Original Research Article

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Evaluation of a multiplex polymerase chain reaction for the diagnosis of infectious diarrhea in intensive care unit patients in Upper Egypt

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Abstract

The rapid diagnosis of infectious diarrhea is lifesaving for intensive care unit (ICU) patients. This study evaluated a commercially available multiplex polymerase chain reaction (PCR) (BioFire FilmArray) for the diagnosis of parasitic and bacterial infections in ICU patients with secretory diarrhea in comparison to other traditional methods. This cross-sectional study included 50 subjects with infectious diarrhea. Their stool samples were subjected to macroscopic and microscopic examinations, concentration techniques, permanent staining techniques, stool culture, identification of bacterial infection by the Vitek 2 Compact 15 System, and molecular diagnosis of bacterial or parasitic infections by BioFire FilmArray multiplex PCR. Parasitological examination showed that the sensitivity and specificity of BioFire FilmArray multiplex PCR in the diagnosis of *Cryptosporidium* oocysts were 83.33% and 100.0%, respectively compared with 100% and 92.5% in diagnosis of *G. lamblia* cysts. Bacteriological examination showed that the sensitivity and specificity of BioFire FilmArray multiplex PCR in the diagnosis of *E. coli* and *salmonella* were 100% and 100.0%, respectively. The BioFire FilmArray multiplex PCR gastrointestinal (GI) panel assay was more sensitive and specific in the diagnosis of bacterial infections than parasitic infections. The BioFire FilmArray multiplex PCR GI panel assay was less sensitive in the detection of *Cryptosporidium* oocysts than traditional methods. In conclusion, the BioFire FilmArray multiplex PCR may be useful for rapid diagnosis of ICU patients with infectious diarrhea.

Keywords: FilmArray, multiplex PCR, parasitic, bacterial, secretory diarrhea.

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Introduction

Diarrhea in children under 5 years old is considered the second important cause of death in developing countries. It is responsible for 2.3 billion sicknesses and millions of deaths worldwide.[1] In Egypt approximately 30 million cases are reported annually.[1]

There are many causes of diarrhea including bacterial, viral, and parasitic infections. *Cryptosporidium* spp. and *Giardia duodenalis*

are the common causes of parasitic diarrhea in humans and animals globally. Both organisms are water-borne, may be transmitted by close contact, and are often associated with epidemics of diarrhea in daycare facilities. Infection with *Cryptosporidium* spp. has a higher prevalence in tropical regions, reaching infection levels of 20% to 50%, especially in rural areas.[2, 3] Bacterial diarrhea is the most severe form of acute diarrhea. The most common worldwide organisms causing bacterial diarrhea are *Escherichia coli* and *Salmonella*.[4]

Accurate detection of the etiologic agent is a very important step for diarrheal disease surveillance and control activities.[5] The used conventional diagnostic tests include identification of disease etiology, concentration techniques, permanent staining techniques, and molecular diagnosis of diarrheal diseases.

The BioFire® FilmArray® Gastrointestinal (GI) Panel is a commercially available qualitative multiplex polymerase chain reaction (PCR) produced by BioFire Diagnostics (BioMerieux, USA). It is one of the recent molecular techniques used for diagnosis of secretory diarrhea. The device is rapid, accurate, and with high sensitivity and specificity compared to other traditional methods. The capability of this panel is high for identification of nucleic acids from multiple bacteria, viruses, and parasites at the same time.[6] This test requires a stool sample preserved in Cary Blair transport medium.[7]

This study was designed to evaluate the BioFire FilmArray multiplex PCR for the diagnosis of infectious diarrhea caused by parasitic and bacterial infections in intensive care unit (ICU) patients in Upper Egypt. In particular we intended to determine the sensitivity and specificity of the BioFire FilmArray multiplex PCR, therefore we compared its results to other traditional diagnostic methods.

Subjects and Methods

Study settings

This cross-sectional study was carried out at the Microbiology Unit, Clinical Pathology Department, Assiut University Hospital and Parasitology Department, Faculty of Medicine, Assiut University, from February to October 2020. The study included 50 patients who were presented with secretory diarrhea (acute and persistent). They were of two age categories: children who attended the outpatient laboratory of Assiut University Pediatric Hospital or admitted in the Pediatric Hospital (Gastroenterology Department) and adult patients who attended the outpatient laboratory of Assiut University Hospital or admitted in the Gastroenterology (Liver Transplant Unit), Nephrology, and Oncology Departments of the hospital. Patients diagnosed with inflammatory bowel syndrome, ulcerative colitis, Crohn's disease, and coeliac disease were excluded from the study.

Sample collection

Stool sample collection was done according to the following standard operating procedures. A total of 50 fresh stool samples were collected and divided into two cups: one with formalin (ParaTest kits, Diagnosteck, Brazil) for parasitological examination and the second without preservatives (clean, plastic, wide-mouth containers with a tight-fitting lid) for stool culture and molecular diagnosis by multiplex PCR. Stool samples were transported immediately to the laboratory within one hour. The samples were refrigerated if there was any delay. All 50 stool samples were subjected to parasitological examination, stool culture, bacterial identification by the Vitek 2 Compact 15 System (BioMerieux, USA), and molecular diagnosis of bacteria or parasitic infections.

Parasitological examination

Direct stool examination used the commercially available ParaTest kit (Diagnosteck, Brazil) that contained one ParaTest container with 5% buffered formalin solution and one collection spoon. The consistency of the stool specimen (soft or liquid), color, odor, presence of mucus, and blood in stool were determined by macroscopic examination. Wet-mount microscopy was examined at $\times 10$ and $\times 40$ magnifications for the detection of *Cryptosporidium* and *Giardia* oocysts, RBCs, WBCs, fungi, bacteria, and plant cells. The

concentration techniques were used to separate *Cryptosporidium* and *Giardia* oocysts from fecal debris by centrifugation. The sedimentation concentration technique included formalin-ether sedimentation. The flotation concentration technique included Sheather's sugar flotation.[8] Permanent stained smears by the modified Ziehl-Neelsen (MZN) stain kits (Core Diagnostics, India) were used for the detection of *Cryptosporidium* (cold method).[9, 10]

Bacteriological examination

The stool sample was plated onto xylose lysine deoxycholate agar, *Salmonella Shigella* agar, blood agar, and MacConkey agar (Oxoid, United Kingdom). A loopful of stool sample was inoculated into Selenite-F broth. The samples were processed as per the BioMerieux standard protocol for culture and sensitivity. All isolates were identified by the Vitek 2 Compact 15 System (BioMerieux, USA), according to the manufacturer's instructions.

Molecular diagnosis

The BioFire FilmArray GI Panel (BioMerieux, USA) was used for the identification of nucleic acids from multiple bacteria and parasites

directly from stool samples preserved in 45 % saline, according to the manufacturer's instructions. Due to logistical difficulties, the use of Cary Blair transport medium was not incorporated for sample preservation.[11]

Statistical Analysis

Data were analyzed using the Statistical Package for Social Science (SPSS) version 26.0 for Windows. The mean and standard deviation were used to express quantitative data, whereas qualitative data were presented by frequencies and percentages. The Chi-square test and Fisher's exact test were used to compare the proportion between two different groups. The degree of agreement was measured by Cohen's κ between comparative methods and BioFire FilmArray multiplex PCR in the diagnosis of parasitic and bacterial infections. The sensitivity and positive percent agreement (PPA) were calculated as 100 × [no. of TP/(no. of TP + no. of FN)], where TP is true positive and FN is false negative. The specificity and negative percent agreement (NPA) were calculated as 100 × [no. of TN/[no. of TN + no. of FP)], where TN is true negative and FP is false positive. PPA and NPA were calculated in the same manner as sensitivity and specificity, respectively, and were used instead of sensitivity and specificity to indicate that no gold standard test was used for the comparator method. Significance was considered at $p<0.05$.

Results

Diagnosis of parasitic infections

Diagnosis of parasitic infections by microscopic examination

Of the 50 stool study samples, microscopic stool examination revealed presence of *Cryptosporidium* oocysts in 6 (12%) samples and *Giardia* in 10 (20%) samples (Figures 1-3).

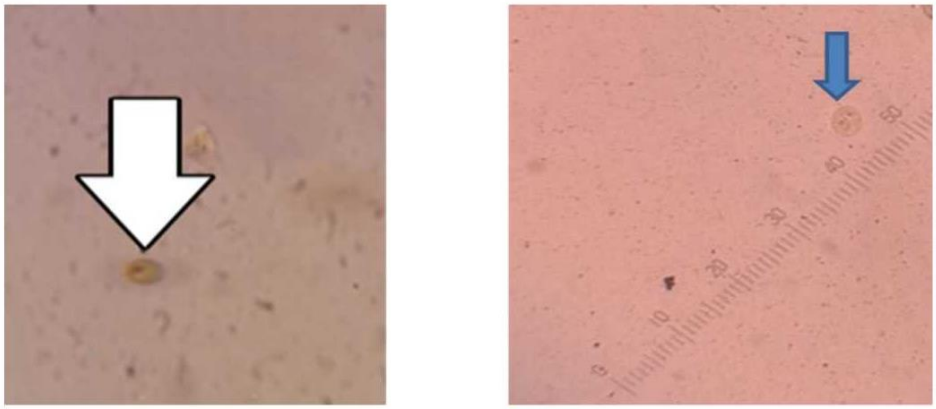

Figure 1. Microscopic image (100 high-power field) of *Cryptosporidium* oocysts (~5 μm) prepared from stool samples using the flotation technique.

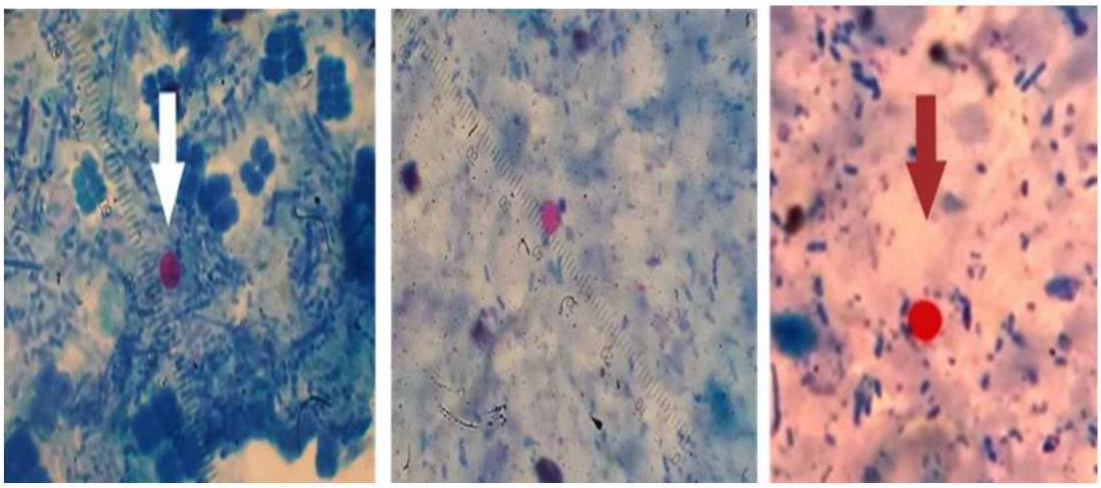

Figure 2. Microscopic image (100 high-power field) of *Cryptosporidium* oocysts (~5 μm) stained with the modified Ziehl-Neelsen stain technique.

Figure 3. Microscopic image (100 high-power field) showing *G. lamblia* cysts prepared from stool sample using the flotation concentration technique.

Diagnoses of parasitic infections by BioFire FilmArray multiplex PCR

Of the 50 stool study samples, the BioFire FilmArray multiplex PCR detected parasitic infection (*Cryptosporidium* oocysts and *G. lamblia* cysts) in 17 (34%) samples. *G. lamblia* oocysts were detected in acute and persistent diarrhea (28.6% and 20%, respectively), whereas *Cryptosporidium* oocysts were detected only in the persistent type (33.3%).

Comparison between microscopic examination and BioFire FilmArray multiplex PCR for detection of parasitic infections

Microscopic stool examination had a higher ability to diagnose *Cryptosporidium* oocysts [12% (6/50)] than the BioFire FilmArray multiplex PCR [10% (5/50)]. However, the BioFire FilmArray multiplex PCR had a higher ability to diagnose *G. lamblia* cysts [26% (13/50)] than microscopic stool examination [20% (10/50)], (kappa = 0.9, p <0.001) (Figure 4).

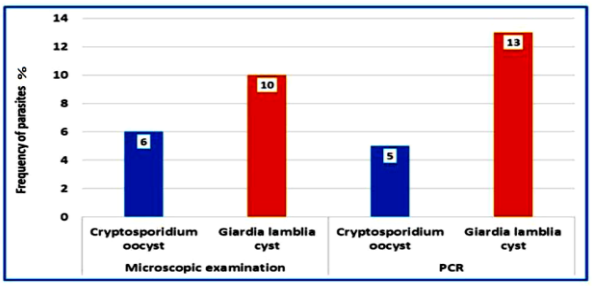

Figure 4. Frequency of parasites detected by microscopic examination and BioFire FilmArray multiplex PCR, according to the causative agent of diarrhea.

Positive percent agreement (PPA) and negative percent agreement (NPA) of the BioFire FilmArray multiplex PCR

The frequencies of the agreement between the two methods (microscopic examination and PCR) are shown in Tables 1 and 2. The degree of agreement was measured by Cohen's κ. The two tests showed perfect agreement in the detection of *Cryptosporidium* oocysts and *G. lamblia* cyst (consistency; κ = 0.9, p< 0.001). BioFire FilmArray multiplex PCR results were considered true positive (TP) or true negative (TN) only if they agreed with the results of the comparator method (microscopic stool examination). The sensitivity (PPA) of BioFire FilmArray multiplex PCR in the diagnosis of *Cryptosporidium* oocysts was 83.33% (5/6), whereas the specificity (NPA) was 100.0% (44/44). The overall percentage of agreement was 98.0% (5 + 44/50). However, the sensitivity (PPA) of BioFire FilmArray multiplex PCR in the diagnosis of *G. lamblia* cysts was 100% (10/10), whereas the specificity (NPA) was 92.5% (37/40). The overall percentage of agreement was 94.0% (10 + 37/50), as shown in Table 3.

Table 1. Relation between detection of *Cryptosporidium* oocyst determination in the patients with infectious diarrhea using BioFire FilmArray multiplex PCR and microscopic stool examination.

Cryptosporidium oocysts			
BioFire FilmArray multiplex PCR	Microscopic stool examination		
	Positive	Negative	Total
Positive	5	0	5
Negative	1	44	45
Total	6	44	50

Table 2. Relation between detection of *G. lamblia* cyst in the 50 patients with infectious diarrhea using BioFire FilmArray multiplex PCR and microscopic stool examination.

G. lamblia cysts			
Polymerase chain reaction	Microscopic stool examination		
	Positive	Negative	Total
Positive	10	3	13
Negative	1	37	37
Total	10	40	50

Table 3. Diagnostic value of BioFire FilmArray multiplex PCR in the diagnosis of *Cryptosporidium* oocysts and *G. lamblia* cysts compared to direct examination of stool in the 50 patients with infectious diarrhea.

BioFire FilmArray multiplex PCR	Sensitivity (PPA)	Specificity (NPA)	Accuracy
Cryptosporidium oocysts	83.33%	100.0%	98.0%
G. lamblia cyst	100.0%	92.5%	94.0%

Sensitivity is the positive percent agreement (PPA). Specificity is the negative percent agreement (NPA). Accuracy is the overall percentage of agreement.

Diagnosis of bacterial infections

Stool culture and identification by the Vitek 2 Compact 15 System showed that *E. coli* was positive in 38/50 (76.0%) and *Salmonella* positive in 2/50 (4.0%). The BioFire FilmArray multiplex PCR showed that *E. coli* was positive in 76.0% (38/50) and *Salmonella* was positive in 4% (2/50).

Comparison between stool culture and BioFire FilmArray multiplex PCR

The BioFire FilmArray multiplex PCR and stool culture and identification by the Vitek 2 Compact 15 System had the same ability for the diagnosis of *E. coli* [76.0% (38/50)] and *Salmonella* [4% (2/50)], as shown in Figure 5.

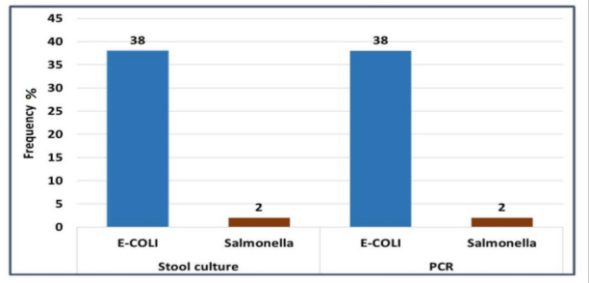

Figure 5. Frequency of Bacteria in stool culture and BioFire FilmArray multiplex PCR according to types of secretory diarrhea.

Positive percent agreement (PPA) and negative percent agreement (NPA) of BioFire FilmArray multiplex PCR

The frequencies of the agreement between the two methods (microscopic examination and PCR) are shown in Table 4. The degree of agreement was measured by Cohen'sκ. The two tests showed perfect agreement in the detection of *E. coli* and *Salmonella* (consistency; $\kappa = 0.9$, $p < 0.001$). BioFire FilmArray multiplex PCR results were considered TP or TN only if they agreed with the results of the comparator method (stool culture). The sensitivity (PPA) of BioFire FilmArray multiplex PCR in the diagnosis of *E. coli* was 100% (38/38), whereas the specificity (NPA) was 100% (12/12). The overall percentage of agreement was 100% (38 + 12/50). The sensitivity (PPA) of BioFire FilmArray multiplex PCR in the diagnosis of *Salmonella* was 100% (2/2), whereas the specificity (NPA) was 100% (48/48). The overall percentage of agreement was 100% (2 + 48/50), as shown in Table 5.

Table 4. Consistency of frequencies for *E. coli* and *Salmonella* determination using BioFire FilmArray multiplex PCR and stool culture.

E. coli			
Polymerase chain reaction	Stool culture		
	Positive	Negative	Total
Positive	38	0	38
Negative	0	12	12
Total	38	12	50
Positive	2	0	2
Negative	0	48	48
Total	2	48	50

Table 5. Diagnostic value of BioFire FilmArray multiplex PCR in the diagnosis of *E. coli* and *Salmonella* compared to stool culture in the 50 patients with infectious diarrhea.

BioFire FilmArray multiplex PCR	Sensitivity	Specificity	Accuracy
E. coli	100.0%	100.0%	100.0%
Salmonella	100.0%	100.0%	100.0%

PCR: Polymerase chain reaction

Discussion

Diarrheal diseases are considered a serious global public health challenge, especially as the second leading cause of death in children. According to the report of the World Health Organization (WHO), there are over 1.7 billion cases of diarrheal disease worldwide every year.[7] This study compared the results of parasitological microscopic examination and BioFire FilmArray multiplex PCR of 50 samples.

In the present study, the overall percentage of agreement in the diagnosis of Cryptosporidium oocysts was 98.0% (5 + 44/50). This finding agreed with that of a study by Mergen et al., 2020,[12] reported that the GI Panel of PCR detected about 82.5% positive Cryptosporidium samples validated by a real-time PCR laboratory-developed test, although not all specimens in their study were received in the recommended preservative. However, our results did not agree with those reported by Abdel Gawad et al., 2018, who reported that molecular diagnosis done by nested PCR had a detection rate of 21.0% compared to 9.5% by MZN staining method.[13] The study by Parcina et al., 2018, also reported that the BD MAX™ Enteric Parasite Panel provides a highly sensitive (100%) and specific (100%) tool for the laboratory diagnosis of Cryptosporidium.[14]

The FN results in this study were attributed to factors such as the presence of DNA inhibitors in stool samples, sample preservation conditions (Cary Blair transport medium was not used in this study), or the fact that Cryptosporidium is a protozoan that can cause autoinfection (oocysts may appear as ghost free from DNA in microscopic examination).[15] The sensitivity (PPA) of BioFire FilmArray multiplex PCR in the diagnosis of G. lamblia cysts in this study was 100% (10/10), whereas the specificity (NPA) was 92.5% (37/40). The overall percentage of agreement was 94.0% (10 + 37/50). These results agreed with those of Parcina et al., 2018, who reported that the BD MAX™ Enteric Parasite Panel provides a highly sensitive and specific tool for the laboratory diagnosis of predominant protozoan parasites causing enteritis16. The sensitivity and specificity were 97.8% (95% CI, 93.3%–99.4%) and 100% (95% CI, 97.4%–100%), respectively, for G. duodenalis, inconsistent with Ahmad et al., 2020, who reported that microscopic examination for G. duodenalis identified 40 (24.2%) of the 165 cases as positive for giardiasis infection. However, molecular detection for G. duodenalis was positive in only 35 cases (87.5%).[17] This discrepancy may be due to improper DNA processing and preservation of their samples.

In contrast, bacteriological examination showed that BioFire FilmArray multiplex PCR and stool culture and identification by the Vitek 2 Compact 15 System had the same ability in the diagnosis of *E. coli* 76.0% (38/50) and Salmonella 4% (2/50). This finding agreed with that of Valenzuela et al.,[14] who reported that the most frequently detected microorganisms were diarrheagenic *E. coli*, followed by Salmonella spp. The pathogen distribution in order of frequency was enteropathogenic *E. coli* (EPEC) 34 (15.4%), enteroaggregative *E. coli* (EAEC) 26 (11.8%), and Salmonella spp. 14 (6.3%). They also agreed that diarrheagenic *E. coli* [EAEC, EPEC, enterotoxigenic *E. coli* (ETEC), and entero-invasive *E. coli* (EIEC)] were most frequently associated with coinfections in 88.6%, 75%, 75%, and 69.2% of detection, respectively.[18]

The sensitivity (PPA) of BioFire FilmArray multiplex PCR in the diagnosis of *E. coli* was 100%, whereas the specificity (NPA) was 100%. The overall percentage of agreement was 100%. The sensitivity (PPA) of BioFire FilmArray

multiplex PCR in the diagnosis of Salmonella was 100%, whereas the specificity (NPA) was 100%. The overall percentage of agreement was 100%. In agreement with our study findings, Hannet et al.,[18] reported that the BioFire FilmArray GI Panel in comparison with conventional stool culture demonstrated a sensitivity (PPA) of 100% for Plesiomonas shigelloides, Salmonella spp., Yersinia enterocolitica, ETEC, STEC, *E. coli* O157, Cryptosporidium spp., Cyclospora cayetanensis, *G. lamblia*, astrovirus, and rotavirus A.

Our study had some limitations such as the sample size which was small due to the high cost of the BioFire FilmArray multiplex PCR test. The performance of this test has only been validated with human stool collected in Cary Blair transport medium, according to the manufacturer's instructions. However, the transport medium was not used in the current study which may have affected cryptosporidium frequency results.

In conclusion, our data indicated that the BioFire FilmArray GI Panel is a rapid, sensitive, and specific test that can be used in the diagnosis of bacterial infections rather than parasitic infections. The BioFire FilmArray multiplex PCR GI Panel assay is less sensitive in the detection of Cryptosporidium oocysts than comparative methods. As a rapid test, it helps to start appropriate treatment immediately after diagnosis, decreasing both hospital stay and cost.

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Author Contributions

Authors contributed equally to this work.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Ethical Committee of the Faculty of Medicine, Assiut University, Egypt (dated October 2019).

Informed consent

An informed written consent was obtained from each study patient (or their parents) before being included in the study.

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