ORIGINAL ARTICLE INFECTIOUS DISEASES

How well does physician selection of microbiologic tests identify *Clostridium difficile* and other pathogens in paediatric diarrhoea? Insights using multiplex PCR-based detection

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Abstract

The objective of this study was to compare the aetiologic yield of standard-of-care microbiologic testing ordered by physicians with that of a multiplex PCR platform. Stool specimens obtained from children and young adults with gastrointestinal illness were evaluated by standard laboratory methods and a developmental version of the FilmArray Gastrointestinal (GI) Diagnostic System (FilmArray GI Panel), a rapid multiplex PCR platform that detects 23 bacterial, viral and protozoal agents. Results were classified according to the microbiologic tests requested by the treating physician. A median of three (range 1–10) microbiologic tests were performed by the clinical laboratory during 378 unique diarrhoeal episodes. A potential aetiologic agent was identified in 46% of stool specimens by standard laboratory methods and in 65% of specimens tested using the FilmArray GI Panel ($p < 0.001$). For those patients who only had *Clostridium difficile* testing requested, an alternative pathogen was identified in 29% of cases with the FilmArray GI Panel. Notably, 11 (12%) cases of norovirus were identified among children who only had testing for *Clostridium difficile* ordered. Among those who had *C. difficile* testing ordered in combination with other tests, an additional pathogen was identified in 57% of stool specimens with the FilmArray GI Panel. For patients who had no *C. difficile* testing performed, the FilmArray GI Panel identified a pathogen in 63% of cases, including *C. difficile* in 8%. Physician-specified laboratory testing may miss important diarrhoeal pathogens. Additionally, standard laboratory testing is likely to underestimate co-infections with multiple infectious diarrhoeagenic agents.

Keywords: Acute gastroenteritis, children, *Clostridium difficile*, FilmArray, gastrointestinal illness
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Introduction

Despite advances in sanitation, food safety, and immunization, diarrhoeal diseases continue to cause substantial disease and mortality in children living in both high- and low-resource settings [1,2]. A 2011 report from the United States Centers for Disease Control and Prevention estimated that 178.8 million acute diarrhoeal illnesses occur annually in the United States, resulting in 473 832 hospitalizations and 5072 deaths [3]. The changing epidemiology and ongoing outbreaks of old and emerging pathogens including *Clostridium difficile*, *Cryptosporidium* spp., *Cyclospora* spp. and diarrhoeagenic *Escherichia coli* underscore the need for accurate diagnostics and improved surveillance for infectious diarrhoea [4,5].

Correctly diagnosing the aetiology of infectious diarrhoea can improve clinical care and public health surveillance [6]. One major obstacle in diagnosing infectious diarrhoea is the large

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and growing number of viruses, bacteria, and protozoa that are recognized to cause diarrhoea. Testing for specific pathogens is complex, requiring a variety of methods. Clinicians must choose the appropriate test, but this is challenging because of substantial overlap in clinical and epidemiologic features. Testing therefore can be expensive and inefficient. For some important pathogens such as diarrhoeagenic *Escherichia coli*, norovirus and sapovirus, testing is not readily available. To address this, there has been interest in developing multiplex platforms that can simultaneously detect a range of diarrhoeal pathogens [7–12].

The objective of this study was to compare the diagnostic yield of standard microbiologic testing ordered by the treating physician with the FilmArray Gastrointestinal Diagnostic System (FilmArray GI Panel, BioFire Diagnostics Inc., Salt Lake City, UT), a multiplex polymerase chain reaction (PCR) system that simultaneously detects 23 diarrhoeal pathogens. We hypothesized that physician-specified microbiologic testing would not accurately predict the pathogens present.

Methods

Human subjects protection

This study was approved and granted a waiver of informed consent by the University of Utah and Intermountain Healthcare (Intermountain) Institutional Review Boards (IRB #45464).

Setting and study population

Stool samples were collected from children and young adults 1–25 years of age with symptoms of acute gastrointestinal illness (e.g. fever, vomiting, abdominal pain, and diarrhoea) who received medical care at Primary Children's Hospital. Stool specimens that conformed to the shape of the cup were submitted for standard laboratory testing at the request of the treating physician, and residual samples were stored frozen at −80°C until tested with the FilmArray GI Panel. We collected specimens from August 2010 through December 2012.

Of 1504 diarrhoeal episodes, 378 episodes were selected for this study. This convenience sample was based on adequate residual specimen volume and was enriched for those who had multiple standard laboratory tests, including *C. difficile* testing, and for patients in whom a pathogen was detected. We designed a sampling scheme based on the number of standard laboratory tests ordered. We randomly selected 125 specimens that had one standard laboratory test ordered, 118 specimens that had two or three tests ordered, and 135 that had four or more tests ordered. A single stool specimen from each of the 378 episodes was tested with the FilmArray GI Panel. All microbiologic results from physician-ordered tests performed within ±72 hours of the collection of the specimen tested using the FilmArray GI Panel were included in the analysis. The selection of specimens for inclusion in this study was designed to evaluate the potential impact of a multiplex, PCR-based diagnostic platform in comparison with physician-specified standard laboratory testing and not to describe the epidemiology of diarrhoea.

Standard laboratory testing

Standard laboratory testing was performed at the discretion of the treating physician. Routine stool cultures identified *Salmonella* spp., *Shigella* spp., *Aeromonas* spp., *E. coli* O157:H7 and O121, *Bacillus cereus*, and *Campylobacter jejuni* and *Campylobacter coli* using trypticase soy agar, MacConkey II agar, sorbitol MacConkey agar, Hektoen Enteric agar, and Campy cefoperazone, vancomycin, and amphotericin B (CVA) agar. When *Yersinia enterocolitica* testing was ordered, stool was plated on cefsulodin–irgasan–novobiocin (CIN) agar. *C. difficile* was detected using the Illumigene *C. difficile* amplification assay (Meridian Bioscience, Inc., Cincinnati, OH). Rotavirus and adenovirus F 40/41 were detected using commercial immunoassays (Immunocard STAT!® Rotavirus and Meridian Premier™ Adenoclone®, Meridian Bioscience, Inc.). Shiga toxin-producing *E. coli* (STEC) was sought for all bloody specimens and when requested using a rapid immunoassay for Shiga toxin (Meridian Premier™ EHEC, Meridian Bioscience, Inc.) on specimens grown 24 hours in nutrient broth. *Giardia lamblia* and *Cryptosporidium parvum* were detected using the MERIFLUOR antigen detection immunoassay (Meridian Bioscience, Inc.). Other protozoa were identified by routine ova and parasite examination when requested. Laboratory testing for norovirus by PCR was introduced during the last 6 months of the study; however, norovirus testing was not ordered by the physician for any of the specimens evaluated in this study. No standard laboratory tests were available for the detection of astrovirus or sapovirus.

FilmArray GI panel pathogen detection

The FilmArray rapid multiplex PCR platform [13,14] used in this study was a developmental version of the FilmArray GI Panel, which simultaneously detects 23 diarrhoeagenic bacterial, viral, and protozoal agents in <1 hour (Table 1) using pathogen-specific virulence genes or gene signatures in housekeeping genes. Identification of *C. difficile* was based upon detection of the genes that encode an enterotoxin (*tcdA*) and a cytotoxin (*tcdB*). The pathotypes of pathogenic *E. coli* were identified using pathotype-specific genetic markers: STEC by detection of Shiga toxin 1 or 2 genes (*stx1* or *stx2*), enteropathogenic *E. coli* (EPEC) by detection of the intimin gene (*eae*),

TABLE 1. Detection of bacterial, viral, and parasitic diarrhoeal pathogens from 378 paediatric stool specimens evaluated by standard laboratory methods and the FilmArray GI Panel

Organism	Standard laboratory methods No. positive/No. tested (%)	FilmArray GI panel No. positive/No. tested (%)
Bacterial pathogens		
Clostridium difficile	77/273 (28%)	83/378 (22%)
EPEC	NA	37/378 (10%)
All STEC	19/193 (10%)	30/378 (8%)
Escherichia coli O157	12/193 (7%)	18/378 (5%)
Non-O157	7/193 (4%)	12/378 (3%)
Salmonella spp.	24/189 (13%)	27/378 (7%)
Campylobacter spp.	6/189 (3%)	15/378 (4%)
Shigella/EIEC	5/189 (3%)	11/378 (3%)
EAEC	NA	10/378 (3%)
Aeromonas spp.	2/189 (1%)	9/378 (2%)
ETEC	NA	7/378 (2%)
Yersinia enterocolitica	0/36 (0%)	2/378 (1%)
Plesiomonas shigelloides	0/0 (0%)	1/378 (<1%)
Vibrio cholerae	0/0 (0%)	1/378 (<1%)
Viral pathogens		
Norovirus GI/GII	0/0 (0%)	43/378 (11%)
Adenovirus F 40/41	6/73 (8%)	16/378 (4%)
Rotavirus A	17/98 (17%)	16/378 (4%)
Sapovirus	NA	11/378 (3%)
Astrovirus	NA	8/378 (2%)
Parasitic pathogens		
Giardia lamblia	11/125 (9%)	18/378 (5%)
Cryptosporidium spp.	2/118 (2%)	6/378 (2%)
Cyclospora cayetanensis	0/0 (0%)	0/378 (0%)
Entamoeba histolytica	0/0 (0%)	0/378 (0%)

EAEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; GI, gastrointestinal; NA, standard laboratory testing not available; STEC, Shiga toxigenic *E. coli*.

enterotoxigenic *E. coli* (ETEC) by genes encoding heat-labile (*lt*) or heat-stable (*st*) enterotoxin, enteroinvasive *E. coli* and *Shigella* by the invasion plasmid antigen H gene (*ipah*) and enteroaggregative *E. coli* (EAEC) by pAA virulence plasmid carried genes encoding the aggregative adhesion fimbria (AAF) biogenesis transcription regulator (*aggR*) or outer membrane protein (*aatA*). The FilmArray GI Panel identifies *Salmonella* spp., *Aeromonas* spp., *Cryptosporidium* spp., and pathogenic species of *Vibrio* and *Campylobacter* (*V. cholerae*, *V. parahaemolyticus, V. vulnificus, V. mimicus, V. alginolyticus, V. fluvialis, C. jejuni, C. coli* and *C. upsaliensis*). Extensive performance evaluation of the developmental version of the FilmArray GI Panel was conducted on 1721 clinical specimens and has been reported previously [15]. FilmArray testing was performed while blinded to standard testing results and patient characteristics.

Statistical analysis

To characterize findings by physician ordering practice, we divided patients into three groups for the analysis: i) those for whom the physician ordered testing only for *C. difficile*; ii) those for whom testing was ordered for *C. difficile* and other pathogens; and iii) those for whom testing was ordered only for pathogens other than *C. difficile*. Descriptive statistics were used to characterize the study population and microbiologic testing results. Rates and proportions were compared using the χ^2 test or Fisher's exact test, as appropriate. Nonparametric data were compared using the Wilcoxon rank sum test. Alpha was set equal to 0.05; all p-values are two-sided. Analyses were performed in Stata 11.2 (StataCorp LP, College Station, TX) and R 3.0 (R Foundation for Statistical Computing, Vienna, Austria).

To assess the concordance between standard laboratory testing and the FilmArray GI Panel, we evaluated the sensitivity, specificity, positive predictive value, and negative predictive value for *C. difficile*, rotavirus, *Salmonella* and STEC using specimens tested by both methods.

Results

Patient characteristics

FilmArray testing was performed on stool specimens submitted during 378 diarrhoeal episodes at Primary Children's Hospital from August of 2010 through December of 2012. There were 339 unique patients with a median age of 6 (interquartile range (IQR) 3–12) years (40% were <5 years of age); 58% were male. The majority were White (77%), Hispanic (11%), and Black (4%). Patient encounters occurred in the outpatient setting (68%), inpatient setting (20%), and the emergency department (12%).

Standard laboratory testing

A median of three (range 1–10) tests were ordered for each diarrhoeal episode. A gastrointestinal pathogen was detected by standard laboratory methods in 175 (46%) of 378 diarrhoeal episodes (Fig. 1). Co-infections were identified in 1.6% of diarrhoeal episodes by standard laboratory methods. The most commonly detected pathogens included: *C. difficile* (77 of 273 tested; 28%), *Salmonella* (24/189; 13%), rotavirus (17/98; 17%) and STEC (19/193; 10%).

The number of tests ordered varied according to the setting. Outpatient encounters had the lowest number of tests ordered (median 2; IQR 1–3), followed by emergency department visits (median 3; IQR 2–4) and inpatient encounters (median 3; IQR 1–5). A pathogen was detected by standard laboratory methods in 44% of outpatient encounters, 50% of emergency department visits, and 46% of inpatient encounters. *C. difficile* was detected by standard laboratory testing in 22 of 64 (34%) outpatients, 7 of 47 (15%) emergency department patients, and 48 of 162 (30%) inpatients.

FilmArray GI panel

When *C. difficile* testing was performed upon the same specimen using both standard laboratory methods and the FilmArray GI Panel, the sensitivity was 95% (95% CI 87–99%) and the specificity was 99% (95% CI 96–100%).

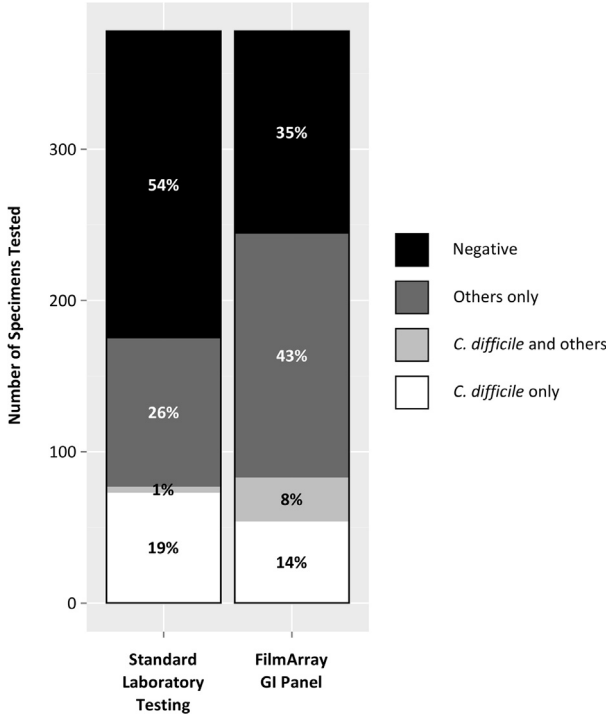

FIG. 1. *Clostridium difficile* and other diarrhoeal pathogens detected from pediatric stool specimens tested by standard laboratory methods and the FilmArray GI Panel. GI, gastrointestinal.

TABLE 2. Diarrhoeal pathogens detected from paediatric stool specimens collected in outpatient, emergency, and inpatient settings with the FilmArray GI Panel

	FilmArray GI Panel		
Pathogen	**Outpatient (*n* = 100)**	**Emergency (*n* = 76)**	**Inpatient (*n* = 202)**
Bacterial pathogens			
Clostridium difficile	25 (25%)	9 (12%)	49 (24%)
EPEC	10 (10%)	10 (13%)	17 (8%)
All STEC	7 (7%)	10 (13%)	13 (6%)
E. coli O157	4 (4%)	5 (7%)	9 (4%)
Non-O157	3 (3%)	5 (7%)	4 (2%)
Salmonella spp.	3 (3%)	14 (18%)	10 (5%)
Campylobacter spp.	5 (5%)	5 (7%)	5 (2%)
Shigella / EIEC	3 (3%)	4 (5%)	4 (2%)
EAEC	0 (0%)	6 (8%)	4 (2%)
Aeromonas spp.	3 (3%)	5 (7%)	1 (1%)
ETEC	2 (2%)	3 (4%)	2 (1%)
Yersinia enterocolitica	0 (0%)	0 (0%)	2 (1%)
Plesiomonas shigelloides	0 (0%)	0 (0%)	1 (1%)
Vibrio cholerae	1 (1%)	0 (0%)	0 (0%)
Viral pathogens			
Norovirus GI/GII	13 (13%)	9 (12%)	21 (10%)
Adenovirus F 40/41	4 (4%)	5 (7%)	7 (3%)
Rotavirus A	1 (1%)	3 (4%)	12 (6%)
Sapovirus	3 (3%)	4 (5%)	4 (2%)
Astrovirus	1 (1%)	1 (1%)	6 (3%)
Parasitic pathogens			
Giardia lamblia	9 (9%)	4 (5%)	5 (2%)
Cryptosporidium spp.	3 (3%)	2 (3%)	1 (1%)
Negative	37 (37%)	16 (21%)	81 (40%)

Numbers presented within cells are the number of positive specimens, and in parentheses are the percentages for each diarrhoeal pathogen. Pathogens with no detections in all three clinical settings are not featured in this table.
EAEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; GI, gastrointestinal; NA, standard laboratory testing not available; STEC, Shiga toxigenic *E. coli*.

A pathogen was identified in stool specimens from 244 of 378 (65%) diarrhoeal episodes (Table 1, Fig. 1). Multiple pathogens were detected in 77 (20%). The most common pathogens detected included *C. difficile* (83/378; 22%), norovirus (43/378; 11%), enteropathogenic *E. coli* (37/378; 10%) and STEC (30/378; 8%).

The aetiologic agents detected varied based upon the setting of the encounter (Table 2). *C. difficile* was more frequently detected among outpatients (25/100; 25%) and inpatients (49/202; 24%) than from patients in the emergency department (9/76; 12%) ($p = 0.02$). However, the overall proportion of specimens with a pathogen identified did not vary between the outpatient, emergency department, and inpatient settings ($p = 0.8$).

Use of the FilmArray improved the diagnostic yield from 46% to 65% as compared to standard laboratory testing methods ($p < 0.001$). Similar improvements were identified among patients who had one, two or three, or four or more standard laboratory tests ordered (18%, 23%, and 14%, respectively).

Findings by physician test selection

Only C. difficile *testing requested.* Standard laboratory testing for *C. difficile* was the only test ordered in 91 episodes (Fig. 2). *C. difficile* was detected by standard laboratory methods in 42 episodes (46%) and by the FilmArray GI Panel in 40 (44%) ($p = 0.8$). An additional pathogen was detected by FilmArray in 26 (29%), 11 (12%) as a co-infection with *C. difficile*. Co-detections among the 11 patients with *C. difficile* included seven with norovirus, one with *Salmonella*, and one with *Campylobacter* spp. Pathogens detected among the 15 children who tested negative for *C. difficile* included norovirus ($n = 4$), astrovirus ($n = 2$), sapovirus ($n = 1$), and *G. lamblia* ($n = 1$). In this group, the proportion of episodes without an identified pathogen declined from 54% to 40% using the FilmArray GI Panel ($p = 0.05$).

The majority (59%) of stool samples in which only *C. difficile* testing was ordered were collected during inpatient encounters. Of these, 50% tested positive for *C. difficile* by standard laboratory methods. Similarly, 46% were positive for *C. difficile* using the FilmArray GI Panel, although an additional 19% had another pathogen detected.

Among children who only had *C. difficile* testing requested, the greatest increase in pathogen detection was among children 1–4 years of age ($n = 42$). In this sub-group, 20/42 (48%) children had *C. difficile* detected by standard laboratory testing methods, while 30/42 (71%) had a pathogen detected by the FilmArray GI Panel ($p = 0.03$). The additional pathogens detected included: norovirus ($n = 7$), EPEC ($n = 3$),

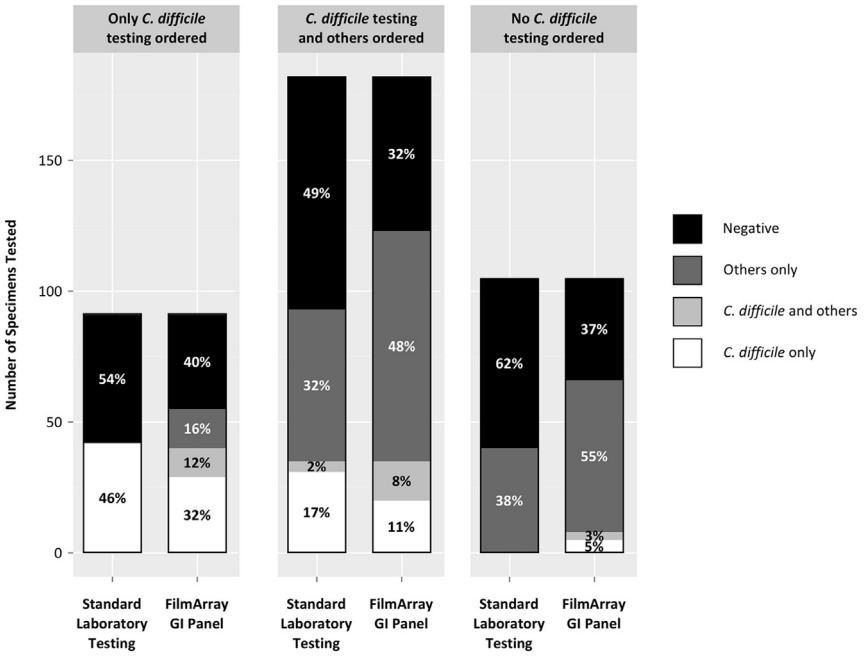

FIG. 2. Physician ordering patterns and their impact upon diarrhoeal pathogen detection from pediatric stool specimens tested with standard laboratory methods and the FilmArray GI Panel. GI, gastrointestinal.

Campylobacter (*n* = 2), sapovirus (*n* = 2), *G. lamblia* (*n* = 1) and *P. shigelloides* (*n* = 1).

C. difficile *requested in combination with other tests.* In 182 of 273 (67%) episodes, *C. difficile* testing was requested along with one or more additional tests. Of these, 51% had one or more pathogens detected by standard laboratory tests, as compared with 68% when tested with the FilmArray GI Panel ($p < 0.001$) (Fig. 2). The most common pathogens detected using standard laboratory tests were *C. difficile* (35; 19%), *Salmonella* (15; 9%), STEC (13; 7%) and rotavirus (9; 5%). Co-infections were detected in four (2%) specimens. Using the FilmArray GI Panel, *C. difficile* was detected in 35 (19%), norovirus in 28 (15%), EPEC in 22 (12%), STEC in 20 (11%) and *Salmonella* in 16 (9%). In 15 of 35 (43%) patients in whom *C. difficile* was detected, an additional pathogen was also identified. The most common co-infecting pathogens were norovirus (*n* = 7), EPEC (*n* = 5), *Campylobacter* spp. (*n* = 2) and *Salmonella* (*n* = 1). When *C. difficile* was not detected, the FilmArray GI Panel detected a pathogen in 60% (88/147), including norovirus (21/147; 14%), STEC (20/147; 14%), *Salmonella* (15/147; 20%) and EPEC (17/147; 12%). When compared with standard laboratory methods, the proportion of specimens with more than one diarrhoeal pathogen identified rose from 2% to 25% ($p < 0.001$).

Only tests other than C. difficile *requested.* In 105 (28%) episodes of diarrhoea, stool specimens were submitted for standard laboratory testing for one or more diarrhoeal pathogens, but did not have *C. difficile* testing. Of these, 40 (38%) had a pathogen detected using standard laboratory testing methods. With the FilmArray GI Panel, a pathogen was detected in 63% ($p < 0.001$); multiple pathogens were detected in 18% (*n* = 19).

The most common pathogens detected by standard laboratory testing methods were *G. lamblia* (10/105; 10%), *Salmonella* (8/105; 8%), rotavirus (7/105; 7%) and STEC (6/105; 6%). Using the FilmArray GI Panel, *G. lamblia* (11/105; 10%), *Salmonella* (10/105; 10%), STEC (10/105; 10%), *C. difficile* (8/105; 8%) and rotavirus (7/105; 7%) were the most common. The proportion of specimens with multiple pathogens identified rose from 2% to 18% ($p < 0.001$). The proportion of specimens that had a pathogen identified did not significantly differ by age ($p = 0.07$) or patient location ($p = 0.1$).

Discussion

Correctly diagnosing the aetiology of infectious diarrhoea depends on both the physician's decision to order the correct test and the sensitivity of the testing method. In this study, we compared the detection of infectious pathogens by physician-selected standard tests to a multiplex PCR assay that simultaneously detects 23 bacterial, viral and protozoal pathogens. In this sample, the identification of a pathogen increased from 46% of episodes to 65%. Co-detection of multiple diarrhoeal pathogens increased from 2% to 20%. Applying the FilmArray GI Panel to patents that clinicians had decided warranted diagnostic testing identified 72 additional viral infections, including 43 cases of norovirus, 13 additional protozoal pathogens and 100 additional potential bacterial infections. The bacterial

infections that were not detected by physician-selected testing included many with clear clinical importance, including: 11 additional STEC, nine *Campylobacter*, seven *Aeromonas*, seven ETEC, six *Shigella*, and six *Salmonella*. This study demonstrated some limitations of physician-specified testing for patients with diarrhoea. Among children in whom *C. difficile* testing was not ordered, 8% had *C. difficile* detected using the FilmArray GI Panel. Conversely, when only *C. difficile* was sought, additional pathogens were detected in 28%.

C. difficile is an important and frequent cause of nosocomial and antibiotic-associated diarrhoea in adults and is increasingly recognized as an important pathogen among children [16–19]. Kim *et al.* reported a 53% increase in the incidence of *C. difficile* among 22 freestanding children's hospitals across the United States from 2001 to 2006 [20]. In a recent propensity-matched cohort study, the morbidity and costs attributable to *C. difficile* infections in hospitalized children were substantial; $18 900 per episode of community-onset *C. difficile* infection and $93 600 for an episode of hospital-onset *C. difficile* infection [21]. These findings underscore the importance of detecting *C. difficile* in a variety of clinical settings. It is noteworthy that we detected *C. difficile* in 8% of diarrhoeal episodes where *C. difficile* testing was not sought.

To be useful, diagnostic tests for diarrhoeal pathogens should positively affect clinical care, infection prevention and public health [6]. Routine stool cultures identify a diarrhoeal pathogen infrequently, with estimates from several studies ranging from 1.5% to 5.8% [22–24]. Use of the FilmArray GI Panel identified a substantial number of additional bacterial infections for which treatment may be helpful (nine *Campylobacter*, seven ETEC, seven *Aeromonas*, six *Shigella*, two *Y. enterocolitica* and one *Plesiomonas shigelloides*) and where the use of antibiotics may be undesirable (11 STEC and three *Salmonella*) (6).

Norovirus is the leading cause of foodborne disease outbreaks and is increasingly recognized as a nosocomial pathogen, sometimes mimicking *C. difficile* [25–27]. With multiplex testing, we identified norovirus in 11% of diarrhoeal episodes. Interestingly, using the same CDC definitions designed for *C. difficile*, three hospital-onset cases of norovirus were identified, defined as detection more than 72 hours after admission. Routine detection of norovirus could facilitate infection control efforts in the hospital and outbreak detection in the community [27].

Limited data exist describing the extent to which co-infections complicate the clinical presentation of paediatric diarrhoea [28]. Tvede *et al.* evaluated 32 Swedish children who were hospitalized with *C. difficile* infection and found that 44% were concurrently infected with another bacterial pathogen, including *Campylobacter* spp., *Salmonella*, *Y. enterocolitica* and *E. coli* [29]. In this study, 20% of specimens tested were positive for two or more diarrhoeal pathogens using the FilmArray GI Panel. Among patients with *C. difficile* infection, 35% had at least one additional diarrhoeal pathogen identified, including norovirus in 17%. It is unclear if these co-infections impacted disease severity.

We frequently detected genes associated with EPEC and EAEC; however, detection of these genes may not prove that they are present in a single organism. The complex molecular pathogenesis and overlapping virulence genes complicates the interpretation of pathogenic *E. coli* detection [30]. However, in a case control study in a paediatric emergency room in Seattle, Denno *et al.* demonstrated that EAEC was significantly associated with acute diarrhoea [31].

The results of this study are subject to several limitations. First, this was a convenience sample of diarrhoeal stool specimens designed to look at test ordering patterns and was not a random or sequential sample of diarrhoeal episodes. Therefore, the detection of pathogens by standard methods was higher in this study than would be expected in routine testing. For example, in our clinical laboratory, a pathogen is identified in about 12% of all specimens by routine physician-selected testing, as compared with 46% in this study. Second, this study was laboratory based and analysed de-identified samples; therefore, limited clinical data were available for review. Most importantly, no control group of asymptomatic patients was included, making it impossible to establish whether detection of a specific pathogen or co-infections with multiple diarrhoeal pathogens was associated with disease.

The diagnosis and management of paediatric diarrhoea is complicated, and current testing strategies are expensive and inefficient. Our data support the use of the FilmArray GI Panel or other multiplex testing platforms to simultaneously detect a wide spectrum of diarrhoeal pathogens. Future studies will need to evaluate the clinical and epidemiologic utility, efficiency, accuracy, and cost-effectiveness of multiplex molecular testing.

Transparency Declaration

M.R., B.H., M.V., R.C., M.P. and S.T. are employees of BioFire Diagnostics, Inc., the maker and manufacturer of the FilmArray GI Panel. This publication contains information regarding assays that have not been cleared by the Food and Drug Administration for *in vitro* diagnostic use.

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Appendix A. Supplementary material

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.cmi.2014.07.011.

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