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Original Research

Evaluation of Diagnostic Performance of Bd Max Ebp Assay in Patients with Diarrheal Illness

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ABSTRACT

Objective: Detection of the etiological agents in patients with acute diarrhea is challenging due to a wide variety of pathogens. The aim of this study is to evaluate the diagnostic performance of BD Max Enteric Bacterial Pathogens (EBP) PCR assay in patients with diarrheal illness.

Methods: Between 1 January 2014 and 31 May 2015, stool samples from pediatric or adult patients with diarrhea submitted for routine analysis of bacterial stool pathogens were included in the study. We compared the BD Max EBP PCR assay to culture for the detection of Salmonella spp., Shigella spp., Campylobacter jejuni, and Campylobacter coli and an EIA for Shiga toxins 1 and 2. Discordant results were adjudicated by either antigen detection methods or Film array GI Panel.

Results: When coinfections were excluded, the positive percent agreement values for the BD Max EBP assay (PPA) was 100% and negative percent agreement (NPA) was between 98.0%-99.7%, when compared with culture and EIA. After discrepant analysis, the PPA values for the BD Max EBP assay was 100% and NPA was between 99.5%-100%.

Conclusion: The BD Max EBP assay showed a high correlation rate with conventional and molecular methods for the detection of stool pathogens.

Key words: Osteoarthritis, knee joint, talocrural joint, transverse tarsal joint

INTRODUCTION

Infectious diarrheal diseases cause substantial morbidity and mortality worldwide. A wide variety of pathogens lead to infectious diarrhea, which makes the diagnosis of bacterial pathogens particularly challenging given the large amounts of background normal gastrointestinal flora (1,2).

Viral agents such as the noroviruses are responsible for most of the acute infectious diarrhea, while bacteria are responsible for most cases with more aggresive and inflamatory diarrhea. (3) *Salmonella*, *Campylobacter*, *Shigella*, and Shiga toxin-producing *Escherichia coli* (STEC) are the most common diarrheagenic bacteria and routine stool culture is designed to detect these pathogens in most laboratories (4).

Detection and identification of the pathogens of acute diarrhea are important for both individual patient care and public health investigation. Furthermore, some infectious diarrheal pathogens can lead to long-term complications such as Hemolytic uremic syndrome, Guillain-Barr syndrome (5).

Conventional stool culture is the gold standard for the diagnosis of bacterial gastroenteritis (6). On the other hand stool cultures are either insensitive or labor intensive with long turn around time. For the diagnosis of bacterial diarrhea, a wide variety of culture protocols involving multiple selective media and reagents are available in the microbiological laboratory (1,7). However, the use of antibiotics affects the culture result and frequently causes low yield for identification of enteropathogens (7). Molecular methods can increase sensitivity and specificity compared to stool culture (8).

The aim of this study is to evaluate the diagnostic performance of BD Max Enteric Bacterial Pathogens (EBP) assay in patients with diarrheal illness.

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METHODS

Between 1 January 2014 and 31 May 2015, stool samples from pediatric or adult patients with diarrhea submitted for routine analysis of bacterial stool pathogens were included in the study. Duplicate specimens from the same patient were not enrolled. Culture and Enzyme Immunoassay (EIA): Fresh stool specimens were inoculated onto Mac Conkey agar, XLD agar for *Salmonella* and *Shigella*, and incubated at 37°C for 24 hours in an aerobic incubator. Lactose, xylose nonfermenting colonies with or without black centers on these media were screened phenotypically on triple sugar iron agar, motility medium, urea agar, Simmon's citrate agar and lysine iron agar. Suspected colonies were tested with Wellcolex™ Color Salmonella Rapid Latex Agglutination Test Kit and Wellcolex™ Color Shigella (ThermoFisher, UK).

E. coli Shiga toxin was detected using by EIA (ProSpecT Shiga Toxin *E. coli* Microplate Assay,Remel,UK), according to the manufacturer's instructions.

Screening for *Campylobacter spp* in stool was performed with *Campylobacter* selective agar and incubated under microaerobic condition at 42°C for 5 days. Suspected colonies were identified by Gram stain examination of the colony along with oxidase test and MALDI-TOF MS.

BD Max EBP automated PCR: The BD Max enteric bacterial panel (EBP) is a multiplex nucleic acid amplification assay which detects DNA from *Campylobacter* spp. (*jejuni* and *coli*), *Salmonella* spp., *Shigella* spp. / Enteroinvasive *E. coli* (EIEC), Shiga toxin1(stx1)/Shiga toxin2(stx2) genes in stool specimens with the BD Max system less than three hours. (BD Diagnostics, Baltimore, MD, USA) (Harrington). The BD MAX™ System is a fully-automated, closed system which allows for simultaneous processing of up to 24 individual tests.

Fresh stool samples were tested daily with the BD Max EBP assay, according to the manufacturer's instructions.

Interpretation

We accepted conventional culture as the reference method for the detection of *Shigella* spp., *Campylobacter spp* and *Salmonella* spp. and EIA as the reference method for the detection of Shiga toxins for the calculation of NPA and PPA of BD Max EBP assay. In addition, BD Max EBP assay positive and conventional method negative results were adjudicated by either antigen detection method (*Campylobacter* EIA) or Film array GI Panel.

Main Points:

- Culture remain the method of choice for diagnosis of bacterial enteritis. On the other hand, nucleic acid amplification tests offer rapid results and markedly improve the detection and identification of stool pathogens.
- In our study, BD Max EBP assay showed excellent performance for the detection of Salmonella spp., Shigella spp., Campylobacter spp and Shiga toxins.
- The BD Max EBP assay showed a high correlation rate with conventional and molecular methods for the detection of stool pathogens.
- The BD Max EBP assay detect DNA and not necessarily viable organisms which may lead to increased appreciation of asymptomatic infections and prolonged shedding.

Stool samples with discordant results between Campylobacter culture and the BD Max EBP assay were tested by using an enzyme immunoassay (RIDASCREEN® Campylobacter,r-biopharm,-Germany) according to the manufacturer's instructions. Samples that gave different results between the BD MAX EBP assay and *Campylobacter* EIA were subject to FilmArray Gastrointestinal (GI) Panel (BioFire -BioMérieux, France).

Samples with discordant results between *Salmonella* and *Shigella* culture or Shiga toxin EIA and the BD Max EBP assay were tested by FilmArray GI Panel following the manufacturer's instructions.

Statistical analysis: Positive percent agreement (PPA) and Negative percent agreement (NPA) and their 95% confidence intervals were calculated, as reported previously (9) .

The method used in the study is a routinely applied method in our hospital. Informed consent was not obtained from the patients because it is not necessary to obtain informed concent for archieve material collected from patient stool. However, data usage permission has been obtained. Ethics committee application was made and ethics committee approval was obtained.

RESULTS

One thousand two hundred twenty four stool samples were included in the study, 46 of which were excluded due to inhibition by BD Max EBP assay.

Culture and Shiga toxin EIA results**:** 14 (1.19%) specimens were positive for *Campylobacter* spp, 22 (1.87%) were positive for *Salmonella* spp and two (0.17%) were positive for Shigella/EIEC by culture. 21 (1.78%) were positive for Shiga toxins by EIA. These were also positive with BD Max EBP assay. Coinfection was not detected by culture. The positivity rate of investigated pathogens was 5.01% (59/1178) by culture and EIA.

Of the 1178 samples, 30 had *Salmonella*, 6 had *Shigella / EIEC*, 37 had *Campylobacter spp*, and 38 had Shiga-like toxin genes (*stx1* and / or *stx2*) by BD Max EBP assay. In addition, BD Max EBP assay identified coinfections in two samples (in one sample *Salmonella* + Shiga-like toxin genes and in another *Campylobacter* + Shiga-like toxin genes).

When coinfections were excluded, the NPA of the BD Max EBP assay was 99.3% for *Salmonella*, 99.7 % for *Shigella* / EIEC, and 98.0% for *Campylobacter* when compared with culture. NPA was 98.5% for Shiga toxins using EIA as a reference method. PPA was 100% for all targets (Table 1).

Results after discrepant analysis: *Campylobacter* spp was isolated from culture in 14 out of 37 samples that were positive by BD Max EBP assay. In 9 out of 23 samples that were found to be incompatible by BD Max EBP assay and culture, the enzyme immunoassay

Table1: Performance of BD Max EBP assay when compared with the reference method (stool culture and Shiga toxin EIA)

	No. of pathogens with BD Max EBP result with reference to culture/EIA				Total number of samples	PPA (95% confidence interval)	NPA (95% confidence interval)
Target type	True Positive	False Negative	False Positive	True Negative			
Salmonella	22	0	8	1146	1176	100	99,31 (98,83-99,78)
Shigella/EIEC	2	0	4	1170	1176	100	99,66 (99,33-99,99)
Shiga toxins	21	0	17	1138	1176	100	98,53 (97,84-99,22)
Campylobacter	14	0	23	1139	1176	100	98,02 (97,22-98,82)

Not:Coinfections were excluded in two samples
EBP: Enteric Bacterial Pathogens, EIA: Enzyme Immun Assay, PPA: Positive Percent Agreement, NPA: Negative Percent Agreement, EIEC: Entero Invasive Eschercia coli

Table 2: Performance of BD Max EBP assay when compared with the reference method (Campylobacter EIA and Film Array GI panel)

	No. of pathogens with BD Max EBP result with reference to EIA and Biofire Film Array GI Panel				Total number of samples	PPA (95% confidence interval)	NPA (95% confidence interval)
Target type	True Positive	False Negative	False Positive	True Negative			
Salmonella	25	0	5	1146	1176	100	99,57 (99,19-99,94)
Shigella/EIEC	6	0	0	1170	1176	100	100
Shiga toxins	32	0	6	1138	1176	100	99,48 (99,06-99,89)
Campylobacter	33	0	4	1139	1176	100	99,65 (99,31-99,99)

Not:Coinfections were excluded in two samples
EBP: Enteric Bacterial Pathogens, EIA: Enzyme Immun Assay, GI: Gastrointestinal, PPA: Positive Percent Agreement, NPA: Negative Percent Agreement, EIEC: Entero Invasive Eschercia coli

(RIDASCREEN® *Campylobacter*, r-biopharm, Germany) was found to be positive. *The remaining 14 discordant samples were studied with* FilmArray GI Panel *and Campylobacter spp was positive in 10 samples. NPA was found as 99.7% for Campylobacter spp.*
Discrepant results between culture or EIA and the BD Max EBP assay for *Salmonella* spp., *Shigella* spp., and Shiga-like toxin genes (*stx1* and/or *stx*2) were tested by using FilmArray GI Panel. The *NPA* of the BD Max EBP assay was 99.6% for *Salmonella*, 100% for *Shigella* / EIEC, and 99.5% for Shiga toxin.

After analysis of discrepant results, use of BD Max EBP assay identified an additional 37 pathogens, thereby increasing the frequency to 8.2% (96/1176), when coinfections were excluded (Table 2).

When the samples with coinfection were examined, there was no growth in culture and EIA tests results were negative. When these samples were studied with FilmArray GI Panel, *Salmonella* and Shiga-like toxin genes were found to be negative in one sample and only *Campylobacter* gene was positive in another sample with *Campylobacter* + Shiga-like toxin genes.

DISCUSSION

Detection of the etiological agents in patients with acute diarrhea is important for appropriate therapy and public health interventions. Culture remain the method of choice for diagnosis of bacterial enteritis. On the other hand, nucleic acid amplification tests offer rapid results and markedly improve the detection and identification of stool pathogens. The use of FDA-approved culture-independent diagnostics in addition to traditional methods is supported by recent research (10).

Harrington et al (9) conducted a multicenter evaluation of the BD Max EBP assay in comparison to culture for the detection of *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, and *Campylobacter coli* and an EIA for Shiga toxins 1 and 2 with stool culture for fresh and preserved stool specimen. Following discrepant analysis, PPA and NPA values were 97.3% and 99.8% for *Salmonella* spp. 99.2% and 100% for *Shigella* spp. 97.5% and 99.0% for *C.jejuni* and *C. coli*, and 100% and 99.7% for Shiga toxins, respectively. They concluded that, the BD Max EBP assay with superior sensitivity compared to conventional methods and excellent specificity, may improve the detection of bacterial stool pathogens and time to reporting of results.

In a prospective study including 971 stool samples, the PPA of the BD MAX EBP assay and stool culture or enzyme immunoassay was **97%** for *Campylobacter* spp. **75%** for *Salmonella* spp., **100%** for *Shigella* spp., and **88%** for Shiga toxins. Furthermore, a NPA of **98%** for *Campylobacter* spp. **99%** for *Salmonella* spp. **99%** for *Shigella* spp. and **99%** for Shiga toxins has been demonstrated.

They found that the use of the BD MAX EBP increased the overall detection rate from 5.26% to 8.06%. Their study highlighted the superior detection rate of molecular assays compared to conventional diagnostic procedures (1)

Biswas et al (11), evaluated the diagnostic accuracy and laboratory turnaround time of three molecular assays. When the prospective samples were evaluated, the sensitivity and specificity of BD MAX EBP assay for Salmonella spp., Shigella spp., and Campylobacter spp. were found to be 99.7-100%.

Anderson et al (2), investigated the performance of the BD MAX EBP in preserved stool specimens that were artificially spiked with pathogen strains at different concentrations. The EBP panel demonstrated superior sensitivity and reliably detected Salmonella, EHEC O157, Shigella, and Campylobacter at concentrations 1 to 2-log10 lower than those needed for culture detection.

Mortensen et al (12), evaluated 86 stool samples with culture and BD Max EBP. Approximately 20% of cultures required additional process steps to exclude potential pathogens. Negative result reporting time with conventional culture was found to be approximately 41-54 hours.

In our study, BD Max EBP assay showed excellent performance for the detection of Salmonella spp., Shigella spp., Campylobacter spp and Shiga toxins. The NPA of BD MAX EBP in our study was similar to previous reports. Since we did not have a BD MAX EBP negative but the reference test positive sample, PPA of BD MAX EBP in our study was slightly higher than previous studies. The reasons for this difference may be due to interlaboratory technical variance, specimen transport and processing practices such as unemployment of enrichment broth.

This is a single center, laboratory-based, prospective study with a high number of samples. The limitation of our study is that the clinical features of patients were not included and also the study was done only in fresh stool samples but not Cary Blair-preserved specimens.

CONCLUSION

We concluded that, the BD Max EBP showed a high correlation rate with conventional and molecular methods for the detection of stool pathogens. In addition, our detection rates increased with BD Max EBP which has high PPV and NPV. On the other hand, BD Max EBP assay detect DNA and not necessarily viable organisms which may lead to increased appreciation of asymptomatic infections and prolonged shedding. For this reason the results should be interpreted with consideration of clinical information.

Ethical Considerations: This study was approved by the Akdeniz University School of Medicine Ethical Committee of Clinical Research (Decision number: 471).

Peer-review: Externally peer-reviewed.

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Conflicts of Interest: The authors declare no conflict of interest.

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