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Original Article
Bangladesh Journal of Infectious Diseases
December 2025, Volume 12, Number 2, Page 243-249
ISSN (Online) 2411-670X
ISSN (Print) 2411-4820
NLM ID: 101761093
DOI: https://doi.org/10.3329/bjid.v12i2.88420

Molecular Detection of Pathogenic Microorganisms in Gastrointestinal Infection Patients Using Real-Time Polymerase Chain Reaction

Rafeef Yousif Rashid[1], Anmar L. Talib[2], Mustafa A. Al-Sheakh[3], Shamsuldhuha K. Ramadhan[4]

[1]Lecturer, Department of Microbiology, College of Science, Al-Karkh University for Science, Baghdad, Iraq; [2]Lecturer, Department of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq; [3]Assistant Professor, Department of Microbiology, College of Science, Al-Karkh University for Science, Baghdad, Iraq; [4]Bachelor of Science, Department of Microbiology, College of Science, Al-Karkh University for Science, Baghdad, Iraq

Abstract

Background: Microbiota are defined as the organisms living within the gastrointestinal tract that coexists in the intestine. Its play an important role in dietary action. This is about bacteria, but there are other species of microorganisms that are causing multiple diseases, ranging from those that are treated quickly to those that are very dangerous and need a long time to treat. **Objectives:** The study aimed to detect and quantify gastrointestinal (GI) tract microbiota using RT-PCR. **Methodology:** This was a cross-sectional study. A total of 60 stool samples were collected from individuals presenting with gastrointestinal symptoms at Yarmouk Hospital in Baghdad, between October 2023 to May 2024. Fifty stool samples were taken from patients and 10 from healthy people. **Results:** The results were recorded 37 samples diagnosed with various bacteria, 6 samples with parasites, and 6 samples with viruses. The use of Real-Time Polymerase Chain Reaction (RT-PCR) represents one of the most accurate and sensitive approaches for detecting microorganisms constituting the gastrointestinal microbiota. **Conclusion:** This technique allows not only the qualitative detection of microbial DNA but also provides a semi-quantitative assessment through the Cycle Threshold (Ct) value. Ct is inversely related to the microbial load; lower Ct = higher DNA concentration, whereas higher Ct = lower microbial load. [*Bangladesh Journal of Infectious Diseases, December 2025;12(2):243-249*]

Keywords: Gastrointestinal tract; Microbiota; RT-PCR; GastroFinder 2SMART; Gut bacteria diagnosis

Correspondence: Doctor. Rafeef Yousif Rashid, Department of Microbiology, College of Science, Al-Karkh University for Science, Baghdad, 10001 Iraq; **Cell No:** +9647707828149; **Email:** rafeef.rashid@kus.edu.iq; **ORCID:** https://orcid.org/0009-0009-5747-5043

Introduction

One of the best ways to find infected microorganisms in the gut is to use the technique of polymerase chain reaction (PCR)[1]. Due to its ease and quickness to find bacterial, viral and parasite infections in clinical samples like faeces when you use this molecular method. With standard methods that use bacteria, it is not possible to find diseases when there are not many of them. PCR can achieve this. It also helps you find more than one stomach issue at the same time, which leads to a worse

result[2]. PCR is a very important way to find stomach problems early, treat them correctly and keep them from getting worse.

There are microorganisms in our digestive tract, such bacteria, viruses, parasites, and yeast. They make up the microbiota in the gut[3]. These microbes are necessary for good health because they control metabolism, influence the immune system, and stop pathogens from spreading[4]. The gut microbiota and the host interact in complicated ways that have a big effect on homeostasis, immune responses, and health in general[5]. Recent discoveries about the gut microbiome have led individuals to pinpoint certain organisms linked to numerous maladies, especially digestive problems like inflammatory bowel disease (IBD)[6]. Inflammatory bowel disease (IBD) is a condition that causes long-term inflammation of the digestive tract. Examples of this are ulcerative colitis and Crohn's disease[7]. The exact cause of IBD is still unknown, however changes in the types of bacteria that live in the gut have been suggested as possible causes[8]. Certain bacterial types and species experience modifications in persons afflicted with inflammatory bowel disease (IBD)[9]. This causes the intestinal cells to be inflamed for a long time.

Studies show that using machine learning to look at the makeup of microbes can better predict the presence of inflammatory bowel disease (IBD) than conventional signs of inflammation in stool[10]. Changes in the gut flora can help doctors figure out what is wrong with a patient and how likely they are to get better[11]. New methods like RT-PCR make it easier for us to find bacteria that are linked to gastrointestinal diseases by using the diagnostic power of gut microbes[12]. This method is better than other diagnostic methods since it is more sensitive and detailed. New diagnostic tools, including RT-PCR, can help us learn more about how microorganisms cause inflammation in the digestive tract[13]. The study was aimed to detect and quantify GI tract microbiota using wit the aid of RT-PCR.

Methodology

Study Settings and Population: A total of 60 stool samples were collected from individuals presenting with gastrointestinal symptoms at Yarmouk Hospital in Baghdad, between October 2023 to May 2024. 50 stool samples were taken for patients and 10 for healthy people. Each sample was collected in a sterile, leak-proof container and immediately stored at −20°C until processing. All samples were anonymized, and informed consent was obtained from the participants.

DNA Extraction: Microbial DNA was extracted directly from the stool samples using the GastroFinder 2SMART DNA extraction module, following the manufacturer's instructions. Briefly, approximately 200 mg of stool sample was mixed with lysis buffer provided in the kit and processed using mechanical disruption to ensure effective cell lysis. The samples were then subjected to spin-column purification to isolate total DNA. The quality and concentration of extracted DNA were measured using a NanoDrop spectrophotometer (Thermo Scientific, USA), and samples with A260/A280 ratios between 1.8 and 2.0 were considered pure and suitable for downstream analysis[14].

Real-Time PCR (RT-PCR) Using GastroFinder 2SMART Kit: The GastroFinder 2SMART is a multiplex Real-Time PCR diagnostic kit designed to detect a panel of gastrointestinal pathogens, including both bacteria and viruses, in a single reaction. The kit includes lyophilized primers and probes specific to each target microorganism, along with internal controls and PCR mastermix[14]. Each PCR run included positive Control, Supplied by the kit for validation of amplification. Negative Control, Nuclease-free water to rule out contamination. Internal Control, Included in each sample reaction to monitor inhibition

Microorganisms Detected by the GastroFinder 2SMART Kit: The GastroFinder 2SMART kit is capable of detecting multiple gastrointestinal pathogens. The targeted microorganisms include *Adenovirus, Clostridium difficile Tox A, Campylobacter species, Entamoeba histolytica, Enterotoxigenic Escherichia coli, Giardia lambila,* Rota virus *and Salmonella typhi*.

Statistical Analysis: Cycle threshold (Ct) values were automatically calculated by the PCR software. A Ct value of more than 38 was considered positive for the corresponding microorganism, as recommended by the kit manufacturer. Samples with no amplification of either the target or internal control were marked as invalid and repeated. Positive results were recorded for each target, and their prevalence among the tested samples was analyzed. The results were also interpreted in clinical context when available[14]. Data were statistically analyzed using the program Statistical Analysis System[15] and compared the significant differences between the averages using the Duncan test (1955) polynomial[16].

Ethical Consideration: All procedures of the present study were carried out in accordance with

the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee. Participants in the study were informed about the procedure and purpose of the study and the confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and were analyzed using the coding system.

Results

A total of 60 stool samples were analyzed using the GastroFinder 2SMART multiplex Real-Time PCR kit to detect a panel of eight gastrointestinal pathogens. These included both bacterial and viral agents, as well as protozoa commonly associated with gastrointestinal infections. All samples were successfully processed, and no invalid results were observed. Internal controls amplified correctly in all reactions, confirming the quality of DNA extraction and the absence of PCR inhibition.

Detection Rates of Targeted Microorganisms: Out of 60 samples, 50 samples (81.6%) tested positive for at least one of the eight targeted pathogens. The remaining 10 samples (18.3%) were negative for all tested targets. The most frequently occurring isolate among the isolates is *Enterotoxogenic E. coli* .The isolates that appeared in second place were *E. Histolytica,* While *G. lambila and Salmonila typhi* same ratio. These are the most important results**.** The prevalence of each pathogen is summarized below in tables and figures.

Table 1: Prevalence of Each Pathogen Detected by The Gastrofinder 2SMART Kit

Pathogen	Positive Samples	Prevalence
Adenovirus	3	5.0%
Clostridium difficile Tox A	3	5.0%
Campylobacter species	5	8.3%
E. Histolytica.	8	13.3%
Enterotoxigenic E. coli	15	25.0%
G. lambila	6	10.0%
Rota virus	3	5.0%
Salmonella typhi	6	10.0%

Note: Some Samples Were Positive For More Than One Microorganism

Table 2: Relationship between Type of Microorganism and Ct Average with Number of Isolate Obtain

Type of Microorganism	No.	Mean±Std. Error (Ct Average)
Adenovirus	3	49.33 ± 0.33^{e}
Clostridium difficile Tox A	3	76.37 ± 0.54^{a}
Campylobacter species	5	52.89 ± 0.90^{ed}
E. Histolytica	8	54.44 ± 0.25^{d}
Enterotoxigenic E. coli	15	58.82 ± 1.28^{c}
G. lambila	6	62.01 ± 0.60^{bc}
Rota virus	3	62.19 ± 0.06^{bc}
Salmonella typhi	6	64.82 ± 0.28^{b}
Significant		**

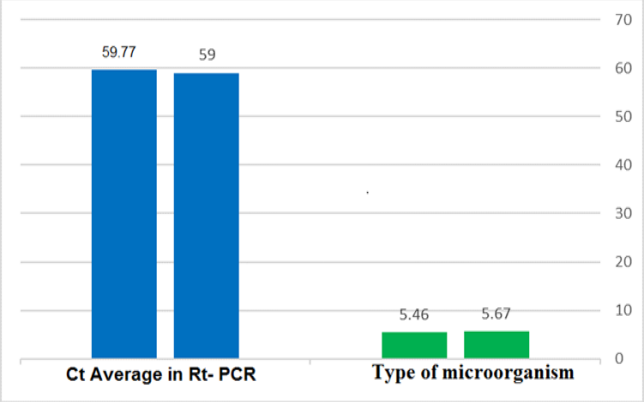

Figure I: Bar chart showing the Frequency of Each Detected Microorganism

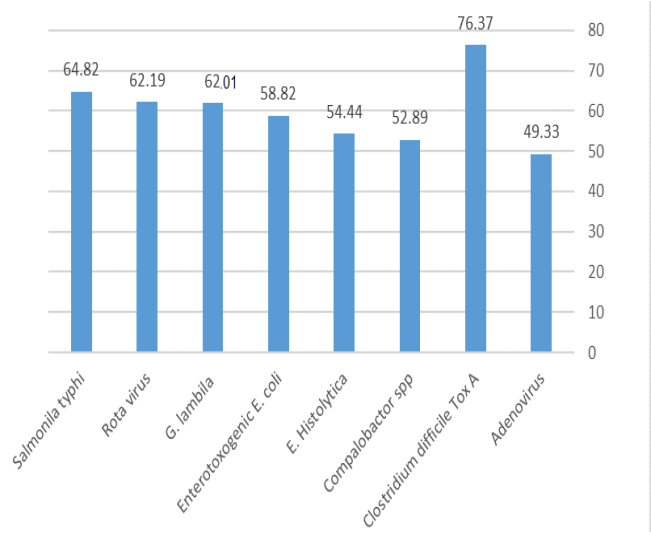

Figure II: Ct Value Distribution for Major Pathogens

Cycle Threshold (Ct) Values: The number of isolated males is greater than that of females wwithout significant differences in readings Ct values.

Table 3: **Relationship Between Ct Average, Type of Microorganism, and Patient Gender**

Gender	No.	Mean±Std. Error (Type of microorganism)	No.	Mean±Std. Error (Ct Average)
1 (Male)	34	5.67± 0.38[a]	28	59.00±1.33[a]
2 (Female)	26	5.46±0.49[a]	21	59.77±1.42[a]
Significant		n.s.		n.s.

n.s.= No Significant; Gender:1= male / gender:2= female

Table 3: Relationship Between Type of Microorganism and Ct Average, depending on age

Age	No.	Mean±Std. Error (Type of microorganism)	No.	Mean±Std. Error (Ct Average)
1	10	6.30±0.76[a]	6	56.99± 1.70[a]
2	17	5.29±0.64[a]	14	58.94±1.91[a]
3	20	5.30±0.57[a]	17	60.66±1.94[a]
4	13	5.84±0.45[a]	12	59.06±1.64[a]
Significant		n.s.		n.s.

n.s.= No Significant; Age:1= (14-27) / Age:2= (28-41) / Age:3= (42-57) / Age:4= (58-71)

The use of GastroFinder 2SMART successfully detected a wide range of gastrointestinal pathogens from stool samples with high sensitivity. Without significant differences in readings between isolates. Age does not significantly affect either as in table and figure below. The number of microbial species detected (microbial diversity), or the Ct values (microbial load).

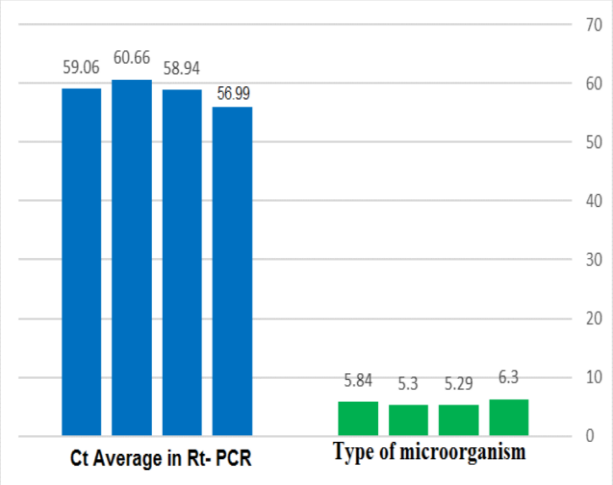

Figure III: Relationship between Type of Microorganism and Ct Average

The microbiota profile appears stable across the different age categories in this study sample (Figure III). These findings indicate that factors other than age, such as diet, medication, health status, or environment, are more influential in shaping the gut microbiota.

Discussion

Modern molecular methods for diagnosing microorganisms, including pathogenic ones, are increasingly being used. Numerous studies have been conducted on this, including a study of large stool samples using important molecular detection methods for intestinal pathogens identified in the study samples. Samples were identified as containing viruses, intestinal bacteria, and parasites[17]. Previous studies relied on sample culture, which is essential to support clinical decisions regarding the patient's condition, especially regarding bacterial pathogens. This condition requires antibiotic sensitivity testing. The study demonstrated sensitivity to detect enteric bacteria such as Shigella, Salmonella, and Campylobacter species. These bacterial organisms are fragile and may fail to grow in culture media. This effect is due to the different, less-than-ideal transport conditions for the organism's growth. The result suggests a reduced role for bacteria in causing gastrointestinal infections[17].

Studies have indicated that the number of parasites in the studied samples constitutes 20.96%, including the De Fragales parasite, which was significantly elevated in the parasite panel[8,9]. This finding aligns with other studies that have demonstrated the widespread presence of these parasites, despite their inability to cause disease[10-11]. Based on previous studies, the most prevalent parasitic agents causing diarrhea were *Entamoeba histolytica* and Giardia lambilia[18]. This study

employed advanced molecular methods, which provided a clearer picture of their role in parasite diagnosis compared to light microscopy[13]. This result is consistent with the presented study, where the percentages were similar for the samples used. The reason for this conclusion is that methods relying solely on light microscopy cannot be entirely depended upon due to the similarity between Entamoeba histolytica and the non-pathogenic Entamoeba histolytica parasite, making it difficult to distinguish between them under a light microscope[19].

Gastrointestinal infections often coexist with other, nonspecific diseases, with clinical overlap between parasitic, bacterial, and viral infections, which can complicate diagnosis and treatment due to the similar symptoms they present. A study demonstrated that when the identified pathogen was not initially confirmed by the physician as a possible cause of gastrointestinal inflammation, cases of Giardia and one case of Entamoeba histolytica were recorded[20].

RT-PCR has been shown to be effective in detecting bacteria in the gastrointestinal tract, as demonstrated by a number of case studies that have offered illustrative examples. The administration of an oral recombinant Lactobacillus strain that expressed SOD enzyme resulted in a reduction in the inflammation scores of mice that had been stimulated with TNBS in certain studies[6]. There are a lot of challenges and difficulties to overcome when using real-time polymerase chain reaction (RT-PCR) for diagnosing bacteria in the gastrointestinal tract. This is mostly because of the complex structure of the gut microbiome[4].

RT-PCR can be a very challenging method to utilize when trying to identify specific pathogens because the gut is home to a wide variety of bacteria, viruses, and parasites. A reliable diagnosis of infections using RT-PCR alone is difficult to achieve due to the vast number and variety of bacteria that are present. The purpose of this research was to discover whether or whether gender has an effect on the microbial variety or microbial load that occurs within the gastrointestinal tract by analyzing the association between Ct Average, Type of Microorganism, and Patient Gender. The Relationship Between the Type of Microorganism and the Gender of the Patient Based on the findings, the following: It can be deduced from the fact that there was no discernible difference between males and females that the quantity of different types of microorganisms found in both groups is comparable[5].

A statistically significant difference ($p < 0.01$) was found in the study of Ct averages among the bacteria that were found in the gastrointestinal tract. This indicates that there is a major variation in the quantities of microbial DNA among the different pathogens discovered. As a result of having the highest nucleic acid load and the lowest Ct value (49.33), adenovirus was shown to be the most active and intensely infected virus. Entamoeba histolytica and Campylobacter spp[21]. Both had Ct values that were moderately low, which is in line with the relatively high organism loads found in positive samples. Enterotoxigenic E. coli, on the other hand, had a Ct value that was in the middle of the range, which pointed to a moderate amount of bacterial abundance. Giardia lamblia and Rotavirus both had somewhat higher Ct values, which corresponded to lower pathogen concentrations[22]. It could be contributed to the connection with sporadic shedding or milder phases of infection.

The Ct value for Salmonella typhi was greater, which indicates that there was a comparatively low amount of bacteria present in the stool samples. It is important to note that Clostridium difficile toxin A had the highest Ct value (76.37), which indicates that there is very little DNA that can be detected[23]. This may be an indication of subclinical colonization rather than really producing the toxin. The data as a whole demonstrate that every bacterium possesses a unique Ct profile, which highlights the sensitivity of real-time polymerase chain reaction (RT-PCR) in detecting differences in microbial load within gastrointestinal illnesses[24].

Conclusion

The study shows that the gender of the patient doesn't have a big effect on the microbial diversity or microbial load in RT-PCR samples from the gastrointestinal tract. RT-PCR is a very useful diagnostic tool for finding microorganisms in the gut. However, it is important to understand Ct values to combine the data with clinical information-like symptoms, food, drug history and diseases that are already present. The results show that culture, age and medical problems, among other things, probably have a bigger impact on the make-up and quantity of microbiota than gender does. This shows the real need for larger studies that include more samples with longer period of investigation.

Acknowledgements
We would like to thank the Department of Laboratory in Al-Yarmouk Hospital in Baghdad, Iraq for allowing us the opportunity to collect the samples.

Conflict of Interest
The author has no conflicts of interest to disclose

Financial Disclosure
This study has been performed without any funding from outside else.

Authors' contributions
Conception and design by Rafeef Yousif Rashid, Acquisition, analysis, and interpretation of data by Anmar L. Talib, Manuscript drafting and revising it critically by Mustafa A.Al-Sheakh and Statistical sand approval of the final version of the manuscript was done by Shamsuldhuha K. Ramadhan.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

How to cite this article: Rashid RY, Talib AL, Al-Sheakh MA, Ramadhan SK. Molecular Detection of Pathogenic Microorganisms in Gastrointestinal Infections Patients Using Real Time Data among Iraqi Population. Bangladesh J Infect Dis 2025;12(2):243-249

ORCID
Rafeef Yousif Rashid: https://orcid.org/0009-0009-5747-5043
Ammar L. Talib: https://orcid.org/0009-0008-0274-6486
Mustafa A.Al-Sheakh: https://orcid.org/0000-0002-9320-9633
Shamsuldhuha K. Ramadhan: https://orcid.org/0009-0007-7215-5860

Article Info
Received on: 1 September 2025
Accepted on: 20 October 2025
Published on: 1 December 2025

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