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Evaluation of a culture-independent gastrointestinal multiplex PCR panel for detection of gastrointestinal pathogens detection of gastrointestinal pathogens

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Abstract

Objective: All across the world, gastrointestinal (GI) infections are an important cause of morbidity and mortality, especially in young children, patients in intensive care units, and patients with weakened immune systems. In this study we aimed to assess the Gastroenteritis RT-qPCR MX-24T Panel's utility as a standard technique for identifying gastrointestinal pathogens.

Methods: In this study, 76 stool samples from intensive care patients were tested for bacterial, viral, and parasitic pathogens using the Bio-Speedy® Gastroenteritis RT-qPCR MX-24T Panel kit.

Results: In this study, 31 out of 76 samples gave positive results. Eight bacterial (*Salmonella* spp., *Campylobacter* spp., *Shigella*/Enteroinvasive *Escherichia coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), and *Clostridium difficile* binary toxin A/B), three viral (Astrovirus, Norovirus (GI/GII and Rotavirus (A)) and two parasitic (*Cryptosporidium* spp., and *Giardia lamblia*) agents were detected from the stool samples of intensive care patients. While only a single agent was detected in the 22 samples, multiple agents were detected in 9 (30%). The most detected agent was EAEC (n=11), followed by *Campylobacter* spp. (n=7). EAEC and *Campylobacter* spp. were detected in 3 samples with multiple agents.

Conclusion: The GI panel can minimize the need for additional diagnostic testing and unnecessary antibiotic use by rapidly identifying a wide range of infections detectable only by molecular methods, as well as agents detectable by traditional conventional diagnostic methods. In this way, it may lead to a shorter hospital stay. In addition, we think that further studies should be conducted to determine whether the simultaneous detection of multiple pathogens in a sample in our study is clinically important.

Keywords: Gastrointestinal, infection, molecular panel, multiplex polymerase chain reaction.

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Gastrointestinal patojenlerin tespiti için kültürden bağımsız gastrointestinal multipleks PCR panelinin değerlendirilmesi

Öz

Amaç: Gastrointestinal (Gİ) enfeksiyonlar tüm dünyada özellikle küçük çocuklarda, yoğun bakımdaki hastalarda ve bağışıklık sistemi zayıf olan hastalarda önemli bir morbidite ve mortalite nedenidir. Bu çalışmada Gastroenterit RT-qPCR MX-24T Panelinin gastrointestinal patojenlerin tanımlanmasında standart bir teknik olarak kullanılabilirliğini değerlendirmeyi amaçladık.

Yöntemler: Bu çalışmada yoğun bakım hastalarından alınan 76 dışkı örneği Bio-Speedy® Gastroenteritis RT-qPCR MX-24T Panel kiti kullanılarak bakteriyel, viral ve paraziter patojenler açısından test edildi.

Bulgular: Bu çalışmada 76 örnekten 31'inde patojen mikroorganizma tespit edildi. Yoğun bakım hastalarının dışkı örneklerinden Sekiz bakteriyel (Salmonella spp., *Campylobacter* spp., *Shigella*/Enteroinvaziv *Escherichia coli* (EIEC), Enteroagregatif *E. coli* (EAEC), Shiga toksin üreten *E. coli* (STEC), Enteropatojenik *E. coli* (EPEC), Enterotoksijenik *E. coli* (ETEC) ve *Clostridium difficile* ikili toksin A/B), üç viral (Astrovirus, Norovirus (GI/GII ve Rotavirus (A)) ve iki parazitik (*Cryptosporidium* spp. ve *Giardia lamblia*) etkenleri tespit edildi. Örneklerin 22'sinde tek etken tespit edilirken, 9'unda (%30) birden fazla etken tespit edildi. En çok tespit edilen ajan EAEC (n=11) olurken, onu *Campylobacter* spp. takip etti. (n=7). EAEC ve *Campylobacter* spp. 3 örnekte birden fazla ajanla birlikte tespit edildi.

Sonuç: GI paneli, yalnızca moleküler yöntemlerle tespit edilebilen çok çeşitli enfeksiyonların yanı sıra geleneksel konvansiyonel teşhis yöntemleriyle tespit edilebilen ajanları hızlı bir şekilde tanımlayarak ek teşhis testlerine ve gereksiz antibiyotik kullanımına olan ihtiyacı en aza indirebilir. Bu sayede hastanede kalış süresinin daha kısa olmasına neden olabilir. Ayrıca çalışmamızda bir örnekte birden fazla patojenin aynı anda saptanmasının klinik açıdan önemli olup olmadığının belirlenmesi için ileri çalışmaların yapılması gerektiğini düşünüyoruz.

Anahtar kelimeler: Gastrointestinal, enfeksiyon, moleküler panel, multipleks polimeraz zincir reaksiyonu.

INTRODUCTION

Gastroenteritis is an illness marked by inflammation of the mucous lining of the gastrointestinal (GI) system. Gastrointestinal infections can be classified into three categories: gastritis, enteritis, and gastroenteritis. Gastritis refers to the inflammation of the stomach's protective lining and can be categorized as either acute or chronic[1,2]. Enteritis refers to the inflammation specifically limited to the small intestine. Gastroenteritis is characterized by inflammation of the stomach and intestines. It is commonly referred to as infectious diarrhea and is the main illness linked to gastrointestinal infections. The passing of three or more loose stools in a day is referred to as diarrhea[3]. Diarrhea is a global health issue that causes a high number of outpatient visits, a heavy load on inpatients, and a decline in quality of life in both domestic and international travelers[2]. An estimated four to six million children perish annually from diarrheal illnesses, with Asia and Africa being the most affected developing regions[4]. Developed nations, including the United States, have documented cases of infectious enteritis and foodborne disease in around 1.3 million individuals who have been diagnosed with enteritis or GI symptoms[5].

Swift and precise diagnosis is not only essential, but also highly significant for the prevention and management of infectious illnesses, administration of suitable antibiotic or antiparasitic medications, and analysis of epidemiological data. Nowadays, the use of syndromic panel-based tests is increasing to determine the factors that cause gastroenteritis. These tests are particularly used in adult and pediatric patients with weakened immune systems, individuals where identifying the organism is crucial due to clinical symptoms

like bloody diarrhea, or dehydration, high fever, and high-risk patient groups who need to be hospitalized[6,7]. A single reaction can detect bacteria, viruses, and occasionally parasites simultaneously with molecular testing panels, which are becoming increasingly prevalent. These extremely specific and sensitive methods of diagnosis reduce the need for laborious and time-consuming traditional diagnostic techniques[8-10]. Additionally, rapid molecular test panels are not meant to be used for individual patient diagnosis, but rather for public health objectives such as determining the origins of disease outbreaks, as per the 2017 guidelines published by the Infectious Diseases Society of America (IDSA). Although advanced algorithms for the clinical use of these tests are currently lacking, several studies and guidelines consistently emphasize their significant role in antimicrobial stewardship programs in multiple countries[11,12].

This investigation was conducted to evaluate the value of the Gastroenteritis RT-qPCR MX-24T Panel as a standard technique for gastrointestinal pathogen detection. This work was presented in part at the 34th European Congress of Clinical Microbiology and Infectious Diseases held on 27-30 April 2024 in Barcelona, Spain.

METHODS

In this study, 76 stool samples taken from patients hospitalized in Dicle University Faculty of Medicine intensive care units were tested for bacterial, viral and parasitic pathogens. After getting informed consent from patients or their legal representatives, the treating physician requested a stool polymerase chain reaction (PCR) panel test. Each patient's feces sample weighing around thirty grams was transferred to a sterile container and brought to the clinical microbiology laboratory within thirty minutes. After the samples were examined directly microscopically, they were kept cold until the PCR analysis at +4 °C. In this study, stool samples taken from patients were tested for bacterial, viral and parasitic pathogens using the Bio-Speedy® Gastroenteritis RT-qPCR MX-24T Panel kit.

RESULTS

In this study, 8 bacterial (*Salmonella* spp., *Campylobacter* spp., *Shigella*/Enteroinvasive *Escherichia coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Shiga toxin producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), and *Clostridium difficile* binary toxin A/B) (Table 1), 3 viral (Astrovirus, Norovirus (GI/GII), and Rotavirus (A)) (Table 2) and 2 parasitic (*Cryptosporidium* spp., and *Giardia lamblia*) (Table 3) agents were detected from the stool samples of intensive care patients by RT-qPCR. In our study, 31 out of 76 samples gave positive results. While a single agent was detected in 22 samples, multiple agents were detected in 9 (30%) of them (Table 4). The most detected agent was EAEC (n=11), followed by *Campylobacter* spp. (n=7). EAEC and *Campylobacter* spp. were detected in 3 samples with multiple agents. The maximum number of different agents with which EAEC and *Campylobacter* spp were detected together in a sample was determined to be 4. In one sample, *Shigella*/EIEC, *Campylobacter* spp., EAEC, EPEC. and ETEC were detected as multiples agents.

Table I: Bacterial agents detected with Gastroenteritis RT-qPCR MX-24 Panel

Bacterial agents detected by the kit	Bacterial agents detected in the study			
	Alone (n)	**With more one agents (n)**	**With two or more than agents (n)**	**Total (n)**
Salmonella spp.	1	ND	ND	1
Campylobacter spp.	4	ND	3	7
Shigella/Enteroinvasive E. coli (EIEC)	2	2	1	5
Enteroaggregative E. coli (EAEC)	5	3	3	11
Shiga toxin producing E. coli (STEC)	ND	1	ND	1
Enteropathogenic E. coli (EPEC)	2	2	2	6
Enterotoxigenic E. coli (ETEC)	1	1	2	4
*Clostridium difficil*e binary toxin A/B	1	ND	ND	1
Vibrio parahaemolyticus	ND	ND	ND	ND
Vibrio cholerae	ND	ND	ND	ND
Yersinia enterocolitica	ND	ND	ND	ND
Plesiomonas shigelloides	ND	ND	ND	ND
Clostridium difficile	ND	ND	ND	ND
Clostridium difficile toxin A	ND	ND	ND	ND
Clostridium difficile toxin B	ND	ND	ND	ND

ND: not detected

Table II: Viral agents detected with Gastroenteritis RT-qPCR MX-24 Panel

Viral agents detected by the kit	Viral agents detected in the study			
	Alone (n)	**With more one agents (n)**	**With two or more than agents (n)**	**Total (n)**
Astrovirus	ND	1	ND	1
Norovirus (GI/GII)	2	ND	1	3
Rotavirus (A)	2	1	ND	3
Sapovirus *(GI/GII/GIV/GV)*	ND	ND	ND	ND
Adenovirus	ND	ND	ND	ND

ND: not detected

Table III: Parasitic agents detected with Gastroenteritis RT-qPCR MX-24 Panel

Parasitic agents detected by the kit	Parasitic agents detected in the study			
	Alone (n)	**With more one agents (n)**	**With two or more than agents (n)**	**Total (n)**
Cryptosporidium spp.	1	ND	ND	1
Giardia lamblia	1	ND	ND	1
Entamoeba histolytica	ND	ND	ND	ND
Cyclospora cayetanensis	ND	ND	ND	ND

ND: not detected

Table IV: Distribution of multiple causative agents

Multiple agents	**n**
Norovirus, *Enteropathogenic E. coli* (EPEC) and *Campylobacter* spp.	1
Campylobacter spp., *Enteroaggregative E. coli* (EAEC) and *Enterotoxigenic E. coli* (ETEC)	1
Enteroaggregative E. coli and Rotavirus	1
Enteroaggregative E. coli and Astrovirus	1
Shigella/Enteroinvasive E. coli (EIEC), *Campylobacter* spp., *Enteroaggregative E. coli* (EAEC), *Enteropathogenic E. coli* (EPEC) and *Enterotoxigenic E. coli* (ETEC)	1
Shigella/Enteroinvasive E. coli (EIEC) and *Enteroaggregative E. coli* (EAEC)	1
Enteropathogenic E. coli (EPEC) and Shiga toxin producing *E. coli* (STEC)	1
Shigella/Enteroinvasive E. coli (EIEC) and *Enterotoxigenic E. coli* (ETEC)	1
Enteroaggregative E. coli (EAEC) and *Enteropathogenic E. coli* (EPEC)	1

DISCUSSION

For the infectious disease laboratory, automated processes utilizing molecular platforms are becoming more and more crucial as they provide faster turnaround times and higher sensitivity for target pathogen identification[13]. Manual culture and phenotypic identification assays are still used to accomplish "classical" microbiological stool diagnoses. These approaches are time demanding and have limitations in sensitivity and specificity[14]. In this investigation, 76 stool samples from critical care unit patients were analyzed for bacterial, viral, and parasite pathogens using the Bio-Speedy® Gastroenteritis RT-qPCR MX-24T Panel kit.

Gastroenteritis may be caused by infectious or non-infectious factors [15]. Several bacteria, including *Campylobacter* spp., *Shigella* spp., *Salmonella* spp., *Yersinia* spp., *Aeromonas* spp., intestinal pathogenic strains of *C. difficile*, and *E. coli*, as well as protozoa, including *Entamoeba histolytica*, *Giardia intestinalis*, and *Cryptosporidium* spp., viruses, including Norovirus, Rotavirus, enteric Coronavirus, enteric Adenovirus, and Astrovirus, and several types of fungi are the primary agents responsible for these infectious diseases[15-17]. In our study, we detected 8 bacterial (*Salmonella* spp., *Campylobacter* spp., *Shigella*/EIEC, EAEC, STEC, EPEC, ETEC, and *C. difficile* binary toxin A/B), 3 viral (Astrovirus, Norovirus (GI/GII and Rotavirus (A)), and 2 parasitic (*Cryptosporidium* spp., and *G. lamblia*) agent strains from the stool samples of intensive care patients using RT-qPCR.

In a recent one-year retrospective study with multiplex PCR by Mohtar et al.[18] in Lebanon, In all, enteropathogens were reported in 71% of the included cases; of these, 46% were diagnosed as single and 54% as multiple infections in patients. It was reported that bacteria were found in 48% of the samples, parasites in 12% and viruses in 11%. Bacteria were found to be identified as the most common agents in all age groups. EAEC (26.5%), ETEC (23.2%) and EPEC (20.3%) were reported as the most frequently identified agents. In our study, enteropathogens were detected in 40.8% (31/76) of the samples. We detected single infection in 64.5% (20/31) of positive samples and mixed infection in 35% (11/31). In our study, bacterial agents were mostly detected. Consistent with the literature, EAEC was the

most frequently identified agent in our study. It was followed by *Campylobacter* spp.

In another study carried out in Istanbul and Kocaeli cities in Türkiye [15], stool samples from 86 acute gastroenteritis patients were examined by multiplex real-time PCR using the viral&bacterialgastroenteritis kit. Among the 86 samples tested in the research, a single agent was identified in 41 samples, but co-infection was detected in 5 samples. The predominant bacterial agents identified in the research were *Salmonella* spp., *Shigella* spp./EIEC, and *Campylobacter coli/jejuni*, whereas the most commonly found viral agents were reported as Norovirus G1/G2, Rotavirus, Astrovirus, and Adenovirus. In accordance with their studies, the predominant viral agents identified in our investigation were Norovirus (GI/GII) and Rotavirus (A), whereas the most often found bacterial agents included EAEC, *Campylobacter* spp, *Shigella*/EIEC, and ETEC.

In our investigation, two or more causative organisms were detected in 9 positive samples (29%). In another study conducted in Türkiye, this rate was reported as 5 samples (11%)[15]. In their study, only two causative agents were detected in one sample, whereas in our study, out of 9 samples in which more than one agent was detected, 3 agents were detected together in 2 and 5 agents were detected together in 1 sample. In the current study, two causative agents were detected together in the remaining 6 samples. Conversely, co-infection was found in the majority of diarrheal stool samples in research by Eibach et al.[8] that involved adult patients in Ghana. The researchers attributed the finding to the elevated levels of exposure to environmental pathogens among asymptomatic children living in unsanitary and unhealthy conditions. Additionally, they inquired about the effectiveness of extremely sensitive multiplex PCR methods in sub-Saharan Africa for the diagnosis of intestinal diseases. Comparing reported coinfection rates to our study, Italy reported similar rates (28.2%)[19], whereas the USA reported lower rates (27.0%)[20].

Our study has a few limitations. First, since our study was retrospective, we did not compare GI panel and conventional tests. Therefore, we could not verify whether the GI panel and conventional test results were concordant. This limited the assessment of false positive or negative cases, especially when multiple determinations were made. Additionally, a significant disadvantage is the absence of a control group consisting of patients who did not exhibit any symptoms. Comparing colonization of identified pathogens in symptomatic patients with asymptomatic individuals may provide valuable information about the clinical significance of the pathogen and its potential to cause disease.

CONCLUSION

In conclusion, The GI panel can reduce the need for extra diagnostic testing and inappropriate antibiotic usage by quickly identifying a wide range of pathogens that can only be detected using molecular approaches, as well as agents that can be detected using standard diagnostic methods. This might result in a reduced length of stay in the hospital. We also believe that further research has to be done to evaluate the clinical significance of multiple infections being detected simultaneously in a sample in our investigation. There is a need for studies in which new algorithms are created to evaluate test results, taking into account CT values in which multiple factors are seen in the tests performed. For this purpose, more studies should be done to create new algorithms based on factor-specific clinical data and CT values where the agent is detected.

Ethics Committee Approval: The Siirt University Non-Interventional Clinical Research Ethics Committee approved this study on March 29, 2024 (Meeting number: 101760, decision no: 2024/3/03/03).

Informed consent: All subjects and/or their guardian(s) gave their informed consent.

Conflict of Interest: The authors declared no conflicts of interest.

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