UC extent	n	[%]	n	[%]	n	[%]	n	[%]	n	[%]	n	[%]	0.280*
	28	93.3	3	75.0	8	100.	2	6.7	1	25.0	0	0.0	
CD loca-	L1: I	leal	L2: 0	Colonic		lleoco- onic	11		L2: (Colonic		leoco- onic	
tion	n	[%]	n	[%]	n	[%]	n	[%]	n	[%]	n	[%]	0.630
	8	100.0	10	100.0	19	95.0	0	0.0	0	0.0	1	5.0	-

^{*}P value less than 0.05 was considered as statistically significant

Source: compiled by the authors of this study

negative for glucose fermentation. This preliminary diagnosis therefore further designated the bacteria as *C. jejuni,* according [20]. This distribution of *Campylobacter* species is consistent with findings from earlier studies conducted in industrialized and developing nations, including Out of the 580 stool samples that were gathered from 11 Kibera schools, 294(51%) of them fit the suspicious Campylobacter spp. phenotype. Upon subjecting these isolates to PCR Campylobacter spp. were identified in 106(18%) of the isolates by means of genus- and species-specific primers. Of the 106 isolates, 28(4.8%) and 44(7.6%) were of the type *C. jejuni* [21].

QUANTITATIVE PCR ANALYSIS OF C. JEJUNI

The utilization of a quantitative PCR approach in this study provided a precise assessment of the presence of *C. jejuni*. The results revealed a noteworthy disparity between the cohort of individuals diagnosed with IBD and the control group. Upon conducting in-depth analysis, a statistically significant difference in the occurrence of *C. jejuni* between these two groups was observed (P-value <0.05) (Table 7).

Conversely, the statistical analysis of the tabulated data revealed a lack of statistically significant differ-

Table 10. Signs and Symptoms of IBD Patients in association with C. *jejuni*

	Ci	C. jejuni (copy/mL)				
•	Signs and symptoms	Med	ian	Percentile 05-95		
All : l :	Absent	809.81	177.40	5893.50		
Abdominal pain -	Present	796.68	198.35	4298.66		
	P value		0.906			
Consolidar a	No	851.00	239.60	5893.50		
Smoking -	Yes	607.33	188.09	4298.66		
	P value		0.345*			
Dia advadia sula a	Absent	794.33	198.35	5478.30		
Bloody diarrhea -	Present	813.31	188.09	5893.50		
	P value		0.996			
D: - l +b	No	796.68	188.09	5478.30		
Biology therapy –	Yes	1199.53	198.35	19372.22		
	P value		0.465*			
Dahardan	B2: stricturing	857.17	124.36	36987.00		
Behavior –	B1: nonstricturing, nonpenetrating	831.79	277.30	4396.32		
	P value		0.792			
	E1: Ulcerative proctitis	843.97	323.78	14852.30		
UC extent	E2: Left sided UC	793.19	569.30	1642.04		
_	E3: Extensive UC	420.99	152.07	960.35		
	P value		0.048*			
	L1: lleal	787.32	319.98	5478.30		
CD location	L2: Colonic	696.91	124.36	36987.00		
_	L3: Ileocolonic	892.28	251.20	3595.20		
	P value		0.815			

^{*}P value less than 0.05 was considered as statistically significant Source: compiled by the authors of this study

ence between the two subgroups, Crohn's Disease (CD) and Ulcerative Colitis (UC) within IBD patients (P-value > 0.05), as shown in Table 8.

A COMPARATIVE ANALYSIS OF CLINICAL MANIFESTATIONS BETWEEN IBD PATIENTS AND C. JEJUNI POSITIVE SAMPLES

Based on the conventional method results and the statistical analysis conducted, it was found that there was no significant association between signs and symptoms of IBD with and smoking, ulcerative colitis (UC) extent, bloody diarrhea, abdominal pain, use of biological therapy, behavior, CD (Crohn's disease) location (P-value > 0.05), (Table 9).

However, according to the Real time PCR results, signs and symptoms of IBD with *C jejuni* were not correlated with abdominal pain, smoking, bloody diarrhea, behavior, CD location, while were significantly correlated in UC extent (P-value <0.05), (Table 10).

DISCUSSION

AVERAGE AGE AND GENDER DISTRIBUTION ANALYSIS IN IBD PATIENTS

The exact cause of IBD is not well understood, but it is believed to involve a combination of genetic, environmental, and immune system factors. IBD can affect individuals of any age but often presents itself between the ages of 15-30 years [22]. This is consistent with the findings of our study as the mean age of patient group was 34 years. One of the potential factors could indeed contribute to the increased diagnosis of IBD at older ages is the increased awareness among both patients and healthcare providers. As medical technology and awareness have advanced, more individuals might be seeking medical attention for their gastrointestinal symptoms, which in turn leads to more diagnoses being made across a broader age range [23]. Regarding the distribution between males and females, no statistically significant difference in the number of affected males and females, observed little increase in number of affected males over females. This was consistent with previous studies, which adds credibility to the present study's findings [24-26]. The fact that there is a an increase in the number of males affected with IBD compared to females raises interesting questions about the potential underlying mechanisms and the factors contributing to this gender distribution. While the reasons for this observation might not be fully understood at this point, several hypotheses can be considered. One of them can be the immune response differences between genders which lead to differences in susceptibility to IBD [27]. Exploring the reasons behind such patterns can be crucial for understanding the disease, developing targeted treatments, and improving overall healthcare outcomes.

INTERPRETING THE CLINICAL SIGNIFICANCE OF CONVENTIONAL AND PCR FINDINGS IN IBD PATIENTS WITH C. JEJUNI

The results demonstrated the presence of C. jejuni in a subset of participants, with a notably higher prevalence observed using the real-time PCR method compared to conventional culturing methods. This underscores the importance of employing advanced molecular tools for accurate detection, which might otherwise be missed using traditional approaches [28]. According to the real time PCR results, there was a significant difference in the prevalence of C. jejuni between IBD patients and controls. Several studies also reported an increase in the distribution of C. jejuni in IBD patients comparing with the general healthy population. For example, Kaakoush and colleagues found a substantial correlation between C. jejuni infection and Crohn's disease compared to healthy controls in their meta-analysis study [29]. Similarly, the work of Barker et al. further supports the argument on a relationship between C. jejuni and IBD [30]. Their study demonstrated a higher prevalence of this bacterium in individuals with UC compared to healthy controls. The consistency in results across different studies reinforces the reliability of the observed association between C. jejuni and IBD. Several hypotheses in literature have been provided in how C. jejuni may contribute to the development or exacerbation of IBD, which reinforce the reliability of the observed association between C. jejuni and IBD. For instance, C. jejuni infection has been found to break an exaggerated immune response, represented by pro-inflammatory cytokines and activation of different immune cells. This may disrupt the balance of the gut microbiota and lead to chronic inflammation in infected individuals [31]. Additionally, C. jejuni exhibits surface structures that resemble some host antigens. This mimicry may trigger an autoimmune response, where the immune system attacks both the bacterium

and the host's self-tissues, leading to inflammation and disease [32]. Furthermore, C. jejuni possesses the ability to adhere and invade intestinal epithelial cells, compromising the integrity of the intestinal barrier. This defect in the barrier can help bacterial translocation and trigger an immune response, hence chronic inflammation [33]. Interestingly, the study did not show significant variations in the detection of C. jejuni bacteria between the two types of IBD, UC and CD. This means that the potential contribution of C. jejuni may go beyond specific IBD subtypes, suggesting a wider impact on the disease process itself. According to our study, there is a significant correlation between female sex, shorter exclusive breastfeeding length, lower maternal age, lower maternal education, untreated drinking water, and inadequate sanitation and Campylobacter infections [34]. Although the presence of Campylobacter species is linked to growth deficiencies, birth weight for age is just a marginal predictor for these species.1. The prevalence of Campylobacter was higher in malnourished children from a case control study in Dhaka (6-23 months old) with weightfor-age z score (WAZ) <-2 than in healthy (control) children [weight-for-age z score (WAZ)>-1], but the adjusted effect size was not statistically significant [35]. Using the well-characterized human clinical C. jejuni isolate 81-176, another investigation showed that translocation of noninvasive bacteria is increased in C. jejuni-infected human intestinal epithelium [36]. In [37], we have found campylobacter in 5(5%) of our samples overall, additionally, campylobacter was found throughout Iraq, including in the city of Duhok. Campylobacter is one of four individuals with diarrhea who are segregated [38]. In the city of Mosul, an incidence of 6.3% of patients with diarrhea had Campylobacter. In the western Iragi city of Ramadi, secluded Campylobacter at an 8.92% rate [39]. It is comparable to the findings of a study where the two researchers isolated Campylobacter from the city of Al-Duwaniyah. Using the technique of direct DNA isolation from feces, 100 infected patients had diarrhea, and the percentage of Campylobacter infection was (5.8%) [40] Conversely, a study conducted in India discovered that just 3.40 percent of the 88 participants had diarrhea, which is similar to our findings [41]. Compared to our investigation, current research in New Zealand discovered a negligible amount of Campylobacter, finding only 2.9% of all of her samples (1604) [42]. Our study's findings are different from those of Liang et al., who discovered that the lowest percentage of females (36.31%) and the highest percentage of males 63.69% [43], however, Karikari et al. indicate that 32.4% of men and 67.6% of women experienced diarrhea [44-45].

RELATIONSHIP BETWEEN C. JEJUNI INFECTION AND IBD SYMPTOMS

Current study also reveals a lack of significant correlations between certain signs and symptoms of IBD with *C. jejuni* infection and several features defined by the Montreal classification. This may indicate that *C. jejuni's* effect on IBD is not mainly determined by these particular clinical and categorization parameters. This highlights the multifactorial nature of IBD and the numerous complicated interactions that affect the disease development, progression, and symptoms.

CONCLUSIONS

This study clearly shows how crucial *Campylobacter jejuni* is to gastrointestinal health, especially when it comes to the emergence of post-infectious irritable bowel syndrome (IBS). It draws attention to the bacterium's rising prevalence worldwide, its significant effects on the gut microbiota, and the ensuing medical difficulties. It emphasizes the necessity of better epidemiological surveillance, the creation of targeted therapeutic approaches, and a more thorough comprehension of the host-pathogen relationship's dynamics. Examine how *Campylobacter jejuni* contributes to post-infectious IBS.

REFERENCES

- 1. Yang AZ, Jostins-Dean L. Environmental variables and genome-environment interactions predicting IBD diagnosis in large UK cohort. Sci Rep. 2022;12:10890. doi:10.1038/s41598-022-13222-0. DOI 22
- 2. Soriano A, Beltrami M, Pizarro TT, Soriano M, et al. Inflammatory bowel diseases: Sex differences and beyond. In: Rezaei N (ed). Translational Autoimmunity: Autoimmune Diseases in Different Organs. Academic Press, 2022, pp. 295-308. doi:10.1016/B978-0-12-824466-1.00010-8
- 3. Ananthakrishnan AN, Nguyen GC, Bernstein CN. AGA Clinical Practice Update on Management of Inflammatory Bowel Disease in Elderly Patients: Expert Review. Gastroenterology. 2021;160:445-451. doi:10.1053/j.gastro.2020.08.060.
- 4. Allaith SA, Abdel-aziz ME, Thabit ZA, Altemimi AB, et al. Screening and Molecular Identification of Lactic Acid Bacteria Producing β-Glucan in Boza and Cider. Fermentation 2022 8(8):350. doi:10.3390/fermentation8080350.
- 5. Edo Gl, Mafe AN, Ali A, Akpoghelie PO, et al. Evaluation of different antimicrobial polymeric coatings for food contact surfaces. Discover Food. 2025 Dec;5(1):1-48. doi:10.1007/s44187-025-00487-3.
- 6. AL-Shammri FK, Obeid H., Abbas MS, Mohammed AS, et al. Developing Healthcare using Internet of Things (IoT): A Survey of Applications, Challenges and Future Directions. BIO Web of Conferences 97 2024;97:00004). doi:10.1051/bioconf/20249700004
- 7. Nand P, Drabu S, Gupta RK. Antimicrobial investigation of Linum usitatissimum for the treatment of acne. Nat Prod Commun. 2011;6:1701-1704. doi:10.1177/1934578x1100601133
- 8. Selvamani S, Mehta V, Ali El Enshasy H, Thevarajoo S, et al. Efficacy of Probiotics-Based Interventions as Therapy for Inflammatory Bowel Disease: A Recent Update. Saudi J Biol Sci. 2022;29:3546-3567. doi:10.1016/J.SJBS.2022.02.044
- 9. Okobi OE, Udoete IO, Fasehun OO, Okobi T, et al. A Review of Four Practice Guidelines of Inflammatory Bowel Disease Cureus. 2021 Aug 3;13(8):e16859. doi: 10.7759/cureus.16859.
- 10. Zhang L, Liu F, Xue J, Lee SA, Liu L, Riordan SM. Bacterial Species Associated With Human Inflammatory Bowel Disease and Their Pathogenic Mechanisms. Front Microbiol 2022 Feb 24:13:801892. doi: 10.3389/fmicb.2022.801892.
- 11. Rouhani S, Griffin NW, Yori PP, Olortegui MP, et al. Gut microbiota features associated with campylobacter burden and postnatal linear growth deficits in a Peruvian birth cohort. Clin Infect Dis. 2020;71:1000-1007. doi:10.1093/cid/ciz906.
- 12. Hasan AS, Muhsen TAA, Alabassi HM. Diagnostic study of the most important fungal infections associated with some inflammatory bowel disease in Iraqi patients. Acta Biomed. 2023; 94: 2023138. doi:10.23750/abm.v94i2.15548
- 13. Ghazi HF, Alubaidi GT, Fahad HM. Sero-prevalence of Epstein Barr virus in Iraqi inflammatory bowel disease. Wiad Lek. 2022; 75: 1979-1984. doi:10.36740/WLek202208207.
- 14. Gupta S, Khan A, Biswas P, Mondal K, et al. A combined protocol for isolation of T6SS-positive Campylobacter jejuni and assessment of interspecies interaction. STAR Protoc. 2022;3. doi:10.1016/j.xpro.2022.101368.
- 15. Porte L, Pérez C, Barbé M, Varela C, Vollrath V, Legarraga P, Weitzel T. Campylobacter spp. Prevalence in Santiago, Chile: A Study Based on Molecular Detection in Clinical Stool Samples from 2014 to 2019. Pathogens. 2023 Mar 22;12(3):504. doi: 10.3390/pathogens12030504.
- 16. Kalischuk LD, Inglis GD. Comparative genotypic and pathogenic examination of Campylobacter concisus isolates from diarrheic and non-diarrheic humans. BMC Microbiol. 2011; 11, 53-2180-11-53.
- 17. Ismail Y, Mahendran V, Octavia S, Day AS, et al. Investigation of the enteric pathogenic potential of oral Campylobacter concisus strains isolated from patients with inflammatory bowel disease. PLoS One. 2012;7:e38217.
- 18. Duarte A, Seliwiorstow T, Miller WG, De Zutter L, et al. Discriminative power of Campylobacter phenotypic and genotypic typing methods. J. Microbiol. Methods. 2016;125:33-39. doi: 10.1016/j.mimet.2016.03.004.

- 19. Al-Huseini LMA, Kadhim NJ, Mahdi MS, Ogaili RH, Al-Hammood O. Microbial infection disease diagnosis and treatment by artificial intelligence. Wiad Lek. 2025;78(2):442-447.
- 20. Kadhim NJ, Hafidh RR, Kadhim J, Abdulamir AS. Novel isolation and optimization of anti-MRSA bacteriophages using plaque-based biokinetic methods. J Emerg Med Trauma Acute Care 2024(5):2. doi:10.5339/jemtac.2024.iscncm.2.
- 21. Wang H, Gu Y, Ju C, Li Y, Chen X, Zhou G, Zhang X, Liu C, Chen J, Han Y, Zhang J, Shao Z, Zhang M. Genetic characteristics and potential pathogenic agents in Campylobacter upsaliensis based on genomic analysis. Emerg Microbes Infect. 2024 Dec;13(1): 2294857. doi: 10.1080/22221751.2023.2294857. DOI 2
- 22. Franco J, Bénejat L, Ducournau A, Mégraud F, Lehours P, Bessède E. Evaluation of canpylobacter quick chekTM rapid membrane enzyme immunoassay to detect Campylobacter spp. antigen in stool samples. Gut Pathog. 2021;13. doi:10.1186/s13099-021-00400-0
- 23. Alsafar SH, Abd AR, Mahdi IG, Al-Tameemi TT. Identification and isolation of Campylobacter jejuni and C. upsaliensis from bovine local milk, milk products, and human stool samples by molecular technique in Karbala province. Journal of Kerbala for Agricultural Sciences. 2023 Dec 15;10(4):1-17. doi:10.59658/jkas.v10i4.1289. 00 2
- 24. Malik TA. Inflammatory Bowel Disease. Historical Perspective, Epidemiology, and Risk Factors. Surg Clin North Am. 2015;1105-1122. doi:10.1016/j.suc.2015.07.006.
- 25. Garrett N, Devane ML, Hudson JA, Nicol C, et al. Statistical comparison of Campylobacter jejuni subtypes from human cases and environmental sources. J Appl Microbiol. 2007; 103(6): 2113-2121. doi: 10.1111/j.1365-2672.2007.03437.x.
- 26. Behringer M, Miller WG, Oyarzabal OA. Typing of Campylobacter jejuni and Campylobacter coli isolated from live broilers and retail broiler meat by flaA-RFLP, MLST, PFGE and REP-PCR. J Microbiol Methods. 2011 Feb; 84(2): 194-201. doi: 10.1016/j.mimet.2010.11.016
- 27. Kaplan GG, Windsor JW. The four epidemiological stages in the global evolution of inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2021;56-66. doi:10.1038/s41575-020-00360-x.
- 28. Molodecky NA, Kareemi H, Parab R, Barkema HW, et al. Incidence of primary sclerosing cholangitis: A systematic review and meta-analysis. Hepatology. 2011;53:159-1599. doi:10.1002/hep.24247.
- 29. Saich R, Chapman R. Primary sclerosing cholangitis, autoimmune hepatitis and overlap syndromes in inflammatory bowel disease. World J Gastroenterol. 2008;14:331-337. doi:10.3748/wjg.14.331
- 30. Acharya B, Acharya A, Gautam S, Ghimire SP, Mishra G, Parajuli N, et al. Advances in diagnosis of Tuberculosis: an update into molecular diagnosis of Mycobacterium tuberculosis. Molec Biol Rep. 2020;4065-4075. doi:10.1007/s11033-020-05413-7.
- 31. Kaakoush NO, Sodhi N, Chenu JW, Cox JM, Riordan SM, Mitchell HM. The interplay between Campylobacter and Helicobacter species and other gastrointestinal microbiota of commercial broiler chickens. Gut Pathog. 2014; 6. doi:10.1186/1757-4749-6-18.
- 32. Barker CR, Painset A, Swift C, Jenkins C, et al. Microevolution of Campylobacter jejuni during long-term infection in an immunocompromised host. Sci Rep. 2020; 10. doi:10.1038/s41598-020-66771-7.
- 33. Callahan SM, Dolislager CG, Johnson JG. The host cellular immune response to infection by Campylobacter spp. And its role in disease. Infect Immun. 2021. doi:10.1128/IAI.00116-21
- 34. Burnham PM, Hendrixson DR. Campylobacter jejuni: collective components promoting a successful enteric lifestyle. Nat Rev Microbiol. 2018;551-565. doi:10.1038/s41579-018-0037-9.
- 35. Negretti NM, Gourley CR, Talukdar PK, Clair G, Klappenbach CM, Lauritsen CJ, et al. The Campylobacter jejuni CiaD effector co-opts the host cell protein IQGAP1 to promote cell entry. Nat Commun. 2021;12. doi:10.1038/s41467-021-21579-5.
- 36. Thomrongsuwannakij T, Blackall PJ, Chansiripornchai N. A Study on Campylobacter jejuni and Campylobacter coli through Commercial Broiler Production Chains in Thailand: Antimicrobial Resistance, the Characterization of DNA Gyrase Subunit A Mutation, and Genetic Diversity by Flagellin A Gene Restriction Fragment Length Polymorphism. Avian Dis. 2017 Jun; 61(2):186-197. doi: 10.1637/11546-120116-Reg.1.
- 37. Eberle KN, Kiess AS. Phenotypic and genotypic methods for typing Campylobacter jejuni and Campylobacter coli in poultry. Poult Sci. 2012 Jan; 91(1):255-64. doi: 10.3382/ps.2011-01414.
- 38. Yassin NA, Jubrael JM. Specidic Identification of Camplylobacter Jejuni Using A Pcr Assay Based on The Hip-O Gene. Duhok Med J. 2012;6(2): 1-9.
- 39. Al-Mawla SO, Al-Dalla Ali FJ, Al-Ani MM. The role of Campylobacter species in diarrhea among children under five years of age in Ramadi city, west of Iraq. Al-Anbar Med. 2008;6(1): 76-87.
- 40. AL-Hamadani AH, Saleh ZF. Detection of Campylobacter spp. in children diarrhea by using Polymerase Chain Reaction PCR technique in Al-Diwanyiah Governorate. Al-Qadisiyah J of Vet Med Sci. 2011;10(2):45-54.
- 41. Nachamkin I, Nguyen P. Isolation of Campylobacter species from stool samples by use of a filtration method: assessment from a United States-based population. J Clin Microbiol. 2017 Jul;55(7):2204-2207. doi: 10.1128/JCM.00332-17.
- 42. Rawat N, Maansi DK, Upadhyay AK. Virulence typing and antibiotic susceptibility profiling of thermophilic Campylobacters isolated from poultry, animal, and human species. Vet World 2018;11(12):1698. doi:10.14202/vetworld.2018.1698-1705.

- 43. Nohra A, Grinberg A, Midwinter AC, Marshall JC, Collins-Emerson JM, French NP. Molecular epidemiology of Campylobacter coli strains isolated from different sources in New Zealand between 2005 and 2014. Appl Environ Microbiol. 2016;82(14): 4363-4370. doi: 10.1128/AEM.00934-16. Doi 2016
- 44. Liang H, Wen Z, Li Y, Duan Y, Gu Y, Zhang M. Comparison of the filtration culture and multiple real-time PCR examination for Campylobacter spp. from stool specimens in diarrheal patients. Front Microbiol. 2018;9:2995. doi: 10.3389/fmicb.2018.02995
- 45. Huang Y, Gu D, Xue H, Yu J, Tang Y, Huang J, Zhang Y, Jiao X. Rapid and Accurate Campylobacter jejuni Detection with CRISPR-Cas12b Based on Newly Identified Campylobacter jejuni-Specific and -Conserved Genomic Signatures. Front Microbiol. 2021 Apr 27:12:649010. doi: 10.3389/fmicb.2021.649010.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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