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Original Article

Syndromic testing for increasing diagnostic accuracy in gastrointestinal infection

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Abstract

Introduction: Diarrhea is a global problem that commonly occurs in cases of gastrointestinal infection. The prevalence of diarrhea in Indonesia was 6.8% according to Riskesdas 2018 data. The conventional diagnosis in cases of gastrointestinal infection is limited in sensitivity and time. This may be overcome by gastrointestinal syndromic testing that can detect a number of pathogens simultaneously in one assay. The aim of this study was to determine the role of the gastrointestinal syndromic testing panel in patients with gastrointestinal infection.
Methodology: This retrospective study of stool specimens performed syndromic testing and microbiological cultures at a private hospital in Central Jakarta.
Results: Of the 119 specimens with negative culture test results, syndromic gastrointestinal testing found pathogens in 46 specimens (38.7%), of which 32 specimens contained a single pathogen and 14 specimens had > 1 pathogen. The most frequently found pathogens were enteropathogenic *E. coli*, enteroaggregative *E. coli,* and *C. difficile* A/B toxins.
Conclusions: Syndromic testing can increase the etiologic diagnosis of gastrointestinal infections in a shorter time period than the conventional methods.

Key words: Gastrointestinal panel; gastrointestinal infection; syndromic testing.

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Introduction

Diarrhea constitutes an important global health problem and was estimated to result in 1.6 million deaths in 2016. Diarrhea usually occurs in acute or chronic gastrointestinal infections. In 2019 it was estimated that 294,100 of the population of 17.2 million in the Netherlands visited their family physician in connection with complaints of diarrhea or gastrointestinal symptoms [1]. According to data from the Indonesian Basic Health Research (Riskesdas) for 2018, the prevalence of diarrhea in Indonesia reached 6.8%, on the basis of diagnosis by healthcare workers [2]. The etiologic diagnosis of gastrointestinal infection or infectious gastroenteritis constitutes a considerable challenge, as it may be caused by bacteria, viruses, or parasites, but presents nearly identical clinical symptoms. Gastrointestinal infections are one of the most frequently found diseases in the world. The majority of episodes of infectious gastroenteritis are of short duration and self-limiting [3–6]. However, persistent or severe infections may lead to hospitalization, particularly in infants, elderly, and immunocompromised patients, who are at increased risk of dehydration [5–7]. Infectious gastroenteritis rather frequently develops complications in hospitalized patients that may increase morbidity, mortality, duration of care, and hospital costs [8,9]. Identification of the causative pathogens in cases of gastroenteritis may assist in determining appropriate antibiotic therapy, management, isolation, and further investigation [10,11]. The causative pathogens in cases of gastroenteritis cannot be determined exclusively on the basis of clinical manifestations and therefore laboratory examination becomes essential [12].

The standard laboratory methods, including microbial culture, nucleic acid amplification, and immunoassay, require several days and the number of pathogens examined is limited. Prior to the use of multiplex PCR, in around 80% of cases of acute gastroenteritis, the causative pathogen could not be detected. In comparison with conventional methods, multiplex PCR increases the diagnostic sensitivity and shows shorter examination times, with run times of only 1-2 hours, which is far less than the several days required by conventional methods. The rapidity of the results may be useful for adjusting the therapy to

correspond to the etiology [6,10,11]. Syndromic testing is capable of identifying potential pathogens that cannot be diagnosed with conventional methods, such as enteroaggregative *E.coli*, enterotoxigenic *E.coli*, enteropathogenic *E.coli,* and viruses, that may potentially be the causes of the infections [11]. One of the limitations of stool culture is that it has an exceedingly low probability of detecting the causative pathogens, particularly in the small number of patients with prior antibiotic therapy, which may impact the management of these patients [13,14]. The sensitivity of gastrointestinal syndromic testing is higher than that of microbial culture (68.8% vs. 35.2%[1] and 35.3% vs. 6%[15]). The laboratory examination methods for the diagnosis of infectious gastroenteritis have changed over time, such that most depend on multiplex PCR. Syndromic testing, which allows the simultaneous detection of several targets, can yield a rapid diagnosis that may inform on patient management strategies [16–18]. Syndromic testing is becoming more widely used by clinical microbiological laboratories because it has several advantages, the procedure is relatively easy and rapid, and the targets for detection are comparatively more numerous, including those that are not routinely examined with conventional methods [15,16,19].

In recent years, rapid molecular syndromic testing methods have emerged that can simultaneously detect and identify gastrointestinal pathogenic bacteria, viruses, and parasites, to overcome the limitations of the conventional microbiological culture methods [10,20–22]. The timely and comprehensive detection of gastrointestinal pathogens is essential for guiding targeted antimicrobial therapy, preventing transmission of infections, and improving clinical results [1,23]. The aim of this study was to evaluate the contribution of gastrointestinal syndromic testing to the detection of pathogens in patients with negative microbiological cultures.

Methodology

Study Design

This retrospective study was conducted to determine the effectiveness of gastrointestinal syndromic testing (QIAstat Dx) on stool specimens of adult patients with complaints of gastrointestinal infection, in comparison with conventional microbiological examinations (microscopy, cultures, *Clostridioides difficile* toxin assays) that were conducted in parallel. This study was conducted from November 2021 to April 2022 in a private hospital in Jakarta. The number of specimens obtained by total population sampling was 119. The inclusion criteria were age ≥ 18 years and negative microbiological cultures or cultures with no detectable pathogens. The exclusion criteria were cases under follow-up or therapeutic evaluation. The present study was approved by the ethics committee of the Faculty of Medicine and Health Sciences, UKRIDA, under no. 1267/SLKE-IM/UKKW/FKIK/KE/V/2022.

Diagnostic Methods

The stool specimens were processed in parallel for gastrointestinal panel examination as well as for microscopic examination and microbiological culture for the identification of *E. coli, Campylobacter* spp*., Plesiomonas shigelloides, Salmonella* spp*., Shigella* spp*., Vibrio* spp*.,* and *Yersinia enterocolitica*. The cultures were performed under the standard operating procedures of the laboratory where the study was conducted. Parasites, including *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Entamoeba histolytica,* and *Giardia lamblia* were identified by microscopic observation to determine the morphological characteristics. Detection of *C. difficile* was by simultaneous detection of the *C. difficile* glutamate dehydrogenase (GDH) antigen and toxins A and B. Microscopic observation for parasites and sequential tests for infection with toxigenic *C. difficile* had been requested for some of the specimens by the examining physician. All specimens were also tested in parallel using the QIAstat Dx gastrointestinal panel.

Syndromic testing with the QIAstat Dx gastrointestinal panel (Qiagen, Germany), which is a CE-approved molecular test panel, allows for the one-step detection of 14 bacteria, 4 parasites, and 6 viruses in around 1 hour. The test panel included the following targets: *C. difficile* toxin A/B, enteroaggregative *Escherichia coli* (EAEC), enteroinvasive *E. coli* (EIEC)/Shigella, enteropathogenic *E. coli* (EPEC), LT/ST enterotoxigenic *E. coli* (ETEC), pathogenic *Campylobacter* spp., *Plesiomonas shigelloides*, *Salmonella* spp., Stx1/Stx2 Shiga Toxin-Producing *E. coli* (STEC), Shiga toxin-producing *E. coli* (STEC O:157:H7), *Vibrio cholera, Vibrio parahaemolyticus, Vibrio vulnificus, Yersinia enterocolitica, Cyclospora cayetanensis, Cryptosporidium* spp*., Entamoeba histolytica, Giardia lamblia*, adenovirus F40/41, astrovirus, norovirus GI, norovirus GII, rotavirus A and sapovirus (I, II, IV and V) [6].

Results

The total number of collected specimens was 295 in the course of the study, but 176 specimens were excluded because they had no stool culture results. The

total number of specimens that met the inclusion and exclusion criteria of this study was 119. In this study, the distribution of patients by gender was dominated by 64 women (53.8%). The age range in this study was from 18 to 88 years, with the age range of 18 – 59 years accounting for 65 persons (54.6%) (Table 1). Using the QIAstat DX gastrointestinal panel, among the 119 culture-negative stool specimens, pathogens were found in 46 specimens, among which 32 specimens contained a single pathogen, whereas in 14 specimens multiple pathogens were found to a total of 62 organisms (Table 2). Overall, the total number of specimens that were negative in both the cultures and the gastrointestinal panel was 73 (61.3%). However, there was a discrepancy in the results of the gastrointestinal panel as compared with the *C. difficile* antigen and toxin detection tests, in that 1 specimen was positive for toxin A but negative in the gastrointestinal panel.

With respect to the multiple pathogens found in 14 specimens, in 12 specimens combinations of 2 pathogens were found. Four these 12 specimens contained enteroaggregative *E. coli* and enteropathogenic *E. coli*, whereas the other 8 specimens comprised respectively *Campylobacter* spp. and *Plesiomonas shigelloides*; enteroaggregative *E.coli* and enterotoxigenic *E.coli* (LT/ST); enterotoxigenic *E.coli* (LT/ST) and norovirus GII; *Campylobacter* spp. and enteropathogenic *E.coli*; *C. difficile* toxin A/B and STEC (Stx1/Stx2); *C. difficile* toxin A/B and enteropathogenic *E.coli*; *C. difficile* toxin A/B and enteroaggregative *E.coli*; *Giardia lamblia* and enteropathogenic *E.coli*. In addition, there were 3 pathogens in each of the remaining 2 specimens, namely *Campylobacter* spp., enteroaggregative *E. coli*, and enteropathogenic *E. coli* in one, and enteroaggregative *E. coli*, enteropathogenic *E. coli*, *Plesiomonas shigelloides* in the other.

Table 1. Research subjects' characteristics and examination results (n = 119).

Characteristic	Percentage
Age (mean ± SD)	52.15 ± 15.78
18 – 59 years	54.6%
≥ 60 years	45.4%
Gender	
Male	46.2%
Female	53.8%
Microscopic Parasites	
Positive	0%
Negative	100%
Stool Culture	
Pathogens found	0%
No pathogens found	100%
***C. difficile* antigen and toxins** (n = 37)	
Positive	2.7%
Negative	97.3%
Qiastat DX GI Panel	
Positive	38.7%
Negative	61.3%
Number of Pathogens	
Single pathogen	69.6%
Multiple pathogens	30.4%

Discussion

This study found that the mean age was 52.51 ± 15.78, and that there were more cases of gastrointestinal infection in females (53.8%) than in males (46.2%). Similar results were obtained in the study by Friesema *et al*., who found that 77% of the females had gastrointestinal infections, as compared with 23% of the males [5]. Shen *et al.* also found more gastrointestinal infections in females (56.1%) than in males (43.9%) [24]. However, differing results were obtained in the study by Luo *et al*., where the difference in gender among the cases of gastrointestinal infection was in the larger number of males (52.1%) than of females (47.9%) [25]. The study by Vázquez-Martínez *et al.* states that males are more susceptible to gastrointestinal and respiratory infections, and to sepsis, whereas women are more susceptible to genitourinary infections [26]. In our study, differing results were found, which may have been due to the fact that the specimens in our sample were restricted to those with negative culture results, such that they were not representative of gender. The study by Kim *et al.* found that the relationship between gender and gut microbiota was still unclear [27].

In our study, the positive gastrointestinal syndromic test results accounted for 38.7% of the gastrointestinal infections with negative cultures. These results are similar to those of the study by Bresee *et al*., who found that among 364 stool specimens, the number of

Table 2. Distribution of causative pathogens in gastrointestinal infection.

No.	Type of Pathogen	Total
1	Enteropathogenic *E.coli*	19
2	Enteroaggregative *E.coli*	13
3	*Clostridioides difficile* toxin A/B	7
4	*Plesiomonas shigelloides*	6
5	*Campylobacter* spp.	5
6	STEC *(stx1/stx2)*	4
7	Enterotoxigenic *E.coli* (LT/ST)	2
8	*Salmonella* spp.	1
9	*Cyclospora cayetanensis*	1
10	*Cryptosporidium* spp.	1
11	*Giardia lamblia*	1
12	Norovirus GII	1
13	Sapovirus	1
14	None (negative)	73

pathogens found was 25%, using a comprehensive panel for viruses, bacteria, and parasites [28]. Among several studies that compared the gastrointestinal panel with culture tests was the study by Axelrad *et al.*, who found that the positive results in the gastrointestinal panel were greater (29.2%) than in the cultures (4.1%) [20]. Similar results were obtained in the study by Sobczyk *et al.* on HIV patients as subjects, yielding a positivity rate of 52.5% for the gastrointestinal panel, whereas for cultures the positivity rate was 2.6% [11]. These results show that gastrointestinal syndromic testing can increase the sensitivity of the diagnosis of gastrointestinal infection if compared with conventional tests. Another advantage of gastrointestinal syndromic testing is that one examination can simultaneously detect a number of pathogen targets, in far shorter times than those of cultures [21,22].

The turnaround time for gastrointestinal syndromic testing is ± 70 minutes, whereas cultures need 3-5 days. Syndromic testing is based on molecular testing that has higher sensitivity and specificity than conventional methods because it can detect the nucleic acids of pathogens in minuscule amounts. However, it has the limitation of not being able to differentiate between live and dead organisms, such that the results should be carefully interpreted based on the condition of the patients. On the other hand, one limitation of the conventional methods is that they require a specific request for cultures or detection of parasites to determine the selection of appropriate media and methods. In addition, cultures require trained and experienced staff to differentiate colonization or contamination by normal flora from that of pathogens and to conduct advanced identification procedures against the pathogens. The case is similar in microscopic examination for the detection of ova and parasites in stools, which is of extreme benefit for direct detection, but has low sensitivity because it is exceedingly dependent on the laboratory technique and requires trained and experienced staff. Detection of ova and parasites may be difficult as a result of their low numbers or their intermittent presence [7,16,29].

This study found that there were single pathogens in 69.6% of the specimens and multiple pathogens in 30.4%, with the most frequently found pathogens being enteropathogenic *E. coli* (41.3%), enteroaggregative *E. coli* (28.3%) and *C. difficile* toxin A/B (15.2%). These results are similar to those of the study by Shen *et al.*, who obtained 41.5% for single pathogens and 7% for multiple pathogens, with the most frequent pathogen being *Salmonella* sp, norovirus, and enterotoxigenic *E.coli* [24]. Friesema *et al.* found 62.5% of cases with a single pathogen and 37.5% with multiple pathogens, with the most frequent pathogens being *C. difficile* and norovirus [5]. The study results of the study by Boers *et al.* and Sobczyk *et al.* are similar to those of the present study, in that the most numerous pathogens were enteropathogenic *E. coli* and enteroaggregative *E.coli* [6,11]. A systematic review found that the most frequently found pathogens as causes of diarrhea are enterotoxigenic *E.coli* and *Vibrio cholera* O1/O139 [30]. The study that was conducted by Krumkamp *et al.* in children with gastrointestinal infections in Ghana, found that the most frequent pathogens were rotavirus, *Shigella* spp., or enteroinvasive *E.coli* and norovirus [31]. Similar results were found in a study in Burkina Faso where the most numerous pathogens were rotavirus and pathogenic *E. coli* [32]. The results of another study on children in Chile found that the most numerous pathogens were norovirus, enteropathogenic *E.coli,* and rotavirus [33].

Apart from its strengths in detecting a number of targets simultaneously, syndromic testing also has several limitations, the first being that some of the targets are not always clinically relevant. In addition, there are several targets that do not require special management, such that the greater the number of positive targets, the greater the probability of false positives. Other limitations are the relatively high cost of the examination in comparison with conventional methods, and the fact that it cannot differentiate between live and dead organisms or remains of nucleic acids. The advantages of syndromic testing in detecting multiple pathogens or co-infections are the following. It can minimize repeat specimen collection for further examination, the detected multiple pathogens may provide a clearer picture to clinicians for determining the therapy, it can detect rare pathogens, and is beneficial in certain patient populations, such as transplant patients [7,16,29,34].

As noted previously, in comparing the results of gastrointestinal syndromic testing and determination of *C. difficile* antigens and toxins, there was a discrepancy in 1 specimen, where *C. difficile* was positive for toxin A, whereas the syndromic gastrointestinal panel yielded negative results. This indicates that each of these examinations may be used as an auxiliary for the other, but not as a substitute. If there is clinical suspicion of infection by *C. difficile*, then the *C. difficile* antigen and toxin test may be followed by gastrointestinal syndromic testing. Because overall gastrointestinal syndromic testing is more sensitive and can detect co-

infections with other pathogens, this constitutes a limitation of the conventional methods [1,35].

Syndromic testing is not only crucial for diagnosis but is also beneficial for public health and infection control. Syndromic testing can simultaneously detect pathogens directly from the specimens by a simple procedure. The implications of patient management and the appropriate use of antibiotics may also be determined by syndromic testing. However, in this study, no evaluation was performed on the role of syndromic testing in therapeutic management, especially antibiotic usage, which constitutes another limitation of this study.

Authors' Contributions

Dharmawan A was involved in the planning and supervision of the work, and in the design of the tables. Dharmawan A and Pusparini performed the analysis and drafted the manuscript. Pusparini aided in interpreting the results and worked on the manuscript. Both authors discussed the results and commented on the manuscript.

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