


# Multiplex PCR

## Aid to more-timely and directed therapeutic intervention for patients with infectious gastroenteritis

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### Abstract

**Background:** Multiplex PCR is a sensitive and rapid method compared with conventional methods. Therefore, we use multiplex PCR for the rapid detection of the four major intestinal pathogens causing gastroenteritis (*Shigella* spp., *Campylobacter* spp., *Aeromonas* spp. and *Enterohemorrhagic Escherichia coli* [EHEC]) in stool specimens.

**Materials and methods:** A prospective randomized study using 200 stool samples obtained from patients presented with acute gastroenteritis during the study period (between February 2019 and December 2021). Bacteria in stool samples were identified using conventional culture methods and multiplex PCR for stool samples

**Results:** The identified organisms using conventional cultures; were *Shigella* (27%), *Aeromonas* species (10%) and *EHEC* (*O157*) (8%). Using multiplex PCR. *Shigella* spp. was the most commonly identified pathogen (detected in 40.5% of positive samples), followed by *Aeromonas* spp. (30%), *EHEC* (20%) and *Campylobacter* species was only detected in (1%) of positive samples. The diagnostic evaluation of multiplex PCR in relation to conventional method in diagnosis of *Shigella*, *EHEC* and *Aeromonas* showed, sensitivity of 100% (for each), specificity of 88.5%, 92.4%, 77.8% respectively. However, the diagnostic evaluation of multiplex PCR in relation to conventional method in diagnosis of *Campylobacter* showed specificity of 99% and NPV of 100%.

**Conclusions:** Multiplex PCR is an accurate and rapid method for detection of common intestinal pathogens causing severe gastroenteritis. a rapid method that could be used in outbreaks for diagnosis of the common enteric pathogens causing fatal gastroenteritis.

**Abbreviations:** EHEC = Enterohemorrhagic *Escherichia coli*, HPF = High power field, HS = Highly significant, IG = Infectious gastroenteritis, Multiplex PCR = Multiplex polymerase chain reaction, NPV = Negative predictive value, NS = Non-significant, PPV = Positive predictive value, RBCs = Red blood cells, S = Significant, Spp = species, WBCs = white blood cells, XLD agar = Xylose Lysine Deoxycholate.

**Keywords:** Enterohemorrhagic *E coli* O157 spp., multiplex PCR, *Shigella* spp, stool culture

### 1. Introduction

Infectious gastroenteritis (IG) is one of the most common diseases worldwide, killing millions of individuals each year. In industrialized countries, IG remains a major public health burden, although mortality is low. However, mortality can be found in developing countries, where epidemics of bacterial gastroenteritis may develop.<sup>[1]</sup> *Shigella*, *Campylobacter* and enterohemorrhagic *E coli* are three common intestinal pathogens involved in gastroenteritis and are important to study because of the frequency and severity of symptoms they cause.

EHEC identification would also be critical in the event of food-borne illness outbreaks or agroterrorism, thus the accurate and rapid diagnosis is a must in outbreaks to direct therapeutic intervention.<sup>[2]</sup>

*Aeromonas* causes diarrheal infections, most commonly in children (especially those under 5 years) and in immune-compromised patients and the incidence of gastroenteritis tends to be higher in summer than other seasons. The organism has been also isolated with high frequency from patients with traveler's diarrhea.<sup>[3]</sup> Acute self-limiting diarrhea occurs in children, and chronic gastroenteritis or enterocolitis may occur in children

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The study protocol was approved by South Valley Faculty of Medicine Ethical Committee and written informed consent was obtained from each subject.

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and the elderly. The presentation of gastroenteritis caused by *Aeromonas* includes various combinations of fever, vomiting, and increased fecal leucocytes or erythrocytes.<sup>[4]</sup>

Most medical microbiology laboratories use traditional and conventional methods for detection of infectious cause of diarrhea, among direct microscopic examination, the use of conventional cultures and identification of organisms using biochemical reactions, which take a long time; results can release after 3 to 4 days.<sup>[2]</sup> Furthermore, misidentification of culture increases hands-on time and delay in reporting of a definite negative result. Other problems are the viable but non-culturable state of *Campylobacter jejuni*<sup>[5]</sup> and the limited viability of *Shigella* outside the human body. These may compromise the sensitivity of culture.<sup>[6]</sup> Another value of the accurate and rapid diagnosis of gastroenteritis is the rapid start of therapeutic intervention as just mentioned before. It is known that bacterial gastroenteritis is a self-limiting disease, and antimicrobial therapy is not particularly required. Clinically, it is not possible to distinguish pathogens that will respond to antibiotics from those that will not respond. Antibiotics add to the cost of treatment, put the patient at risk for adverse events, and can encourage development of resistant bacteria.<sup>[7]</sup> However, in certain infections e.g. dysentery, cholera, and for certain cases of persistent diarrhea particularly in infants, elderly people, and immunocompromised individuals who are at higher risk of developing more severe complications, treatment can decrease the duration and severity of illness if it is initiated early in the course of infection.<sup>[8]</sup>

Molecular methods are sensitive and rapid methods compared to conventional methods. However, broad application remains limited due to their assumed high costs, inhibition caused by fecal constituents, and the need for specialized laboratories. Due to the high throughput of stool screening and the number of possible enteric pathogens, implementation of a molecular approach which uses multiplexing of targets is mandatory.<sup>[9]</sup> The objective of this study is to evaluate the multiplex PCR for the rapid detection of four major intestinal pathogens causing gastroenteritis (*Shigella* spp., *Campylobacter* spp., *Aeromonas* spp. and Enterohemorrhagic *Escherichia coli* (EHEC) in stool specimens compared to other conventional culture methods.

## 2. Materials and Methods

### 2.1. Patient selection

A total of 200 stool samples were collected randomly from patients attended Helwan, South Valley and Tanta Universities outpatient clinics with clinical manifestations of acute gastroenteritis; between February 2019 and December 2021. For each stool sample, a portion of the sample was transferred into a sterile tube and stored at  $-20^{\circ}\text{C}$  for molecular diagnostics. The rest was used for stool culture process within 1 hours of receipt. All patients were subjected to full history taking, lying stress on manifestations of gastroenteritis (diarrhea, vomiting, fever and abdominal discomfort), bleeding tendency, hematuria and history of antibiotic intake. All patients aged from 18 to 60 years with clinical manifestations of acute gastroenteritis were included in the study. Patients with history of antibiotics intake, chronic diarrhea of any cause, cancer anywhere in the body including cancer colon, drug intake and immunosuppression due to any cause were excluded from the study.

### 2.2. Microbiological processing of stool samples

**2.2.1. Sample processing.** Two Microbiology doctors with more than 5-year experience were responsible for stool samples processing as a routine test in Microbiology department of Helwan, South Valley and Tanta Universities.

Stool specimen in a wide-necked container was processed within 1 hour from its arrival to the lab. Macroscopy of stool samples will

be done to check for characteristic color, presence of blood and mucous. Wet smear of stool samples was examined microscopically for presence of pus, RBCs and parasitic causes as protozoa trophozoite, ameba cyst. Basic fuchsin stain (10 gm/L) for 10 to 20 seconds was used for rapid examination of *Campylobacter* spp. which appears as small, S-shaped, gram-negative, non-spore forming rods.

**2.2.2. Sample culture.** Using a sterile inoculation loop, a loopful from the stool specimen (from areas with blood or mucous, if present) was inoculated onto a plate of MacConkey agar (Oxoid Discs, Oxoid, North Shore, United Kingdom) for *Enterobacteriaceae*, a plate of XLD agar selective media for *Salmonella* and *Shigella* (Oxoid) and a plate of Skirrow's agar (Oxoid) for *Campylobacter* spp. isolation. The inoculated XLD agar and MacConkey agar were then incubated at  $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 18 to 48 hours under aerobic conditions. The inoculated Skirrow's agar medium was incubated at  $42^{\circ}\text{C} \pm 1^{\circ}\text{C}$  under microaerophilic conditions for 48 to 72 hours. A loopful of the stool specimen was additionally inoculated onto a tube of Selenite F broth, for maximum recovery of *Salmonella* and *Shigella* is obtained when inoculating an enrichment broth in addition to primary direct plating of specimens, and was incubated at  $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$  under aerobic conditions for 18 hours. After 18 hours incubation, a subculture was done from the surface of the Selenite F broth on XLD and MacConkey agar media and the plates were incubated for 48 hours at  $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$  under aerobic conditions. Except for the Skirrow's agar plate, which was left to be examined at the end of the incubation period, other plates were examined daily for the presence of growing microorganisms. Growing colonies were further identified by traditional biochemical reactions (triple sugar iron agar, citrate, urase, oxidase, lysine iron agar and motility indole ornithine) and sub cultured on a blood agar plate for further identification by API 10S (Biomérieux, Marcy-l'Étoile, France). When cases of severe bloody diarrhea were implicated, sorbitol MacConkey media (Liofilchem, Roseto degli Abruzzi, Italy) was used to detect *E coli* O157:H7 (most O157:H7 *E coli* are sorbitol-negative so appear colorless).<sup>[10]</sup>

### 2.3. Multiplex PCR extraction and amplification

A multiplex PCR was performed (by 2 clinical pathology doctors with more than 5 year experience) on all stool specimens for the detection of *Shigella* spp., *Campylobacter* spp., *Aeromonas* spp. and *E coli* O157 DNA. After thawing the samples, 1 mL (for liquid stool) or 1 gram (for solid feces) was used for the extraction of bacterial DNA using the QIA amp DAN Mini Kit supplied by (Qiagen, Germantown, MD) according to the manufacturer's instructions. The bacterial genomes were amplified by multiplex real-time PCR using the Seeplex® Diarrhea ACE Detection kits (Seegene, Republic of [South Korea]) were used for the multiplex PCR test. The bacterial panels included:

a) Diarrhea B1 ACE detection is a multiplex assay that permits the simultaneous amplification of target DNA of: *Shigella* spp. (*S flexneri*, *S boydii*, *S sonnei* and *S dysenteriae*), *Campylobacter* spp. (*C jejuni* and *C coli*) and Internal Control (IC) Figure 1.

b) Diarrhea B2 ACE Detection is a multiplex assay that permits the simultaneous amplification of target DNA of: *Aeromonas* spp. (*A salmonicida*, *A sobria*, *A bivalvium*, *A hydrophila*), *E coli* O157:H7, VTEC family and Internal Control (IC). The target genes were respectively IpaH, Rfb, Hip and Hly for *Shigella*, EHEC, *Campylobacter* and *Aeromonas* Figure 2.

*Shigella dysenteriae* (Shiga) (ATCC® 13313) was used as a positive control and *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™) was used as a negative control

### 2.4. Statistical analysis

Analysis was done using the IBM SPSS statistics (V. 20.0, IBM Corp., Chicago, USA, 2011). Descriptive Statistics: Qualitative

data were presented as counts and percentage. The association between each 2 variables or comparison between 2 independent groups as regards the categorized data was done using the Chi square test or the Fisher exact test. The probability of error at > .05 was considered non-significant, while ≤ .05 was considered significant and < .01 was considered highly significant.

**2.4.1. Ethical approval and consent to participate.** The study has been performed in accordance with the Declaration of Helsinki. The study protocol was approved by South Valley Faculty of Medicine Ethical Committee code number (SVU-MED-GIT023-4-22-9-447).

**2.4.2. Conflict of interest.** We don't receive any financial support or relationships that may pose conflict of interest in this work.

**3. Results**

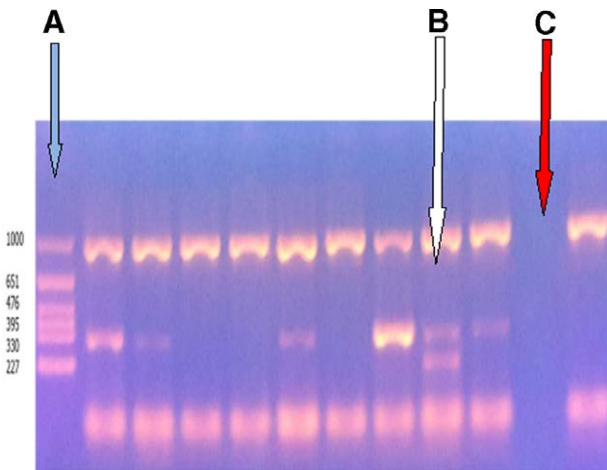
**3.1. Demographic and clinical presentation of patients with gastroenteritis**

In this prospective randomized cohort study, between February 2019 and December 2021, a total of 200 stool samples were collected from patients presented with acute gastroenteritis;

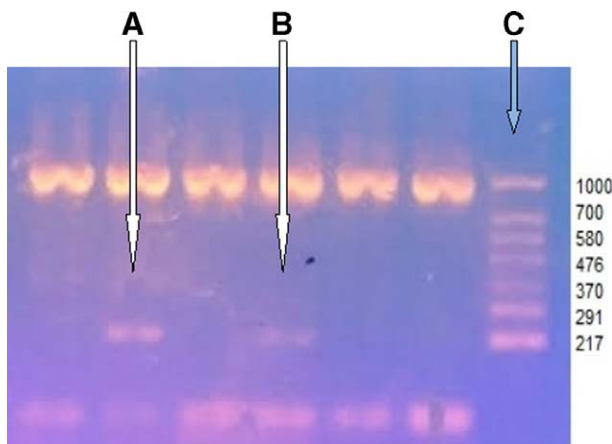
including 120 female samples and 80 male samples with their age ranging from 18 years to 55 years. One hundred eighty (90%) of patients suffered from watery diarrhea while (10%) had bloody diarrhea. ninety patients (45%) had associated abdominal cramps/colic, (50/200, 25%) had vomiting, (40/200, 20%) had dehydration, (20/200, 10%) had steatorrhea and (4/200, 2%) complained from weight loss Table 1.

**3.2. Macroscopic and microscopic examination of the studied stool samples of all included patients**

The macroscopic examination in the studied stool samples showed that (160/200, 80%) of samples were watery in consistency whereas (24/200, 12%) were semi-formed. Regarding the stool color (66%) of the studied samples were brownish, (20/200, 10%) were whitish, (22/200, 11%) were greenish, (12/200, 6%) were reddish, and (14/200, 7%) yellowish. Out of the 200 studied samples, 136 samples (68%) contained visible mucous and 12 samples (6%) were bloody. As regard to the microscopic examination, (40/200, 20%) of the studied samples had a WBCs count < 10 cells/HPF, (90/200, 45%) had a WBCs count 10 to 49 cells/HPF, (40/200, 20%) had a WBCs count 50 to 99 cells/HPF, and (36/200, 18%) had a WBCs count > 100 cells/HPF. Three out of the 200 studied samples (5.5%) contained red blood cells > 100/HPF Table 2.



**Figure 1.** Multiplex PCR using the ACE B1 diarrhea kit (A) ACEB1 marker (B) Combined Shigella and Campylobacter (bp: 330 and 227 respectively), (C) Negative control. PCR = polymerase chain reaction.



**Figure 2.** Multiplex PCR using the ACE B2 diarrhea kit showing (A) and (B) Aeromonas spp. (bp: 217), (C) ACEB2 marker. PCR = polymerase chain reaction.

**Table 1**

**Demographic and clinical presentation of patients with gastroenteritis.**

Variables	N (%)
Age (yr) (Mean ± SD)	49.1 ± 9.8
Sex, N (%)	
Female	120 (60)
Male	80 (40)
Clinical presentation, N (%)	
Watery diarrhea	180 (90)
Bloody diarrhea	20 (10)
Abdominal cramps/colic	90 (45)
Vomiting	50 (25)
Dehydration	40 (20)
Steatorrhea	20 (10)
Loss of weight	4 (2)

**Table 2**

**Macroscopic and microscopic examination of the studied stool samples of all included patients.**

Stool examination		N (%)
Macroscopic examination		
Consistency	Watery	160 (80)
	Loose	16 (8)
Color	Semi-formed	24 (12)
	Brownish	132 (66)
	Whitish	20 (10)
	Greenish	22 (11)
	Reddish	12 (6)
Mucous	Yellowish	14 (7)
		136 (68)
	Blood in stool	12 (6)
Microscopic examination		
WBC/HPF	<10	40 (20)
	10–49	90 (45)
	50–99	40 (20)
	>100	30 (15)
RBCs (>100/HPF)		3 (1.5)

HPF = high power field, RBCs = red blood cells, WBC = white blood cells.

**3.3. Comparison between conventional methods and multiplex-PCR with regard to the recovery of the studied pathogens in the 200 included patients**

Using conventional culture and identification methods, (192 out of 200, 96%) studied stool samples gave positive results. The most commonly identified organisms were *E coli* (118/200, 59%), *Shigella* (54/200, 27%) and *Aeromonas* species (20/200, 10%). *Campylobacter* was not revealed in cultures.

Using specific *E coli* O157 anti-sera, 16 isolates out of 118 identified *E coli* (13.56%) were found to belong to the O157 serotype, and the remaining isolates (102/118, 86.4%) were the normal gut *E coli* microbiota.

Thus, the EHEC represented 8% (16/200) of the total identified pathogens by conventional culture method.

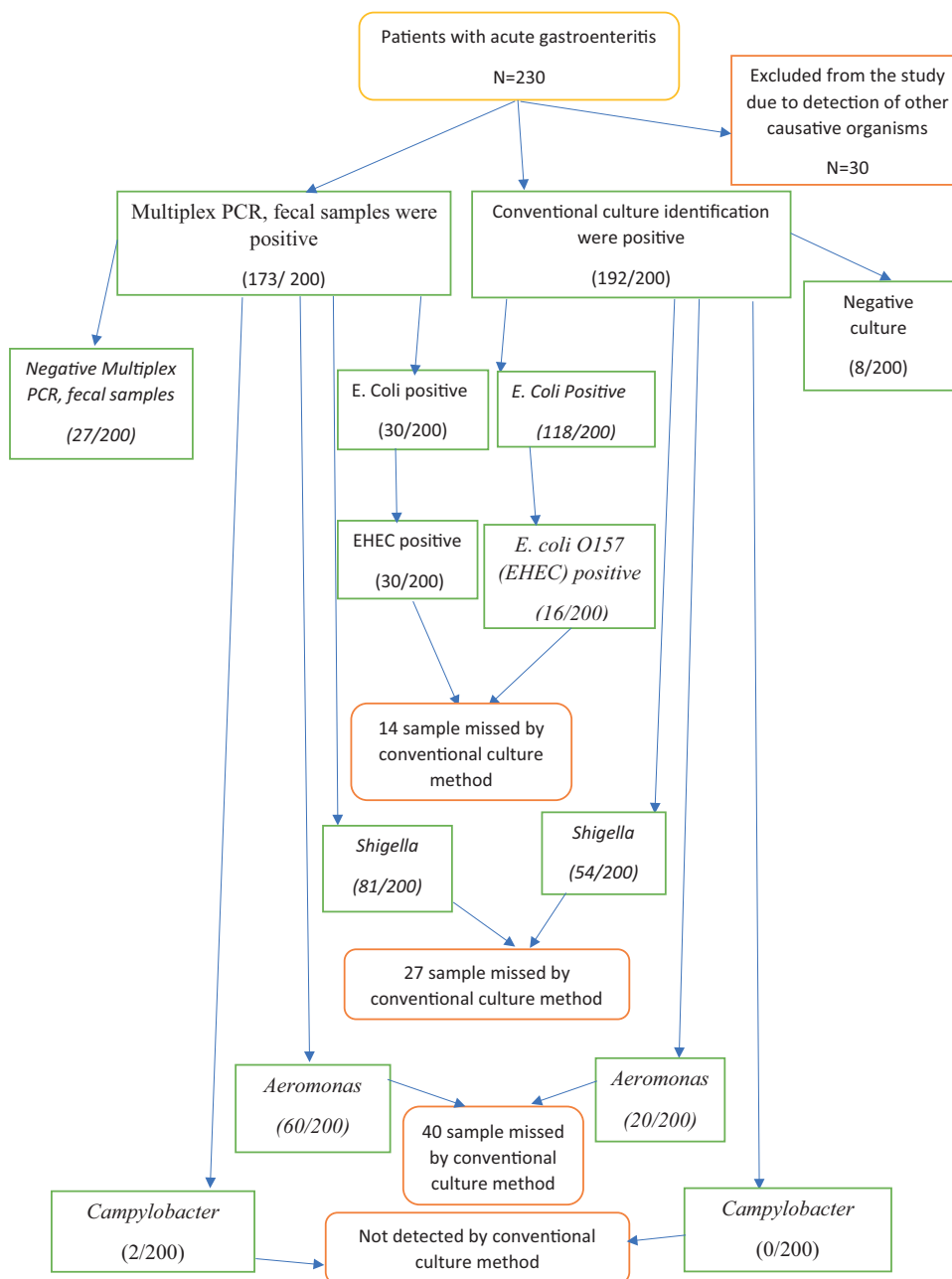
Using multiplex PCR, 173/ 200 fecal samples (86.5%) were positive. *Shigella* spp. was the most commonly identified pathogen as it was detected in (81/200, 40.5%) of the PCR-positive samples; Out of the 81 multiplex PCR-positive *Shigella* spp., 27 samples (27/81, 33.3%) were not detected by conventional methods.

*Aeromonas* spp. was detected in 60 samples (60/200, 30%); out of the 60 positive samples., forty samples (40/60, 66.67%) were missed by the conventional culture methods.

EHEC were detected in (30/200, 15%) samples. Of them 14 samples were missed by conventional culture method.

*Campylobacter* species were also detected in only 2 samples (2/200, 1%); *Campylobacter* spp. isolate was only detected by the multiplex PCR Figure 3.

Finally, Comparison between conventional methods and multiplex-PCR as regard to the recovery of the pathogens in



**Figure 3.** Comparison between conventional culture methods and multiplex-PCR as regard to the recovery of the pathogens in the 200 included patients. EHEC = Enterohemorrhagic Escherichia coli, PCR = polymerase chain reaction.



the 200 included patients. Multiplex-PCR appeared to be more advantageous to conventional detection methods regarding the detection of *Shigella*, EHEC and *Aeromonas* isolates with a statistically significant difference ( $P < .05$ ).

Although, no significant difference was detected between conventional culture methods and Multiplex-PCR regarding detection of *Campylobacter*, but this pathogen was only detected by Multiplex-PCR method Table 3.

### 3.4. Diagnostic evaluation of multiplex PCR in relation to conventional method in diagnosis of studied pathogen

The majority of infections were caused by a single pathogen. However, multiple enteric pathogens in individuals' samples were detected by the multiplex PCR not by conventional methods.

Only three samples contain mixed infections with the four studied pathogen.

The diagnostic evaluation of multiplex PCR in relation to conventional method in diagnosis of *Shigella*, EHEC and *Aeromonas* showed, sensitivity of 100% (for each), specificity of 88.5%, 92.4%, 77.8% respectively, PPV of 66.7%, 53.3%, and 33.3%, respectively and NPV of 100% (for each). However, the diagnostic evaluation of multiplex PCR in relation to conventional method in diagnosis of *Campylobacter* showed specificity of 99% and NPV of 100%; (sensitivity and PPV cannot be calculated as no *Campylobacter* isolate identified by conventional method; which considered as gold standard for diagnosis of bacterial infection) Table 4.

**Table 3**  
Comparison between conventional methods and multiplex-PCR with regard to the recovery of the studied pathogens in the 200 included patients.

		Conventional (N = 200)	Multiplex PCR (N = 200)	$\chi^2$	P value
Shigella	Negative	146 (73%)	119 (59.5%)	10.1	.001 (HS)
	Positive	54 (27%)	81 (40.5%)		
Enterohemorrhagic <i>E coli</i> (EHEC)	Negative	184 (92%)	170 (85%)	4.8	.028 (S)
	Positive	16 (8%)	30 (15%)		
Campylobacter	Negative	200 (100%)	198 (99%)	2.1	.156 (NS)
	Positive	0 (0%)	2 (1%)		
Aeromonas	Negative	180 (90%)	140 (70%)	25	<.001 (HS)
	Positive	20 (10%)	60 (30%)		

PCR = polymerase chain reaction.

$\chi^2$ : Chi-square test.

S: P value < .05 is considered significant.

HS: P value < .001 is considered highly significant.

NS: P value > .05 is considered non-significant.

**Table 4**  
Diagnostic evaluation of multiplex PCR in relation to conventional method in diagnosis of studied pathogen.

Diagnostic evaluation of Multiplex PCR (N = 200)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Shigella	100	81.5	66.7	100
Enterohemorrhagic <i>E coli</i> (EHEC)	100	92.4	53.3	100
Campylobacter	–	99	–	100
Aeromonas	100	77.8	33.3	100

NPV = negative predictive value, PCR = polymerase chain reaction, PPV = positive predictive value.

## 4. Discussion

In Africa, Asia, and South America, diarrhea accounts for 13% of deaths annually.<sup>[11]</sup>

Symptomatic treatment is the aim of therapy of acute gastroenteritis; antibiotic therapy required in specific critical situation; however, one of the great problems is a long duration needed till stool culture results become available, so its impact on treatment is minimal.<sup>[12,13]</sup>

Stool culture is the gold standard for identification of bacteria present in the stool. However, its time consuming and require specific culture agars followed by biochemical, morphologic, and serologic testing to confirm the culture isolate.<sup>[14-18]</sup>

It's known that multiplex PCR, is time saving allow early and multiple gastrointestinal infections identification, this is of great important for early and accurate treatment of acute bacterial gastroenteritis especially in critically ill patients.<sup>[19]</sup>

Therefore, we aim in this study to evaluate efficacy of multiplex PCR method, as a rapid and accurate method for diagnosis of the four common enteropathogenic bacteria responsible for fatal gastroenteritis; that could be missed when using conventional laboratory methods.

To our knowledge, this study is the first one done in Egypt using multiplex-PCR for accurate diagnosis of the four common bacterial pathogens responsible for adults' infectious gastroenteritis.

In this study, using conventional culture; *E coli* were the most commonly identified organisms it is detected in more than half of samples, followed by *Shigella* (27%), finally, *Aeromonas* species which detected in only 10% of samples.

This come in agreement with El Hassan et al,<sup>[20]</sup> who reported that, *E coli* was the most commonly isolated enteropathogen from diarrheal stool; being responsible for about 72% of cases of diarrhea. Followed by *Shigella* spp. which detected in 8% of stool samples.

Yet, our results are much higher than those found in Saudi Arabia by El-Sheikh and El-Assouli<sup>[21]</sup> who detected EHEC in about 2% of cases presenting with diarrhea. This may be related to his study on the prevalence of different causes (viral, bacterial, and parasitic) of acute diarrhea among children with in Jeddah. In addition to different worldwide prevalence of pathogens responsible for infectious gastroenteritis.

Both *Shigella* spp. and *Enterohemorrhagic E-Coli* (EHEC) are intestinal pathogen that presented with dysentery; however, development of fatal complications is commonly reported with *shigella* spp.<sup>[22,23]</sup> So, discrimination between *Shigella* spp. and EHEC is of clinical and epidemiological importance, for rapid and early outbreak control.<sup>[24]</sup>

In our study, using multiplex PCR, 86.5% of fecal samples were positive. *Shigella* spp., was the most commonly identified pathogen as it was detected in about 40% of the PCR-positive samples, with 27 samples detected by using multiplex-PCR and missed by conventional culture methods. Also, *Aeromonas* spp. and EHEC were detected by multiplex-PCR in 60 (30%) and 30 (15%) samples respectively with 40 samples and 14 samples respectively were missed by the conventional culture methods.

Our results are in agreement with O'Leary et al,<sup>[2]</sup> who compared between multiplex PCR and conventional diagnostic methods for accurate detection of bacterial pathogen from feces of patients with acute gastroenteritis; they found that, all positive results by conventional methods were matched by positive results with multiplex PCR with additional 17 positive results detected by multiplex PCR.

Our results come in agreement with Zaki and El-Adrosy<sup>[25]</sup> at Mansoura, Egypt who reported that EHEC was positive by PCR in 25% of patients affected in an outbreak of diarrhea.

Also, Gray et al,<sup>[26]</sup> reported that using multiplex-PCR provide both improved recovery of pathogens and detection of pathogens unable to be tested by conventional tests.

At the same time, Dixit et al,<sup>[27]</sup> by using the same multiplex PCR commercial kit as in our study, *Shigella* spp. was the most detected organism, followed by *Campylobacter* and *E. coli*. Also, Mota et al,<sup>[28]</sup> who investigated the bacterial causes of bloody diarrhea by both conventional and molecular methods, *Shigella* was recovered from most cases, while *EHEC* was detected in 1% of cases.

Likewise, Guan et al and Thiem et al,<sup>[29,30]</sup> who found that multiplex PCR has a higher detection rate for *EHEC* and *Shigella* spp. than conventional culture methods. Also, two previous studies in India<sup>[31]</sup> and Thailand<sup>[32]</sup> suggest that stool culture has a lower sensitivity than PCR in the diagnosis of *Shigella* spp.

*Shigella* spp. frequently escape detection by traditional culture methods. However, detection of the four species of *Shigella* by real-time PCR, targeting the invasion plasmid antigen H (ipaH) gene; can mask the *EIEC* detection in stool by real-time PCR, which carry the same target gene. IpaH is carried by all four *Shigella* species as well as by enteroinvasive *Escherichia coli* (*EIEC*). Because *EIEC* is rare in fecal specimens from patients with diarrhea, it is thought that most organisms detected by ipaH-specific PCR are *Shigella* spp.<sup>[30]</sup> Multiplex PCR can be modified to identify *EIEC* in cultures from ipaH-positive samples by an assay with two sets of multiplex PCR reactions that differentiates *Shigella* and *EIEC* based on the presence/absence of at least two out of six loci, the majority of *Shigella* genomes lacked all six loci, while at least two loci were present in most *EIEC* genomes.<sup>[33]</sup>

Different studies done to confirm the volatility of molecular technique over conventional culture method in discriminating between *Shigella* spp. and diarrheagenic *E-Coli*.

Likewise, Islam et al,<sup>[34]</sup> in Bangladesh, who found that conventional culture methods for the isolation of *Shigella* spp. had a sensitivity of 72% however, when the PCR technique targeting the ipaH gene it was considered as the gold standard. They recommended PCR to be employed in routine diagnosis of dysentery in clinical centers as well as in epidemiologic studies. Also, Dutta et al,<sup>[31]</sup> agreed with that of Islam et al,<sup>[34]</sup> and added that the PCR assays can further identify a number of non-typable *Shigella* strains, which would have remained undiagnosed if PCR had not been used.<sup>[31,34]</sup>

Moreover, Thiem et al and Wang et al<sup>[30,35]</sup> found that real-time PCR targeting the ipaH gene; detected *Shigella* spp. in 58% of randomly selected *Shigella* culture negative specimens and in 97% of *Shigella* culture positive specimens. The authors concluded that the high detection rate of ipaH gene in culture negative specimens through use of real-time PCR suggests that earlier estimates of shigellosis burden measured by conventional culture may have underestimated the true disease burden.

*Campylobacter* species isolate in Egypt was more frequent than *Salmonella*, *Shigella* and other enteric bacterial pathogens.<sup>[36]</sup> Also, WHO reported that *Campylobacter* was the most common bacterial cause of human gastroenteritis in the world; about 3 to 4 times more frequent than *Salmonella* and *E. coli*.<sup>[37]</sup>

One of the great problems of *Campylobacter* infection is its serious long-term complications, including the peripheral neuropathy (Guillain-Barré syndrome and Miller Fisher syndrome) temporary paralysis, arthritis and irritable bowel syndrome.<sup>[38]</sup>

In our study *Campylobacter* spp., was isolated only by using multiplex-PCR in two male patients presented with bloody diarrhea.

This comes with White et al,<sup>[39]</sup> who found that 4% of patients presented with bloody diarrhea, had campylobacteriosis, and considered older patients are more likely to have severe illness and be hospitalized.

Also, Barakat et al,<sup>[40]</sup> reported that multiplex PCR is a valuable method for detection of virulent and resistant genes of *Campylobacter* bacteria.

In this study, the diagnostic evaluation of multiplex PCR in relation to conventional method in diagnosis of *Shigella*, *EHEC* and *Aeromonas* showed sensitivity of 100% each and specificity

of 88.5%, 92.4%, and 77.8% respectively. However, the diagnostic evaluation of multiplex PCR for *Campylobacter* showed specificity of 99% but sensitivity cannot be calculated (as no cases detected by conventional culture method).

This nearly come in agreement with *Rundell et al* who found in his study that, multiplex PCR had a sensitivity of for detection of *Campylobacter*, *EHEC* and *Shigella* spp. of 100%, 96.8%, and 97.6% respectively; and specificity of 100%, 99.5%, and 100% respectively depending on the causative bacteria.<sup>[41]</sup> Also, Our results are in agreement with O'Leary et al,<sup>[2]</sup> who compared between multiplex PCR and conventional diagnostic methods for the simultaneous detection of *Campylobacter* spp., *Shigella* spp., and *E. coli* O157 from feces. The sensitivity for multiplex PCR was found to be 100%, the specificity was 99.3%, the positive predictive value was 91.5%, and the negative predictive value was 100%.

## 5. Conclusion

Multiplex PCR can overcome limitations of culture-based methods as it is a more rapid and to some extent more accurate method with higher sensitivity compared to the traditional cultured based methods for detection of common intestinal pathogens.

### 5.1. Study limitation

1. No reevaluation of patients after antimicrobial therapy as multiplex PCR panels can't differentiate between viable and dead organisms, especially in patients with multiple stools detected organisms, which could be related to colonic colonization with asymptomatic organisms.
2. Since no controls were included in this study and enteropathogens have often been detected in healthy controls, the detection didn't necessarily mean disease association.

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