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ORIGINAL RESEARCH

A Comparative study of Sensitivity, Specificity of Real-time PCR with the Conventional Methods of diagnosis for Patients with Diarrhoea

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ABSTRACT

Background: Diarrhoeal diseases remain a significant global health burden, particularly in low- and middle-income countries. The ability to identify the specific diarrhoeal disease-causing pathogens rapidly and with an appropriate degree of accuracy is crucial both for patient care and preventing the spread of disease. Stool culture is a classic and not as expedient procedure, and does not manage to detect fastidious pathogens effectively. Real-time PCR, which is a fast, reliable, and accurate method for the identification of a wide range of enteric pathogens, increases diagnostic accuracy and facilitates effective antibiotic prescription. **Methods:** This study included 276 patients with symptoms of diarrhoea and 138 healthy controls. Stool samples were tested for the presence of major bacterial pathogens implicated in the gut using conventional culture and real-time PCR. The subjective information regarding individual backgrounds and health status was gathered. The use of PCR assays allowed for the selection of pathogen-specific genetic markers (such as those in *Shigella, Salmonella, Campylobacter, Clostridium difficile*, and *Vibrio cholerae*). Culture procedures complied with the then-laid guidelines in microbiology procedures. Statistical analysis of the results was performed to determine and compare the sensitivity, specificity, and predictive values of individual methods. **Results:** Out of 276 diarrhoeal patients, real-time PCR detected pathogens in 23 cases, significantly higher than conventional culture (5 cases). PCR showed markedly higher sensitivity (96%) compared to culture (21%), with both methods exhibiting 100% specificity and PPV. Mixed infections and less common pathogens like *Clostridium difficile* were only identified via PCR. Antibiotic use significantly declined post-PCR diagnosis. PCR-positive patients had higher rates of blood/mucus in stool, abdominal pain, and fever. Antimicrobial susceptibility patterns revealed resistance to commonly used antibiotics, emphasizing the value of accurate, rapid diagnostics for targeted therapy. **Conclusion:** Based on results from this study, real-time PCR was more sensitive than traditional methods and especially useful for screening immunocompromised or severely afflicted patients. Application of the real-time PCR in clinical settings is likely to deliver better outcomes in patients due to the possibility of making an accurate and timely diagnosis and choosing the appropriate antimicrobial therapy.

Keywords: Diarrhea, Conventional Culture, Real-time PCR, Sensitivity, Specificity

INTRODUCTION

Diarrheal diseases are a common problem, especially in tropical and developing countries, where they are a leading cause of morbidity and mortality among children under the age of five years. As per the World Health Organization (WHO), diarrhea accounts for approximately 1.7 billion cases and 2.2 million deaths annually across the world. Among the total deaths reported, 15% of deaths are among children below the age of five years. [1-3] In India, over 10 million cases are reported each year, and more than ten thousand deaths occur due to diarrhea every year [4, 5]. The major causative organisms for infectious diarrhea are bacterial, viral, and parasitic agents. Bacterial pathogens such as *Escherichia coli*, *Vibrio cholerae*, *Shigella*, *Salmonella*, *Campylobacter*, and *Yersinia enterocolitica* are commonly implicated [3, 6, 7]. The identification of the causative agents remains a

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problem because the clinical features often overlap, and in approximately 80% of the cases etiology of acute diarrhea remains unclear [8]. This underscores the importance of the need for a rapid, accurate, and sensitive diagnostic method. Conventional stool culture is a widely used method despite its several limitations, which include turnaround times, reliance on selective culture media, and dependence on technical expertise for results. Many times, routine culture-based testing fails to detect bacterial organisms like *Campylobacter* and *Yersinia* [9, 10]. Studies have shown that the yield of stool culture is often low. Such results have been reported by Koplan et al. [11], who found a culture positivity rate of just 2.4%, limited to *Salmonella* and *Shigella*. Delays in sample collection and prior empirical antibiotic use can further reduce the diagnostic yield in cultures [12].

Recent advances in molecular diagnostic methods, such as real-time polymerase chain reaction (PCR), offer rapid and precise pathogen identification.Since these techniques are capable of detecting multiple enteric pathogens simultaneously, they can significantly improve the diagnostic sensitivity and specificity compared to the conventional methods [13-15]. Real-time PCR has demonstrated its ability to detect fastidious organisms that are missed by standard culture techniques, and it is capable of providing results within hours, potentially helping in faster therapeutic decisions. Because diagnostic challenges exist in the clinical management of diarrheal diseases, comparative studies for evaluation of the effectiveness of real-time PCR versus conventional diagnostic methods are essential. These comparisons will help to validate molecular assays as the first-line diagnostic tool and can alter the approach for the management of diarrheal diseases.

MATERIALS AND METHODS

This was a hospital-based prospective study conducted in the department of Microbiology in association with the Department of Gastroenterology and Nephrology, SGPGIMS, Lucknow, India, a multi-level Teaching Hospital.

Inclusion criteria

1. Immunocompetent patients with diarrhoea were included in the study.
2. Patient on immunosuppressive therapy for various causes of autoimmune disorders and transplant recipients (patients with diarrhoea who have undergone at least one transplant were included)

Exclusion criteria

1. Patients with active ulcerative colitis
2. Patients with celiac disease

The study included 414 participants divided into three groups:

1. Immunocompetent patients with diarrhoea (n=138)
2. Immunocompromised patients on immunosuppressive therapy or transplant recipients with diarrhoea (n=138)
3. Age- and sex-matched healthy controls without diarrhoea (n=138)

Sample collection and processing: Each patient provided three successive stool specimens. After the samples reached the laboratory, they were processed using the following steps.

Microscopy: Wet mount preparations with saline and iodine were used for identification of motile protozoa, helminths, and cysts. The use of special stains such as Kinyouns acid-fast procedure was used to detect coccidian parasites and microsporidia were found using modified trichrome stain.

Culture methods: After enrichment in appropriate broths, the samples were cultured on various selective and differential media, such as MacConkey agar, XLD, DCA, CIN agar, and, in the case of Vibrio, TCBS, with specific testing on CCDA for Campylobacter.

Isolation identification was performed using common biochemical methods, while serotyping with NICED antigens for *Salmonella, Shigella,* and *V. cholerae* was used for confirmation.

Antimicrobial susceptibility testing: The antimicrobial susceptibility of bacterial isolates was determined using the Kirby-Bauer disc diffusion method in compliance with the recommendations of the CLSI (2022) [16]. Quality control was performed using *E. coli* ATCC 25922 and S. aureus ATCC 25923 as standard reference strains.

Real-Time Multiplex PCR: For molecular detection of diarrhoea pathogens was used.A*multiplex real-time PCR* was performed using the *FTD Bacterial Gastroenteritis Kit* (Fast Track Diagnostics, Siemens Healthineers), a CE-IVD-marked qualitative assay.

Nucleic Acid Extraction: Total nucleic acid (TNA) was extracted from a portion of each stool sample using the *RTP® Pathogen Kit* according to the manufacturer's protocol. Briefly, 400 µL of the stool sample was treated with lysis buffer and incubated at 65°C, followed by sequential binding, washing, and elution steps using spin column-based purification. The final elution was carried out in 60 µL of elution buffer, and extracts were stored at −80°C until PCR analysis.

Real-Time PCR Assay: The extracted TNA was subjected to real-time PCR using pathogen-specific

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primers and dual-labeled fluorescent probes targeting the following organisms:

1. *Salmonella* spp.
2. *Shigella* spp. and Enteroinvasive*E. coli* (EIEC)
3. Verotoxin-producing *E. coli* (VTEC: vtx1+, vtx2+)
4. *Campylobacter* spp. (*C. jejuni, C. coli, C. lari*)
5. *Yersinia enterocolitica*
6. *Clostridium difficile*

An internal control (murine cytomegalovirus, MCMV) was included in each reaction to monitor extraction efficiency and PCR inhibition. Amplification and detection were performed using a real-time PCR thermocycler capable of multiplex detection. Results were interpreted based on the presence or absence of target-specific amplification curves.

RESULTS

The clinical and demographic characteristics of patients included in the study are given in Table 1. A critical analysis of the table shows that the mean age of the patients and controls was comparable (36.5 ± 18.2 vs. 36.9 ± 18.2 years). In both groups, 75% of patients were males and the remaining 25% were females. In the study group, we found 50% (n=138) of the patients were immunocompromised, whereas all controls were immunocompetent. The duration of diarrhoea in patients in the study group showed 39.1% with acute symptoms (<2 weeks), and a greater proportion (60.9%) had chronic diarrhoea (>2 weeks), indicating a higher prevalence of persistent gastrointestinal symptoms in this cohort.

Table 1: Demographic and Clinical Profile of Study Participants

Parameter	Patients (n=276)	Controls (n = 138)
Mean Age (Years)	36.5 ± 18.2	36.9 ± 18.2
Gender		
Male	207 (75%)	104 (75%)
Female	69 (25%)	34 (25%)
Immunocompromised Status	138 (50%)	0(0.0%)
Diarrhea Duration		
Acute (32 weeks)	108 (39.1%)	-
Chronic (>2 weeks)	168 (60.9%)	-

The detection of pathogens by both conventional methods versus their detection by real-time PCR is given in Table 2. A critical analysis of this table shows that the PCR detected pathogens in 23 cases, significantly higher than the five detected by culture. *Shigella spp* was the most commonly identified organism by PCR (14 cases vs. 3 by culture, p<0.001), followed by *Campylobacter spp* (4 vs. 0, p<0.001). Mixed infections and other pathogens were also more frequently detected via PCR. The differences were statistically significant for most organisms; this shows that PCR has superior sensitivity and ability to detect pathogens missed by conventional culture methods.

Table 2: Pathogen Detection by PCR vs. Conventional Methods

Pathogen	PCR-Positive (n=23)	Culture-Positive (n=5)	p-value
Shigella spp	14	3	<0.001*
Campylobacter spp	4	0	<0.001*
Salmonella spp	2	1	0.007*
Clostridium difficile	1	0	0.03*
Vibrio cholerae	0	1	0.12
Mixed Infections	1	0	0.02*

*Significant

The assessment of sensitivity and specificity of real-time PCR versus culture is shown in Table 3. The analysis of the table reveals that PCR demonstrated markedly higher diagnostic performance compared to conventional culture methods. The sensitivity of PCR was 96% as compared to 21% for the conventional culture method, showing its higher ability to detect true positives. Evaluation of the specificity and PPV of both methods showed 100% specificity as well as positive predictive value (PPV), which indicates that there is a lower likelihood of false-positive results. PCR also had a higher negative predictive value (NPV) at 99% compared to the conventional culture value of 92%, further supporting its reliability in ruling out infections. These results confirm the robustness of PCR as a diagnostic tool for diarrhoeal pathogens.

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Table 3: Sensitivity and Specificity of PCR vs. Culture

Metric	PCR	Culture
Sensitivity	96%	21%
Specificity	100%	100%
Positive Predictive Value (PPV)	100%	100%
Negative Predictive Value (NPV)	99%	92%

Antimicrobial Susceptibility of Pathogens was done as per the CLSI (2022) guidelines depicted in Table 4. *Shigella sonnei* was sensitive to ciprofloxacin and ceftriaxone but resistant to ampicillin. *Salmonella Paratyphi B* showed sensitivity to cotrimoxazole and ceftazidime, with resistance to azithromycin. *Vibrio cholerae* was sensitive to tetracycline and doxycycline but resistant to cotrimoxazole and ampicillin. Importantly, *Shigella dysenteriae* exhibited sensitivity only to ciprofloxacin, with resistance to both ceftriaxone and ampicillin.

Table 4: Antimicrobial Susceptibility of Pathogens

Pathogen	Sensitive To	Resistant To
Shigella sonnei	Ciprofloxacin, Ceftriaxone	Ampicillin
Salmonella Paratyphi B	Cotrimoxazole, Ceftazidime	Azithromycin
Vibrio cholerae	Tetracycline, Doxycycline	Cotrimoxazole, Ampicillin
Shigella dysenteriae	Ciprofloxacin	Ceftriaxone, Ampicillin

The analysis of clinical features of PCR-positive patients revealed more severe clinical features compared to those with PCR-negative cases. The analysis of Table 5 revealed that blood or mucus in stool was found in 56.5% of PCR-positive cases, compared to only 7.9% of PCR-negative patients ($p<0.001$). The symptoms of abdominal pain and fever were more common in PCR-positive cases (73.9% and 69.6%, respectively). The p-values were found to be significant. The occurrence of weight loss was reported more in the PCR-positive group (21.7% vs. 9.9%); this difference was not statistically significant ($p=0.09$). These findings suggest a strong association between pathogen presence, as confirmed by PCR, and more pronounced gastrointestinal symptoms.

Table 5: Clinical Features of PCR-Positive Patients

Clinical Feature	PCR-Positive (n=23)	PCR-Negative (n=253)	p-value
Blood/Mucus in Stool	13 (56.5%)	20 (7.9%)	<0.001*
Abdominal Pain	17 (73.9%)	85 (33.6%)	<0.001*
Fever	16 (69.6%)	62 (24.5%)	<0.001*
Weight Loss	5 (21.7%)	25 (9.9%)	0.09

*Significant

The assessment of antibiotic use before and after PCR diagnosis is given in Table 6. The results show a significant change in antibiotic usage patterns following PCR-based diagnosis. Before the PCR diagnosis, empirical treatment was common. After the availability of PCR results, targeted therapy was utilized, marked by a reduction in the use of metronidazole dropped to 8.7%, and ciprofloxacin, to 4.3%. The use of Piperacillin-tazobactam was stopped completely, although vancomycin usage remained unchanged. This shows that molecular diagnostics can guide for appropriate and judicious use of antibiotics, potentially reducing antibiotic resistance and improving the outcomes.

Table 6: Antibiotic Use Before vs. After PCR Diagnosis

Antibiotic	Pre-PCR Use (n=23)	Post-PCR Use (n=23)
Metronidazole	9 (39.1%)	2 (8.7%)
Ciprofloxacin	8 (34.8%)	1 (4.3%)
Piperacillin-Tazobactam	3 (13.0%)	0 (0.0%)
Vancomycin	1 (4.3%)	1 (4.3%)

DISCUSSION

The present study was conducted to analyse the sensitivity and specificity of conventional culture methods versus real-time PCR in detecting enteric pathogens in patients with diarrhea. The results of this study showed that the real-time PCR identified a significantly higher number of pathogens compared to the culture method, and the overall sensitivity of PCR

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was 96% versus 21% for the culture method of identification. The observations of our study are in agreement with previous studies that have emphasized an enhanced sensitivity and rapid turnaround time of molecular methods in identifying gastrointestinal pathogens, especially in polymicrobial and low-load infections [17, 18]. This study found *Shigella spp* was the most commonly identified organism, which was detected in 14 cases by PCR versus 3 cases by the culture method. This shows the limitations of the traditional methods in detecting fastidious or low-viability microorganisms. More importantly, pathogens such as *Campylobacter spp* and *Clostridium difficile* were identified exclusively by PCR. This shows the technique's superiority in detecting anaerobic and fastidious organisms that may be missed by conventional culture-based methods [19]. This is of particular importance in immunocompromised patients who are often affected by diarrheal diseases, and 50% of the cases of our study included immunocompromised patients. The early and accurate pathogen identification can prove vital in the management of this vulnerable group [20]. The analysis of the clinical profile of PCR-positive patients further proves the diagnostic utility of PCR. We found that the patients with PCR-detected infections had higher severity of symptoms such as blood/mucus in stool (56.5% vs. 7.9%), abdominal pain (73.9% vs. 33.6%), and fever (69.6% vs. 24.5%) compared to PCR-negative patients. These results demonstrate that PCR is better at detecting pathogens and is more obviously related to the seriousness of the clinical outcomes, justifying the use of PCR for tailoring treatment strategies [21]. The implementation of the PCR method altered the antibiotic prescribing pattern in our cases. Once diagnosed with a pathogen-specific disease, the use of broad-spectrum antibiotics such as metronidazole and ciprofloxacin was reduced considerably. This reduction demonstrates how PCR can contribute to the improvement of antimicrobial stewardship, whereby unnecessary or unwarranted antibiotics are employed; this is especially important with respect to antibiotic resistance [22]. *Shigella spp* were found to be resistant to ampicillin, yet susceptible to ciprofloxacin and ceftriaxone, suggesting that PCR-based identification could have a significant impact on selecting appropriate therapy. Despite the advantages of PCR, its high cost and limited availability in resource-limited areas may, in fact, prevent its use. However, given the growing burden of antimicrobial resistance and the need for rapid diagnostic devices, the shift to the use of molecular methods in the diagnostic algorithms might significantly improve the quality of care for patients and public health surveillance initiatives [23].

CONCLUSION

In conclusion, based on results from this study, real-time PCR was more sensitive than traditional methods and especially useful for screening immunocompromised or severely afflicted patients. Application of the real-time PCR in clinical settings is likely to deliver better outcomes in patients due to the possibility of making an accurate and timely diagnosis and choosing the appropriate antimicrobial therapy.

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