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# Absence of classical enteric pathogens in IBD-associated dysbiosis: Evidence from real-time PCR

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## Abstract

**Background:** Inflammatory bowel disease (IBD) is commonly associated with intestinal microbial imbalance. Persistent gastrointestinal symptoms in IBD may overlap with active disease, functional bowel symptoms, dysbiosis, or acute enteric infection. Molecular testing can help determine whether classical bacterial pathogens contribute to such symptoms.

**Aim:** The present study aimed to detect selected classical enteric bacterial pathogens in intestinal aspirate samples from IBD patients with suspected dysbiosis using real-time polymerase chain reaction.

**Methods:** This short observational molecular study analyzed 48 jejunal aspirate samples obtained from patients with IBD. Bacterial DNA was extracted and tested using the TRUPCR Gastrointestinal Panel I Kit. The panel targeted *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., and verotoxigenic *Escherichia coli* (VTEC). Positive controls, non-template controls, and internal controls were used to validate assay performance.

**Results:** None of the 48 intestinal aspirate samples showed amplification for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., or VTEC. Positive controls amplified successfully, confirming assay validity. Non-template controls showed no amplification, excluding contamination. Internal controls showed acceptable amplification patterns, supporting reliable interpretation of negative findings.

**Conclusion:** The absence of the tested classical enteric pathogens suggests that IBD-associated dysbiosis in this cohort was not attributable to *Salmonella*, *Shigella*, *Campylobacter*, or VTEC. Persistent gastrointestinal symptoms in IBD patients may therefore reflect altered commensal microbial balance rather than acute infection with these selected pathogens. Broader molecular and microbiome-based studies are recommended to further characterize dysbiosis in IBD.

**Keywords:** Inflammatory bowel disease; Dysbiosis; Real-time PCR; Enteric pathogens; *Salmonella; Shigella; Campylobacter*; Verotoxigenic *Escherichia coli*

## 1. Introduction

Inflammatory bowel disease (IBD), mainly comprising Crohn's disease and ulcerative colitis, is a chronic relapsing inflammatory disorder of the gastrointestinal tract. The pathogenesis of IBD involves complex interactions among genetic susceptibility, environmental triggers, epithelial barrier dysfunction, immune dysregulation, and altered gut

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microbiota [1,2]. Dysbiosis, defined as disruption in the structure or function of the intestinal microbial community, is increasingly recognized as an important component of IBD pathophysiology [3,4].

Patients with IBD often present with abdominal pain, diarrhea, bloating, urgency, fatigue, and altered bowel habits. These symptoms may result from active inflammation, functional bowel disturbance, bacterial overgrowth, dysbiosis, or acute enteric infection. Classical bacterial pathogens such as *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., and verotoxigenic *Escherichia coli* (VTEC) are important causes of infectious gastroenteritis and may clinically mimic or aggravate IBD activity [5,6].

Differentiating acute pathogen-driven infection from dysbiosis is clinically important. If persistent symptoms are misinterpreted as infection, unnecessary antimicrobial therapy may be used. Conversely, if infection is missed and symptoms are attributed only to IBD flare, inappropriate escalation of immunosuppressive therapy may occur. Therefore, targeted molecular detection of enteric pathogens can support clinical interpretation in IBD patients with persistent gastrointestinal symptoms.

Real-time polymerase chain reaction (PCR) is a rapid and sensitive method for detecting pathogen-specific nucleic acids in clinical samples. PCR-based assays are useful because they can detect selected organisms directly from clinical specimens and provide rapid exclusion of targeted pathogens when appropriate controls are valid [7,8].

The present study was conducted to detect selected classical enteric bacterial pathogens in jejunal aspirate samples from IBD patients with suspected dysbiosis. The focus of this paper is limited to PCR-based detection of *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., and VTEC.

## 2. Material and methods

### 2.1. Study design

This was a short observational molecular study conducted on intestinal aspirate samples obtained from patients with confirmed inflammatory bowel disease. The study was designed to evaluate the presence or absence of selected classical enteric bacterial pathogens using real-time PCR.

### 2.2. Study samples

A total of 48 jejunal aspirate samples were included in the study. Samples were collected during upper gastrointestinal endoscopy under aseptic precautions as part of the parent research protocol. The present paper reports only the molecular detection of selected enteric pathogens and does not include breath test or culture-based outcomes.

### 2.3. Inclusion criteria

Adult patients aged 18 years or above with confirmed inflammatory bowel disease were included. IBD diagnosis was based on standard clinical, endoscopic, radiological, and histopathological criteria.

### 2.4. Exclusion criteria

Patients were excluded if they had recent antibiotic use, recent probiotic use, recent bowel preparation, pregnancy, critical illness, or inability to provide informed consent or undergo study procedures.

### 2.5. DNA extraction

Bacterial DNA was extracted from jejunal aspirate samples using standard DNA extraction procedures. Extracted DNA was used as the template for real-time PCR amplification. Internal controls were used to assess sample quality, extraction efficiency, and possible PCR inhibition.

### 2.6. Real-time PCR assay

Real-time PCR was performed using the TRUPCR Gastrointestinal Panel I Kit. The assay was used for qualitative detection of four selected classical enteric bacterial pathogens:

- *Salmonella spp.*
- *Shigella spp.*
- *Campylobacter* spp.

- Verotoxigenic Escherichia coli

The assay was based on pathogen-specific amplification using fluorescent probe-based detection. Fluorescence signals were monitored during amplification, and results were interpreted according to the manufacturer's instructions.

### 2.7. Quality control

Each PCR run included positive controls, non-template controls, and internal controls. Positive controls were used to confirm successful amplification of target pathogen sequences. Non-template controls were used to rule out contamination. Internal controls were used to confirm sample quality and reaction validity.

### 2.8. Data analysis

PCR results were summarized descriptively. The number and percentage of positive and negative samples were calculated for each targeted pathogen. Since the purpose of the study was molecular screening, no inferential statistical analysis was required.

## 3. Results

### 3.1. Detection of targeted enteric pathogens

A total of 48 jejunal aspirate samples were tested using real-time PCR. None of the samples showed amplification for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., or VTEC. The detection rate for each targeted pathogen was therefore 0%.

**Table 1** Real-time PCR detection of classical enteric pathogens in jejunal aspirate samples

| Target pathogen | Samples tested | Positive samples | Negative samples | Detection rate |
|---|---|---|---|---|
| *Salmonella* spp. | 48 | 0 | 48 | 0% |
| *Shigella* spp. | 48 | 0 | 48 | 0% |
| *Campylobacter* spp. | 48 | 0 | 48 | 0% |
| Verotoxigenic *E. coli* | 48 | 0 | 48 | 0% |

### 3.2. Quality-control findings

Positive controls showed expected amplification, confirming that the PCR assay was valid. Non-template controls showed no amplification, indicating absence of contamination. Internal controls demonstrated acceptable amplification patterns, supporting reliable interpretation of the negative results.

**Table 2** Quality-control interpretation of real-time PCR assay

| Control type | Observation | Interpretation |
|---|---|---|
| Positive control | Amplification observed | Assay valid |
| Non-template control | No amplification observed | No contamination detected |
| Internal control | Acceptable amplification pattern | Reaction valid |

### 3.3. Overall molecular interpretation

Across all tested jejunal aspirate samples, there was no molecular evidence of infection with the selected classical enteric bacterial pathogens. The PCR findings suggest that the studied IBD-associated dysbiosis pattern was not explained by *Salmonella*, *Shigella*, *Campylobacter*, or VTEC.

## 4. Discussion

The present study evaluated selected classical enteric bacterial pathogens in jejunal aspirate samples from patients with IBD-associated dysbiosis using real-time PCR. None of the 48 tested samples showed amplification for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., or VTEC. Valid positive controls, negative non-template controls, and acceptable internal-control findings supported the reliability of the PCR results.

These findings are important because IBD symptoms often overlap with infectious gastroenteritis. Diarrhea, abdominal pain, bloating, and urgency can occur in both IBD flare and acute enteric infection. Classical bacterial pathogens may also aggravate intestinal inflammation and complicate disease assessment [5,6]. Therefore, molecular exclusion of selected pathogens can help clinicians consider alternative explanations such as dysbiosis, bacterial overgrowth, functional symptoms, or active inflammatory disease.

IBD-associated dysbiosis does not necessarily represent acute infection. Previous microbiome studies have shown that IBD is often associated with reduced beneficial anaerobic bacteria and expansion of facultative anaerobic or inflammation-associated taxa [3,4,9]. Such changes may reflect altered host–microbiome balance rather than invasion by classical pathogens. The present PCR findings support this interpretation by showing absence of four common bacterial causes of enteric infection.

Real-time PCR provides several advantages in this context. It is rapid, targeted, and capable of detecting pathogen-specific DNA directly from clinical samples [7,8]. Its use in IBD patients with persistent gastrointestinal symptoms may help rule out selected infectious causes before clinical decisions are made. However, PCR panels must be interpreted according to their target range. A negative result for selected pathogens does not exclude all possible infectious agents.

The present study therefore supports a cautious interpretation: the tested samples were negative for *Salmonella*, *Shigella*, *Campylobacter*, and VTEC, but other bacterial, viral, parasitic, fungal, or non-targeted microbial agents were not evaluated. Similarly, PCR testing alone does not describe the complete microbiome structure. Broader sequencing-based studies are required to characterize the full dysbiosis pattern in IBD patients.

### 4.1. Clinical significance

In patients with IBD and persistent gastrointestinal symptoms, targeted PCR testing may help exclude selected classical enteric bacterial pathogens. When PCR results are negative and assay controls are valid, clinicians should consider non-infectious dysbiosis or other IBD-related mechanisms rather than assuming acute bacterial gastroenteritis.

### 4.2. Strengths of the study

The study used real-time PCR on jejunal aspirate samples, which provides direct molecular assessment of upper gut material. The inclusion of positive controls, non-template controls, and internal controls strengthens the validity of the findings.

### 4.3. Limitations of the study

This study has some limitations. First, the PCR panel targeted only four bacterial pathogens. Second, viruses, parasites, fungi, non-targeted bacteria, and antimicrobial resistance genes were not assessed. Third, the study was descriptive and did not evaluate associations with disease activity, symptom severity, treatment history, or clinical outcomes. Fourth, PCR detects targeted nucleic acid and does not provide full microbiome composition. Finally, the sample size was modest, and larger studies are needed to confirm the findings.

## 5. Conclusion

Real-time PCR analysis of 48 jejunal aspirate samples from IBD patients with suspected dysbiosis did not detect *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., or verotoxigenic *Escherichia coli*. Valid assay controls supported the reliability of these findings. The results suggest that IBD-associated dysbiosis in this cohort was not attributable to the tested classical enteric bacterial pathogens. Persistent gastrointestinal symptoms in such patients may reflect altered commensal microbial balance rather than acute infection with these selected organisms.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors declare that there is no conflict of interest.

### *Statement of ethical approval*

Ethical approval was obtained from the institutional ethics committee.

### *Statement of informed consent*

Written informed consent was obtained from all participants before sample collection.

### *Funding*

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### *Data availability statement*

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## References

[1] Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011;474(7351):307–317. doi:10.1038/nature10209.

[2] Ananthakrishnan AN. Epidemiology and risk factors for IBD. Nat Rev Gastroenterol Hepatol. 2015;12(4):205–217. doi:10.1038/nrgastro.2015.34.

[3] Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A. 2007;104(34):13780–13785. doi:10.1073/pnas.0706625104.

[4] Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature. 2019;569(7758):655–662. doi:10.1038/s41586-019-1237-9.

[5] Chassaing B, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. Gastroenterology. 2011;140(6):1720–1728. doi:10.1053/j.gastro.2011.01.054.

[6] Axelrad JE, Joelson A, Nobel YR, Lawlor G, Lichtiger S, Cadwell K, et al. Enteric infection in relapse of inflammatory bowel disease: The utility of stool microbial PCR testing. Inflamm Bowel Dis. 2017;23(6):1034–1039. doi:10.1097/MIB.0000000000001097.

[7] Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE Guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55(4):611–622. doi:10.1373/clinchem.2008.112797.

[8] Ghoshal UC, Ghoshal U, Shah A, Holtmann G. Evaluation of small intestinal bacterial overgrowth. Expert Rev Gastroenterol Hepatol. 2023;17(5):461–467. doi:10.1080/17474124.2023.2207008.

[9] Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe. 2014;15(3):382–392. doi:10.1016/j.chom.2014.02.005.

[10] Bushyhead D, Quigley EMM. Small intestinal bacterial overgrowth: Pathophysiology and its implications for definition and management. Gastroenterology. 2022;163(3):593–607. doi:10.1053/j.gastro.2022.04.002.