

World Journal of *Clinical Cases*

World J Clin Cases 2020 September 6; 8(17): 3621-3919



Contents

Semimonthly Volume 8 Number 17 September 6, 2020

REVIEW

- 3621 Autoimmunity as the comet tail of COVID-19 pandemic
Talotta R, Robertson E
- 3645 Gender medicine: Lessons from COVID-19 and other medical conditions for designing health policy
Machluf Y, Chaïter Y, Tal O

MINIREVIEWS

- 3669 Complexities of diagnosis and management of COVID-19 in autoimmune diseases: Potential benefits and detriments of immunosuppression
Georgiev T, Angelov AK

ORIGINAL ARTICLE

Retrospective Study

- 3679 Incidental anal ¹⁸fluorodeoxyglucose uptake: Should we further examine the patient?
Moussaddaq AS, Brochard C, Palard-Novello X, Garin E, Wallenhorst T, Le Balc'h E, Merlini L'heritier A, Grainville T, Siproudhis L, Lièvre A
- 3691 Emergency surgery in COVID-19 outbreak: Has anything changed? Single center experience
D'Urbano F, Fabbri N, Koleva Radica M, Rossin E, Carcoforo P
- 3697 Somatostatin receptor scintigraphy in the follow up of neuroendocrine neoplasms of appendix
Saponjski J, Macut D, Sobic-Saranovic D, Ognjanovic S, Bozic Antic I, Pavlovic D, Artiko V
- 3708 Efficacy of stool multiplex polymerase chain reaction assay in adult patients with acute infectious diarrhea
Ahn JS, Seo SI, Kim J, Kim T, Kang JG, Kim HS, Shin WG, Jang MK, Kim HY
- 3718 Comparison of gemcitabine plus nab-paclitaxel and FOLFIRINOX in metastatic pancreatic cancer
Han SY, Kim DU, Seol YM, Kim S, Lee NK, Hong SB, Seo HI
- 3730 Shear wave elastography may be sensitive and more precise than transient elastography in predicting significant fibrosis
Yao TT, Pan J, Qian JD, Cheng H, Wang Y, Wang GQ
- 3743 Radioactive ¹²⁵I seed implantation for locally advanced pancreatic cancer: A retrospective analysis of 50 cases
Li CG, Zhou ZP, Jia YZ, Tan XL, Song YY
- 3751 Active surveillance in metastatic pancreatic neuroendocrine tumors: A 20-year single-institutional experience
Gao HL, Wang WQ, Xu HX, Wu CT, Li H, Ni QX, Yu XJ, Liu L

- 3763** Clinical efficacy of tocilizumab treatment in severe and critical COVID-19 patients

Zeng J, Xie MH, Yang J, Chao SW, Xu EL

- 3774** Phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor 1 is a diagnostic and prognostic biomarker for hepatocellular carcinoma

Cai Y, Zheng Q, Yao DJ

Observational Study

- 3786** Awareness and attitude of fecal microbiota transplantation through transendoscopic enteral tubing among inflammatory bowel disease patients

Zhong M, Sun Y, Wang HG, Marcella C, Cui BT, Miao YL, Zhang FM

CASE REPORT

- 3797** Cauda equina arachnoiditis – a rare manifestation of West Nile virus neuroinvasive disease: A case report

Santini M, Zupetic I, Viskovic K, Krznaric J, Kutlesa M, Krajinovic V, Polak VL, Savic V, Tabain I, Barbic L, Bogdanic M, Stevanovic V, Mrzljak A, Vilibic-Cavlek T

- 3804** Portal gas in neonates; is it always surgical? A case report

Altokhais TI

- 3808** Large lingual heterotopic gastrointestinal cyst in a newborn: A case report

Lee AD, Harada K, Tanaka S, Yokota Y, Mima T, Enomoto A, Kogo M

- 3814** Osteochondral lesion of talus with gout tophi deposition: A case report

Kim T, Choi YR

- 3821** Traumatic neuroma of remnant cystic duct mimicking duodenal subepithelial tumor: A case report

Kim DH, Park JH, Cho JK, Yang JW, Kim TH, Jeong SH, Kim YH, Lee YJ, Hong SC, Jung EJ, Ju YT, Jeong CY, Kim JY

- 3828** Autoimmune hepatitis in a patient with immunoglobulin A nephropathy: A case report

Jeon YH, Kim DW, Lee SJ, Park YJ, Kim HJ, Han M, Kim IY, Lee DW, Song SH, Lee SB, Seong EY

- 3835** Diagnosis of an actively bleeding brachial artery hematoma by contrast-enhanced ultrasound: A case report

Ma JJ, Zhang B

- 3841** Lung adenocarcinoma harboring rare epidermal growth factor receptor L858R and V834L mutations treated with icotinib: A case report

Zhai SS, Yu H, Gu TT, Li YX, Lei Y, Zhang HY, Zhen TH, Gao YG

- 3847** Gastroduodenitis associated with ulcerative colitis: A case report

Yang Y, Li CQ, Chen WJ, Ma ZH, Liu G

- 3853** Majocchi's granuloma caused by *Trichophyton rubrum* after facial injection with hyaluronic acid: A case report

Liu J, Xin WQ, Liu LT, Chen CF, Wu L, Hu XP

- 3859** Novel deletion mutation in Bruton's tyrosine kinase results in X-linked agammaglobulinemia: A case report
Hu XM, Yuan K, Chen H, Chen C, Fang YL, Zhu JF, Liang L, Wang CL
- 3867** Multidisciplinary treatment of life-threatening hemoptysis and paraplegia of choriocarcinoma with pulmonary, hepatic and spinal metastases: A case report
Lin YY, Sun Y, Jiang Y, Song BZ, Ke LJ
- 3875** Diagnostic value of ultrasound in the spontaneous rupture of renal angiomyolipoma during pregnancy: A case report
Zhang T, Xue S, Wang ZM, Duan XM, Wang DX
- 3881** Gallbladder sarcomatoid carcinoma: Seven case reports
Qin Q, Liu M, Wang X
- 3890** Surgical strategy used in multilevel cervical disc replacement and cervical hybrid surgery: Four case reports
Wang XF, Meng Y, Liu H, Hong Y, Wang BY
- 3903** Diagnosis and treatment of an elderly patient with 2019-nCoV pneumonia and acute exacerbation of chronic obstructive pulmonary disease in Gansu Province: A case report
He TP, Wang DL, Zhao J, Jiang XY, He J, Feng JK, Yuan Y
- 3911** Diagnosis and treatment of mixed infection of hepatic cystic and alveolar *echinococcosis*: Four case reports
A JD, Chai JP, Wang H, Gao W, Peng Z, Zhao SY, A XR

ABOUT COVER

Editorial board member of World Journal of Clinical Cases, Dr. Elia de Maria is Adjunct Professor of Arrhythmology Lab in the Cardiology Unit, Ramazzini Hospital in Carpi, Italy. He graduated in Medicine and Surgery from the University of Napoli in 1999, continuing on to obtain specialization in Cardiology in 2003. He also holds the distinction of High Degree Master in Electrophysiology and Cardiac Stimulation. Since 2005, he has practiced as a Permanent Consultant Cardiologist in the Italian Public Hospitals, and since 2015 as an External Contract Professor in the Faculty of Medicine and Surgery of University of Verona. His clinical and research interests encompass pharmacological therapy in acute and chronic cardiac conditions, temporary and definitive pacing, thoracentesis and pericardiocentesis, and hemodynamic monitoring. (L-Editor: Filipodia)

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Cases* (WJCC, *World J Clin Cases*) is to provide scholars and readers from various fields of clinical medicine with a platform to publish high-quality clinical research articles and communicate their research findings online.

WJCC mainly publishes articles reporting research results and findings obtained in the field of clinical medicine and covering a wide range of topics, including case control studies, retrospective cohort studies, retrospective studies, clinical trials studies, observational studies, prospective studies, randomized controlled trials, randomized clinical trials, systematic reviews, meta-analysis, and case reports.

INDEXING/ABSTRACTING

The WJCC is now indexed in Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, PubMed, and PubMed Central. The 2020 Edition of Journal Citation Reports® cites the 2019 impact factor (IF) for WJCC as 1.013; IF without journal self cites: 0.991; Ranking: 120 among 165 journals in medicine, general and internal; and Quartile category: Q3.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: Yan-Xia Xing; Production Department Director: Yun-Xiaojuan Wu; Editorial Office Director: Jin-Lai Wang.

NAME OF JOURNAL

World Journal of Clinical Cases

ISSN

ISSN 2307-8960 (online)

LAUNCH DATE

April 16, 2013

FREQUENCY

Semimonthly

EDITORS-IN-CHIEF

Dennis A Bloomfield, Sandro Vento, Bao-Gan Peng

EDITORIAL BOARD MEMBERS

<https://www.wjgnet.com/2307-8960/editorialboard.htm>

PUBLICATION DATE

September 6, 2020

COPYRIGHT

© 2020 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS

<https://www.wjgnet.com/bpg/gerinfo/204>

GUIDELINES FOR ETHICS DOCUMENTS

<https://www.wjgnet.com/bpg/GerInfo/287>

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

<https://www.wjgnet.com/bpg/gerinfo/240>

PUBLICATION ETHICS

<https://www.wjgnet.com/bpg/GerInfo/288>

PUBLICATION MISCONDUCT

<https://www.wjgnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjgnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjgnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

<https://www.f6publishing.com>

Retrospective Study

Efficacy of stool multiplex polymerase chain reaction assay in adult patients with acute infectious diarrhea

Jae Sung Ahn, Seung In Seo, Jinseob Kim, Taewan Kim, Jin Gu Kang, Hyung Su Kim, Woon Geon Shin, Myoung Kuk Jang, Hak Yang Kim

ORCID number: Jae Sung Ahn 0000-0003-1623-359X; Seung In Seo 0000-0003-4417-0135; Jinseob Kim 0000-0002-9403-605X; Taewan Kim 0000-0002-4708-5609; Jin Gu Kang 0000-0002-5332-9251; Hyung Su Kim 0000-0002-2394-1095; Woon Geon Shin 0000-0002-9851-5576; Myoung Kuk Jang 0000-0001-7968-7543; Hak Yang Kim 0000-0002-7459-3473.

Author contributions: Seo SI designed the research study; Ahn JS and Seo SI performed the research; Kim JS contributed statistical analysis; Seo SI, Kim TW, Kang JG and Kim HS analyzed the data; Shin WG, Jang MK and Kim HY provided material support; Seo SI and Ahn JS wrote the manuscript. All authors have read and approve the final manuscript.

Supported by Kangdong Sacred Heart Hospital Fund, No. 2017-07.

Institutional review board statement: The protocol for this study was approved by the institutional review board of Kangdong Sacred Heart Hospital, No. 2020-02-008.

Informed consent statement: We received exemption of informed consent.

Conflict-of-interest statement: All

Jae Sung Ahn, Taewan Kim, Jin Gu Kang, Department of Internal Medicine, Kangdong Sacred Heart Hospital, Seoul KS013, South Korea

Seung In Seo, Hyung Su Kim, Woon Geon Shin, Myoung Kuk Jang, Hak Yang Kim, Department of Internal Medicine, Kangdong Sacred Heart Hospital, Hallym University College of Medicine, Seoul KS013, South Korea

Seung In Seo, Hyung Su Kim, Woon Geon Shin, Myoung Kuk Jang, Hak Yang Kim, Institute for Liver and Digestive Diseases, Hallym University, Chuncheon KS013, South Korea

Jinseob Kim, Department of Epidemiology, School of Public Health, Seoul National University, Seoul KS013, South Korea

Corresponding author: Seung In Seo, MD, Assistant Professor, Department of Internal Medicine, Kangdong Sacred Heart Hospital, Hallym University College of Medicine, 445, Gil-dong, Kangdong-gu, Seoul KS013, South Korea. doctorssi@kdh.or.kr

Abstract

BACKGROUND

Recently, stool multiplex polymerase chain reaction (PCR) tests have been developed for identifying diarrhea-causing bacterial pathogens. Furthermore, fecal calprotectin is a well-known effective marker for intestinal mucosal inflammation.

AIM

To evaluate the efficacy of stool multiplex PCR and fecal calprotectin in acute infectious diarrhea.

METHODS

Overall, 400 patients with acute infectious diarrhea were enrolled from Kangdong Sacred Heart Hospital (January 2016 to December 2018). Multiplex PCR detected 7 enteropathogenic bacteria including *Salmonella*, *Campylobacter*, *Shigella*, *Escherichia coli* O157:H7, *Aeromonas*, *Vibrio*, and *Clostridium difficile*. We reviewed clinical and laboratory findings using stool multiplex PCR.

RESULTS

Stool multiplex PCR test detected considerably more bacterial pathogens than

authors have no any conflicts of interest.

Data sharing statement: No additional data.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Received: March 11, 2020

Peer-review started: March 11, 2020

First decision: April 25, 2020

Revised: June 4, 2020

Accepted: August 1, 2020

Article in press: August 1, 2020

Published online: September 6, 2020

P-Reviewer: Malnick S, Islek A

S-Editor: Ma YJ

L-Editor: A

P-Editor: Wang LL



stool culture (49.2% *vs* 5.2%), with *Campylobacter* as the most common pathogen (54%). Patients with positive stool PCR showed elevated fecal calprotectin expression compared to patients with negative stool PCR (1124.5 ± 816.9 mg/kg *vs* 609 ± 713.2 mg/kg, $P = 0.001$). C-reactive protein (OR = 1.01, 95%CI: 1.001-1.027, $P = 0.034$) and sigmoidoscopy-detected colitis (OR = 4.76, 95%CI: 1.101-20.551, $P = 0.037$) were independent factors in stool PCR-based detection of bacterial pathogens. Sensitivity and specificity of calprotectin were evaluated to be 70.5% and 60.9%, respectively (adjusted cut-off value = 388 mg/kg).

CONCLUSION

Stool multiplex PCR test has increased sensitivity in detecting pathogens than conventional culture, and it is correlated with calprotectin expression. Stool multiplex PCR and calprotectin may be effective in predicting clinical severity of infectious diarrhea.

Key words: Acute infectious diarrhea; Stool multiplex polymerase chain reaction; Calprotectin

©The Author(s) 2020. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Stool multiplex polymerase chain reaction (PCR) test showed improved sensitivity than conventional culture method in detecting bacterial pathogens in hospitalized patients with acute infectious diarrhea. Patients with positive stool PCR showed higher C-reactive protein expression level and colitis on sigmoidoscopy. Fecal calprotectin was found to correlate with the positivity of stool PCR, and the adjusted cut-off value of calprotectin for detecting bacterial pathogens by stool PCR was found to be 388 mg/kg.

Citation: Ahn JS, Seo SI, Kim J, Kim T, Kang JG, Kim HS, Shin WG, Jang MK, Kim HY. Efficacy of stool multiplex polymerase chain reaction assay in adult patients with acute infectious diarrhea. *World J Clin Cases* 2020; 8(17): 3708-3717

URL: <https://www.wjgnet.com/2307-8960/full/v8/i17/3708.htm>

DOI: <https://dx.doi.org/10.12998/wjcc.v8.i17.3708>

INTRODUCTION

Acute diarrhea causes gastrointestinal disease, which increases the economic burden of the health care system. Annually, it has been reported to account for more than 2 million deaths^[1,2]. According to World Health Organization report 2003, the median incidence rate for diarrhea in children less than 5 years was 3.2 episodes per child-year, and there has been no significant change since 1980s^[3]. In the United Kingdom, up to 17 million cases and 1 million general practice consultations have been reported with infectious intestinal disease every year^[4].

Diarrhea is caused by enteric pathogens such as bacteria, viruses, parasites, and fungi. Bacterial diarrhea is predominantly caused by diarrheagenic *Escherichia coli* (*E. coli*), *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Yersinia enterocolitica*, and *Clostridium difficile* (*C. difficile*)^[5,6]. As major diarrheal illnesses are self-limited or viral, and approximately half of them persist for less than a day, microbiological investigation is usually avoidable for patients who are diagnosed within 24 hours after the onset of diarrhea^[1]. However, it is essential to identify the diarrhea-causing pathogen when hospitalized patients have persistent symptoms.

Stool culture and microscopic examination methods are conventional among laboratories. However, they are time consuming and often lack sensitivity and specificity. Recently, there has been substantial development of multiplex molecular assays for the detection and identification of diarrhea-causing pathogens^[7-19]. Stool multiplex polymerase chain reaction (PCR) assay has been suggested to be highly specific and sensitive compared to conventional methods^[10-12,16]. Thus, its efficacy should be evaluated in clinical practice.

Fecal calprotectin is 36.5 kDa calcium- and zinc- binding protein, which accounts for about 60% of cytosolic neutrophil protein^[20,21]. Calprotectin has been reported to be

expressed in the mucosal layer of epithelial cells and absent in the normal intestinal mucosa. Thus, upregulation in calprotectin expression has been suggested as an effective marker for predicting inflammatory conditions^[22-26]. Fecal calprotectin has emerged as an effective marker to quantify mucosal inflammation in inflammatory bowel disease (IBD)^[25]. Furthermore, it has been shown as a reliable marker to identify inflammatory causes of chronic diarrhea^[27]. In children, calprotectin expression has been observed to increase during bacterial infection based on disease severity^[20]. However, the efficacy of fecal calprotectin in acute infectious diarrhea has not been elucidated in adults.

Several studies have investigated the efficacy of stool multiplex PCR assay and calprotectin test in children with acute infectious diarrhea. However, studies on adult patients are limited. In this study, we evaluated the efficacy of stool multiplex PCR test and fecal calprotectin expression in adult patients with acute infectious diarrhea.

MATERIALS AND METHODS

Patients

Overall, 400 patients with acute infectious diarrhea, who were admitted at Kangdong Sacred Heart Hospital from January 2016 to December 2018, were enrolled in the study and assessed with stool multiplex PCR test. We reviewed clinical and laboratory findings and compared the clinical characteristics with positivity of stool PCR. Acute diarrhea was defined as having three or more loose or watery stools per day, which persisted < 15 d. The exclusion criteria included the following: Patients who were less than 18 years old, diagnosed with chronic diarrhea (> 30 d), inflammatory bowel disease, active gastrointestinal malignancy, or non-infectious causes. The protocol for this study was approved by the institutional review board of Kangdong Sacred Heart Hospital.

Stool multiplex PCR assay

Fecal specimens (1-1.5 g) were lysed using Buffer ASL, vortexed for 10-15 s, and centrifuged at 1300 rpm. DNA/RNA were extracted from supernatant using Seegene STARlet IVD and multiplex PCR was performed using Seeplex® Diarrhea-B1 ACE Detection and Allplex™ GI-Bacteria(I) Assay kit (Seegene, Seoul, South Korea)^[13]. Multiplex PCR detected bacterial pathogens belonging to *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., *E. coli* O157:H7, verotoxigenic *E. coli* (VTEC), *Aeromonas* spp., *Vibrio* spp., *Yersinia enterocolitica*, *Clostridium perfringens*, and *C. difficile* toxin B. Sensitivity and specificity of Seeplex detection system were found to be comparable to conventional diagnostic testing algorithm for majority of enteric pathogens^[13].

Fecal calprotectin assay

Fecal calprotectin expression was evaluated in 107 patients and assessed for its correlation with positivity of stool PCR. Fecal specimens were collected in black plastic containers and sent to the laboratory in a refrigerated box. Fecal aliquots of approximately 100-500 mg were homogenized in an extraction buffer. Stool sample was then mixed using vortex and incubated for 10 min. The homogenate was transferred to an Eppendorf tube and centrifuged for 5 min at 3000 g. The supernatant was then assessed for calprotectin using EliA Calprotectin Wells (Thermo Fisher Scientific, Sweden), which were coated with monoclonal antibody to calprotectin. Further, calprotectin of patient's specimen was allowed to bind to pre-coated antibody. Excess components were washed and the calprotectin-conjugate complex was incubated with developing solution. The reaction was stopped and fluorescence in the reaction mixture was measured by Phila250 (Thermo Fisher Scientific)^[28]. The cut-off value for positivity was adjusted at 50 mg/kg.

Statistical analysis

Baseline characteristics were compared using Mann-Whitney *U* test or χ^2 test. Multivariate logistic regression analysis was performed to identify the independent factors that were associated with positivity of stool PCR. Receiver operating characteristic curve (ROC) analysis was performed to determine the cut-off value of calprotectin in detecting bacterial pathogens by stool PCR. *P* < 0.05 was considered as statistically significant. All statistical analyses were performed using SPSS for Windows (Version 24.0; IBM Corp., Armonk, NY, United States).

RESULTS

Baseline characteristics

A total of 400 patients who were admitted with acute infectious diarrhea and assessed with multiplex stool PCR test were enrolled between January 2016 and December 2018. Mean age was observed to be 50.4 ± 20.4 years and hospitalization time was reported to be 6.1 ± 4.7 d. Initial C-reactive protein (CRP) level was found to be 78.3 ± 82.5 mg/L. Mean body temperature was noted as 37.3 ± 0.9 °C and duration of diarrhea was observed to be 4 ± 4.6 days. Fecal calprotectin expression was examined in 107 (26.7%) patients, and mean fecal calprotectin was evaluated to be 902.9 ± 812 mg/kg (Table 1). A total of 138 patients were reported to undergo sigmoidoscopy and 56.5% of them were diagnosed with colitis.

Bacterial pathogens detected by stool PCR and culture

Stool multiplex PCR test detected substantially more bacterial pathogens compared to those detected by stool culture (49.2% *vs* 5.2%). Stool multiplex PCR assay detected 106 *Campylobacter* spp., 43 *E. coli*, 21 *Clostridium* spp., 20 *Salmonella* spp., 8 *Aeromonas* spp., 7 *Shigella* spp., and 6 *Vibrio* spp. (Figure 1). Conversely, stool culture detected 8 *Salmonella* spp., 6 *Clostridium* spp., 4 *Campylobacter* spp., and 3 *Vibrio* spp. (Figure 2). *Campylobacter* was detected as the most common pathogen in stool PCR (106/197, 54%) while *Salmonella* was the most common pathogen in stool culture (8/21, 38.1%). From 21 stool culture-identified pathogens, 76.2% (16/21) were also detected by stool multiplex PCR test.

Comparison of characteristics based on the positivity of stool PCR

Stool PCR-positive patients were found to have elevated CRP expression (109.8 ± 83.5 mg/L *vs* 47.7 ± 68.9 mg/L, $P = 0.0001$), high fever (> 38 °C) [101/197 (51.3%) *vs* 47/203 (23.2%), $P = 0.0001$] and upregulated fecal calprotectin expression (1124.5 ± 816.9 mg/kg *vs* 609 ± 713.2 mg/kg, $P = 0.001$) compared to that expressed by PCR-negative patients. Additionally, sigmoidoscopy revealed that colitis was more prevalent in PCR-positive group than PCR-negative group [49/65 (75.4%) *vs* 29/72 (39.7%), $P = 0.0001$] (Table 2). Multiplex PCR detected 55 enteropathogens in patients with sigmoidoscopy-diagnosed colitis, and 6 of them were found to be double PCR positive. *Campylobacter* was reported to be the most common pathogen (28/55, 35.9%) detected in patients with diarrhea and colitis.

Clinical factors that supported detection of bacterial pathogen by stool PCR

To determine the factors predicting positivity of stool PCR, we performed a multivariate logistic regression analysis. As shown in Table 3, fecal calprotectin and body temperature were not found to be associated with stool PCR positivity. The independent factors that predicted detection of bacterial pathogens by stool PCR were observed to be CRP (OR = 1.01, 95%CI: 1.001-1.027, $P = 0.034$) and sigmoidoscopy-diagnosed colitis (OR = 4.76, 95%CI: 1.101-20.551, $P = 0.037$).

Cut-off value of calprotectin to detect bacterial pathogen

Using ROC analysis, we evaluated the diagnostic efficacy of calprotectin expression in detecting bacterial pathogens by stool PCR. ROC curve revealed an area under curve of 0.699 (95%CI: 0.599-0.798) (Figure 3). Furthermore, sensitivity and specificity of calprotectin were evaluated to be 70.5% and 60.9%, respectively, with an adjusted cut-off value at 388 mg/kg.

DISCUSSION

Acute diarrheal disease is usually self-limited; however, it can be fatal at times. Thus, it is necessary to predict severity of diarrhea during the early phase of diagnosis. Typically, approximately 2 to 3 d are required to acquire the results of traditional stool culture. Moreover, many bacterial species are not clearly identified by the stool culture method. Therefore, new molecular assays including stool multiplex PCR and calprotectin test may be faster method to assess stool for bacterial infection.

To date, several studies have reported the efficacy of multiplex real-time PCR assays in clinical laboratory settings. However, commercial availability of molecular assays for the identification of enteric pathogens remains limited, and majority of studies only consider stool PCR data. Our study analyzed the clinical characteristics including

Table 1 Clinical characteristics of patients

Characteristics	Value
Age, yr	50.4 ± 20.4
Male, <i>n</i> (%)	168 (42)
DM/HTN/CKD/CVD [<i>n</i> (%)]	57 (14.3)/99 (24.8)/8 (2)/18 (4.5)
WBC count (× 10 ³ /μL)	9526.9 ± 4803
CRP (mg/L)	78.3 ± 82.5
Fecal calprotectin (mg/kg)	902.9 ± 812
Initial body temperature (°C)	37.3 ± 0.9
Duration of fever (> 38°C), d	0.58 ± 0.9
Duration of hospitalization, d	6.1 ± 4.7
Duration of diarrhea, d	4 ± 4.6

Data are presented as mean ± SD. DM: Diabetes mellitus; HTN: Hypertension; CKD: Chronic kidney disease; CVD: Cerebrovascular disease; WBC: White blood cell; CRP: C-reactive protein.

Table 2 Comparison of patient characteristics according to stool polymerase chain reaction positivity

	Positive stool PCR	Negative stool PCR	<i>P</i> value
Age (yr)	50.4 ± 20.5	50.5 ± 20.3	0.97
Male [<i>n</i> (%)]	76 (38.6)	92 (45.3)	0.145
WBC count (10 ³ /μL)	9968.8 ± 5165.1	9098.1 ± 4394	0.145
CRP (mg/L)	109.8 ± 83.5	47.7 ± 68.9	0.0001
Fever > 38°C [<i>n</i> (%)]	101 (51.3)	47 (23.2)	0.0001
Hospital days	5.9 ± 4.1	6.2 ± 5.1	0.733
Fever duration (d)	0.7 ± 0.9	0.4 ± 0.9	0.0001
Diarrhea duration (d)	3.8 ± 4.3	4.3 ± 4.8	0.772
Calprotectin (mg/kg)	1124.5 ± 816.9	609 ± 713.2	0.001
Colitis on sigmoidoscopy [<i>n</i> (%)]	49/65 (75.4)	29/72 (39.7)	0.0001

Data are presented as mean ± SD. PCR: Polymerase chain reaction; WBC: White blood cell; CRP: C-reactive protein.

Table 3 Multivariate analysis on independent factors detecting bacterial pathogens by stool polymerase chain reaction

	<i>P</i> value	OR	95%CI
CRP	0.03	1.01	1.001-1.027
Calprotectin	0.68	1	0.999-1.001
Colitis on sigmoidoscopy	0.03	4.75	1.101-20.551
Initial body temperature	0.81	1.1	0.484-2.498

CRP: C-reactive protein.

calprotectin expression level and endoscopic findings based on stool PCR data. Our results showed that patients with stool PCR-detected bacterial pathogens had additional acute clinical characteristics and severe inflammation, which could be detected by sigmoidoscopy.

Previous studies have reported that the detection rate is 20.8% among Korean population and *Campylobacter* is the most common pathogen to be identified^[18]. Our

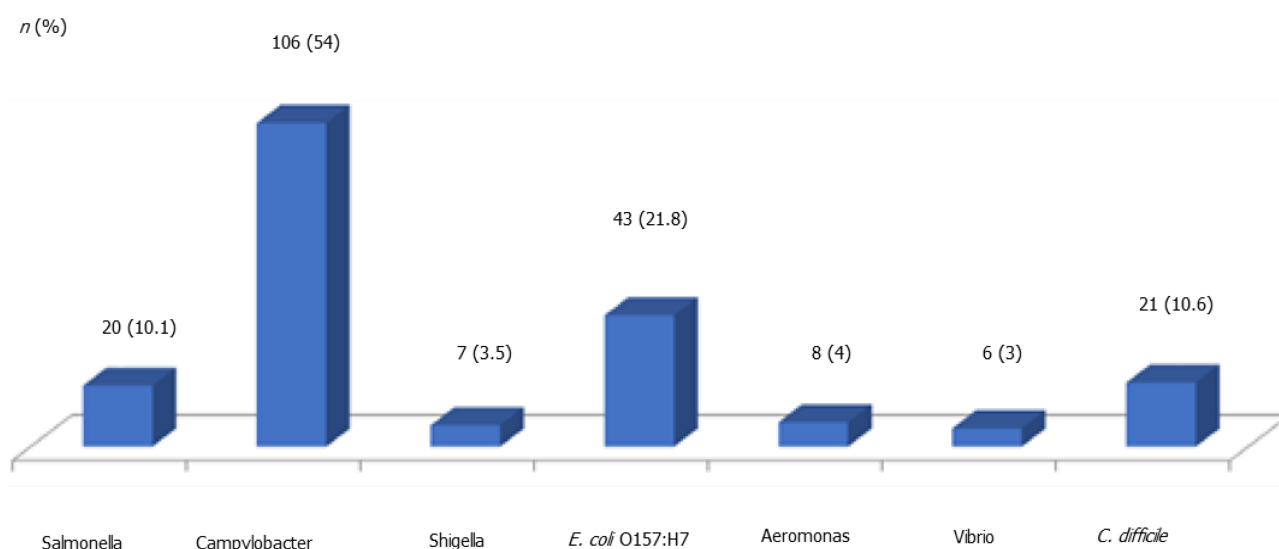


Figure 1 Distribution of detected bacterial pathogens by stool polymerase chain reaction ($n = 197$). *E. coli* *Escherichia coli*; *C. difficile*: *Clostridium difficile*.

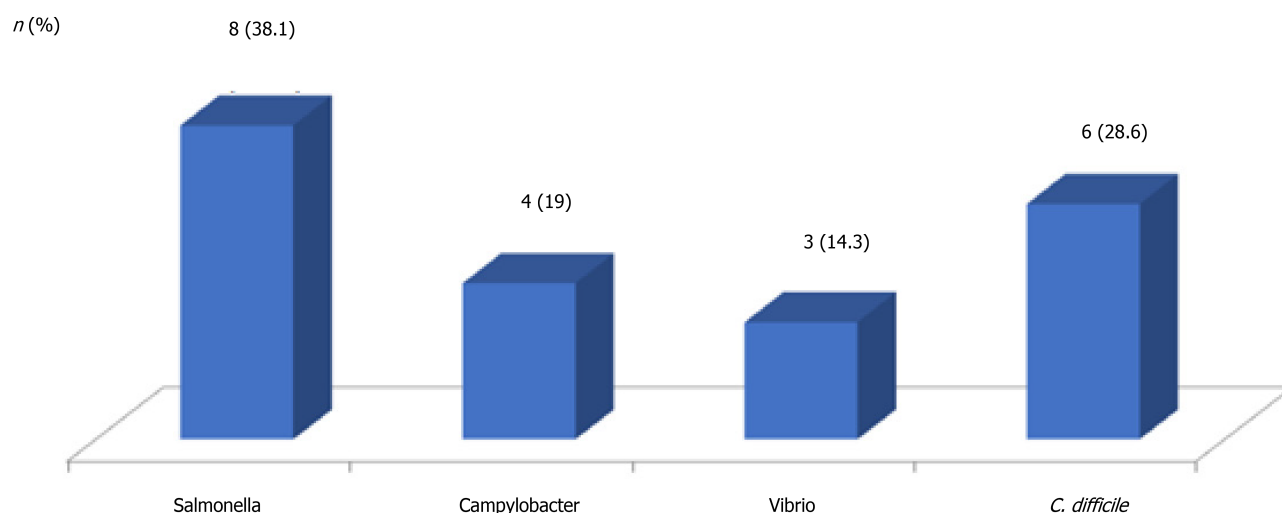


Figure 2 Distribution of detected bacterial pathogens by stool culture ($n = 21$). *C. difficile*: *Clostridium difficile*.

results detected *Campylobacter* as the most common pathogen in stool PCR (106/197, 54%), with a higher detection rate (49.2%). Thus, our study confirmed that *Campylobacter* is the most common pathogen detected by stool PCR in hospitalized patients with acute infectious diarrhea, which was not identified by stool culture method. Fatal *Campylobacter* infections are rare; however, they are more common in severely immunocompromised hosts^[6]. Most guidelines recommend that the empiric antimicrobial therapy in adults should consist of either a fluoroquinolone such as ciprofloxacin or azithromycin, depending on the local susceptibility patterns and travel history^[2,6]. Several randomized controlled trials have demonstrated a minor but significant benefit from antimicrobial therapy by reducing the duration of symptoms due to *Campylobacter gastroenteritis* infection^[29]. In Asian countries, quinolone resistance can be problematic while treating *Campylobacter* infections^[6]. Therefore, early detection of *Campylobacter* spp. by stool PCR could be a valuable method for diagnosing acute infectious diarrhea. Further, potential use of stool PCR for detecting known point mutations resulting in bacterial resistance to antibiotics would be a good complementary method for treatment of severe bacterial infection in the future.

In the present study, the independent factors that predicted detection of bacterial pathogens by stool PCR were found to be high CRP and sigmoidoscopy-detected colitis. This implies that bacterial infection causes severe inflammation and can be evaluated from the laboratory finding of CRP expression or endoscopic mucosal

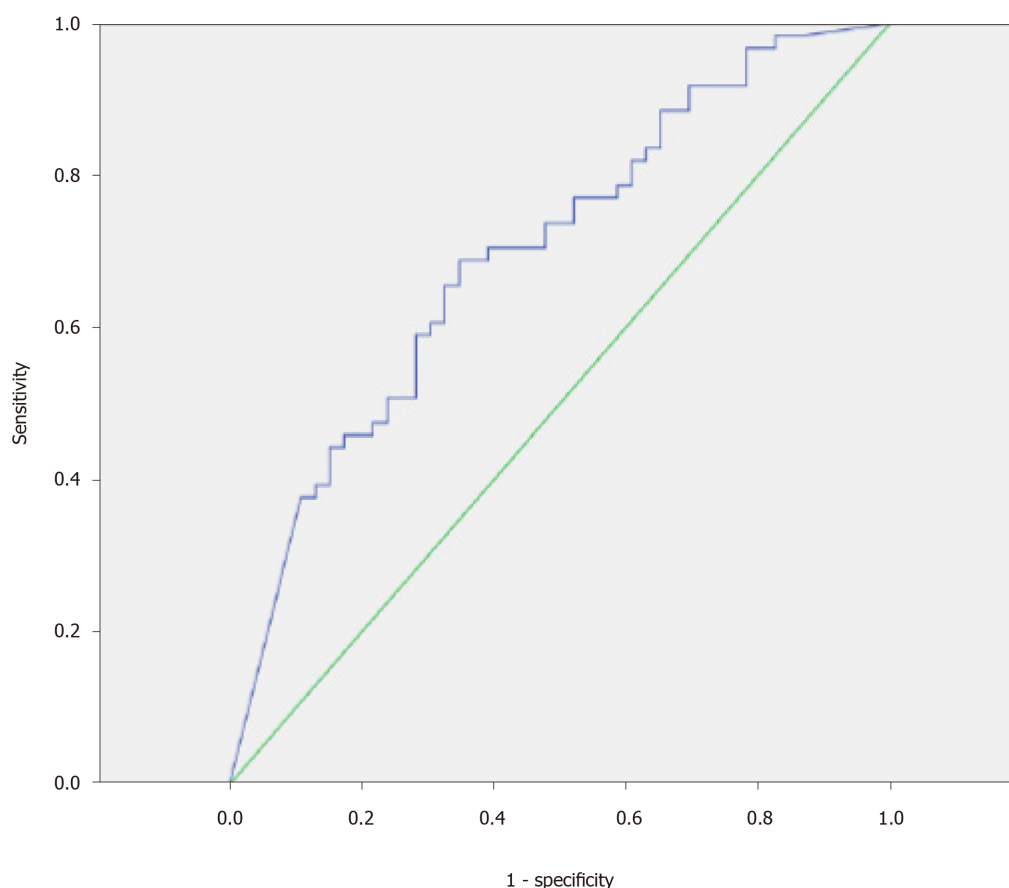


Figure 3 Area under receiver operating characteristic curve of fecal calprotectin for detecting bacterial pathogen by stool polymerase chain reaction (area under the curve = 0.699, 95%CI: 0.599-0.798).

inflammation. Thus, physicians can appropriately predict the presence of bacterial pathogens from high CRP expression or endoscopic findings.

Bacterial pathogens can cause invasive diarrhea. Such pathogens have been reported to possess the capability to invade the mucosa of the distal small intestine and colon, as well as stimulate local and systemic inflammatory responses^[23]. Fecal calprotectin is a well-known significant marker of intestinal mucosal inflammation, and thus, many researchers have investigated its efficacy. Duman *et al*^[23] evaluated the efficacy of fecal calprotectin in children with acute gastroenteritis. Their study suggested that fecal calprotectin can distinguish bacterial gastroenteritis from viral gastroenteritis and the expression levels are significantly higher in patients with microscopy-positive groups, specific for bacterial gastroenteritis^[23]. Our study did not include stool PCR result of viral pathogen, as the sample size of viral pathogen was relatively small.

A prospective multicenter study evaluated fecal calprotectin expression in 2383 adults across Europe with acute bacterial diarrhea^[30]. The study revealed that calprotectin had significant sensitivity and specificity to differentiate between noninfectious and bacterial diarrhea^[30]. Moreover, sensitivity and specificity of calprotectin were reported to be 83% and 87% with a cut-off value of 14.9 mg/L^[30]. Our study showed that the sensitivity and specificity of calprotectin were 70.5% and 60.9%, respectively, with adjusted cut-off value of 388 mg/kg. We assume that the difference might be due to different geographic location (country) and technique of calprotectin assay. In addition, our study included only hospitalized patients, which might have caused the disparity. Our result showed that fecal calprotectin could be elevated in adult infectious diarrhea. It is noteworthy especially in patients with IBD, since monitoring of fecal calprotectin is now routinely performed in IBD. If there is an infectious cause for the diarrhea in IBD patients, the high calprotectin may lead to inappropriate steroid use, it could be harmful.

Our study had some limitations. First, the study design was retrospective and single-centered. The detection of enteric pathogens and magnitude of calprotectin expression could be influenced by the region. Therefore, multicenter prospective study is recommended to validate the efficacy of stool multiplex PCR and fecal calprotectin

in adult patients. Second, the sample size for measuring calprotectin was relatively small, which can influence the results. Third, we did not conduct a cost-benefit analysis of stool PCR. Despite these limitations, the current study proves the efficacy of stool multiplex PCR in correlation with fecal calprotectin expression in relatively large-scaled adult patients.

In summary, stool multiplex PCR test showed improved sensitivity than conventional culture method in detecting bacterial pathogens in hospitalized patients with acute infectious diarrhea. Patients with positive stool PCR showed higher CRP expression level and colitis on sigmoidoscopy. Fecal calprotectin was found to correlate with the positivity of stool PCR, and the adjusted cut-off value of calprotectin for detecting bacterial pathogens by stool PCR was found to be 388 mg/kg. Overall, we suggest that stool multiplex PCR and calprotectin test may improve the diagnosis of acute infectious diarrhea with increased sensitivity for a majority of bacterial pathogens.

ARTICLE HIGHLIGHTS

Research background

Stool multiplex polymerase chain reaction (PCR) assay has been suggested to be highly specific and sensitive compared to conventional methods. Thus, its efficacy should be evaluated in clinical practice. Further, the efficacy of fecal calprotectin in acute infectious diarrhea has not been elucidated in adults.

Research motivation

Several studies have investigated the efficacy of stool multiplex PCR assay and calprotectin test in children with acute infectious diarrhea. However, studies on adult patients are limited.

Research objectives

We aimed to evaluate the efficacy of stool multiplex PCR test and fecal calprotectin expression in adult patients with acute infectious diarrhea.

Research methods

We reviewed clinical and laboratory findings and compared the clinical characteristics according to the positivity of stool PCR. We performed a multivariate logistic regression analysis to determine the factors predicting positivity of stool PCR. Using ROC analysis, we evaluated the diagnostic efficacy of calprotectin expression in detecting bacterial pathogens by stool PCR.

Research results

Stool multiplex PCR test detected considerably more bacterial pathogens than stool culture (49.2% *vs* 5.2%), with *Campylobacter* as the most common pathogen (54%). C-reactive protein (CRP) (OR = 1.01, 95%CI: 1.001-1.027, *P* = 0.034) and sigmoidoscopy-detected colitis (OR = 4.76, 95%CI: 1.101-20.551, *P* = 0.037) were independent factors in stool PCR-based detection of bacterial pathogens. Sensitivity and specificity of calprotectin were evaluated to be 70.5% and 60.9%, respectively (adjusted cut-off value = 388 mg/kg).

Research conclusions

Stool multiplex PCR test showed improved sensitivity than conventional culture method in detecting bacterial pathogens in hospitalized patients with acute infectious diarrhea. Patients with positive stool PCR showed higher CRP expression level and colitis on sigmoidoscopy. Fecal calprotectin was found to correlate with the positivity of stool PCR, and the adjusted cut-off value of calprotectin for detecting bacterial pathogens by stool PCR was found to be 388 mg/kg.

Research perspectives

We suggest that stool multiplex PCR and calprotectin test may improve the diagnosis of acute infectious diarrhea in adults. Future prospective studies are warranted.

REFERENCES

- 1 Thielman NM, Guerrant RL. Clinical practice. Acute infectious diarrhea. *N Engl J Med* 2004; **350**: 38-47 [PMID: 14702426 DOI: 10.1056/NEJMcp031534]
- 2 DuPont HL. Clinical practice. Bacterial diarrhea. *N Engl J Med* 2009; **361**: 1560-1569 [PMID: 19828533 DOI: 10.1056/NEJMcp0904162]
- 3 Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ* 2003; **81**: 197-204 [PMID: 12764516 DOI: 10.1590/S0042-96862003000300010]
- 4 Tam CC, Rodrigues LC, Viviani L, Dodds JP, Evans MR, Hunter PR, Gray JJ, Letley LH, Rait G, Tompkins DS, O'Brien SJ; IID2 Study Executive Committee. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. *Gut* 2012; **61**: 69-77 [PMID: 21708822 DOI: 10.1136/gut.2011.238386]
- 5 DuPont HL. Approach to the patient with infectious colitis. *Curr Opin Gastroenterol* 2012; **28**: 39-46 [PMID: 22080825 DOI: 10.1097/MOG.0b013e32834d3208]
- 6 Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, Langley JM, Wanke C, Warren CA, Cheng AC, Cantey J, Pickering LK. 2017 Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea. *Clin Infect Dis* 2017; **65**: e45-e80 [PMID: 29053792 DOI: 10.1093/cid/cix669]
- 7 Eckbo EJ, Yansouni CP, Pernica JM, Goldfarb DM. New Tools to Test Stool: Managing Travelers' Diarrhea in the Era of Molecular Diagnostics. *Infect Dis Clin North Am* 2019; **33**: 197-212 [PMID: 30712762 DOI: 10.1016/j.idc.2018.10.012]
- 8 Lee S, Park YJ, Lee HK, Kim SY, Kim JY, Lee SY, Yoo JK. Detection of 13 Enteric Bacteria and 5 Viruses Causing Acute Infectious Diarrhea Using Multiplex PCR from Direct Stool Specimens. *Ann Clin Microbiol* 2013; **16**: 33 [DOI: 10.5145/ACM.2013.16.1.33]
- 9 Sjöling Å, Sadeghipoorjahromi L, Novak D, Tobias J. Detection of major diarrheagenic bacterial pathogens by multiplex PCR panels. *Microbiol Res* 2015; **172**: 34-40 [PMID: 25542594 DOI: 10.1016/j.micres.2014.12.003]
- 10 Buchan BW, Olson WJ, Pezewski M, Marcon MJ, Novicki T, Uphoff TS, Chandramohan L, Revell P, Ledebore NA. Clinical evaluation of a real-time PCR assay for identification of Salmonella, Shigella, Campylobacter (Campylobacter jejuni and C. coli), and shiga toxin-producing Escherichia coli isolates in stool specimens. *J Clin Microbiol* 2013; **51**: 4001-4007 [PMID: 24048539 DOI: 10.1128/JCM.02056-13]
- 11 Harrington SM, Buchan BW, Doern C, Fader R, Ferraro MJ, Pillai DR, Rychert J, Doyle L, Laines A, Karchmer T, Mortensen JE. Multicenter evaluation of the BD max enteric bacterial panel PCR assay for rapid detection of Salmonella spp., Shigella spp., Campylobacter spp. (C. jejuni and C. coli), and Shiga toxin 1 and 2 genes. *J Clin Microbiol* 2015; **53**: 1639-1647 [PMID: 25740779 DOI: 10.1128/JCM.03480-14]
- 12 Wohlwend N, Tiemann S, Risch L, Risch M, Bodmer T. Evaluation of a Multiplex Real-Time PCR Assay for Detecting Major Bacterial Enteric Pathogens in Fecal Specimens: Intestinal Inflammation and Bacterial Load Are Correlated in Campylobacter Infections. *J Clin Microbiol* 2016; **54**: 2262-2266 [PMID: 27307458 DOI: 10.1128/JCM.00558-16]
- 13 Coupland LJ, McElarney I, Meader E, Cowley K, Alcock L, Naunton J, Gray J. Simultaneous detection of viral and bacterial enteric pathogens using the Seeplex® Diarrhea ACE detection system. *Epidemiol Infect* 2013; **141**: 2111-2121 [PMID: 23211606 DOI: 10.1017/S0950268812002622]
- 14 Simner PJ, Oethinger M, Stellrecht KA, Pillai DR, Yogev R, Leblond H, Mortensen J. Multisite Evaluation of the BD Max Extended Enteric Bacterial Panel for Detection of Yersinia enterocolitica, Enterotoxigenic Escherichia coli, Vibrio, and Plesiomonas shigelloides from Stool Specimens. *J Clin Microbiol* 2017; **55**: 3258-3266 [PMID: 28878009 DOI: 10.1128/JCM.00911-17]
- 15 Zboromyrska Y, Hurtado JC, Salvador P, Alvarez-Martínez MJ, Valls ME, Mas J, Marcos MA, Gascón J, Vila J. Aetiology of traveller's diarrhoea: evaluation of a multiplex PCR tool to detect different enteropathogens. *Clin Microbiol Infect* 2014; **20**: O753-O759 [PMID: 24621195 DOI: 10.1111/1469-0691.12621]
- 16 Antikainen J, Kantele A, Pakkanen SH, Lääveri T, Riutta J, Vaara M, Kirveskari J. A quantitative polymerase chain reaction assay for rapid detection of 9 pathogens directly from stools of travelers with diarrhea. *Clin Gastroenterol Hepatol* 2013; **11**: 1300-1307.e3 [PMID: 23639597 DOI: 10.1016/j.cgh.2013.03.037]
- 17 Wiemer D, Loderstaedt U, von Wulffen H, Priesnitz S, Fischer M, Tannich E, Hagen RM. Real-time multiplex PCR for simultaneous detection of Campylobacter jejuni, Salmonella, Shigella and Yersinia species in fecal samples. *Int J Med Microbiol* 2011; **301**: 577-584 [PMID: 21855409 DOI: 10.1016/j.ijmm.2011.06.001]
- 18 Cho MC, Noh SA, Kim MN, Kim KM. Direct Application of Multiplex PCR on Stool Specimens for Detection of Enteropathogenic Bacteria. *Korean J Clin Microbiol* 2010; **13**: 162 [DOI: 10.5145/KJCM.2010.13.4.162]
- 19 Schuetz AN. Emerging agents of gastroenteritis: Aeromonas, Plesiomonas, and the diarrheagenic pathotypes of Escherichia coli. *Semin Diagn Pathol* 2019; **36**: 187-192 [PMID: 31036328 DOI: 10.1053/j.semdp.2019.04.012]
- 20 Chen CC, Huang JL, Chang CJ, Kong MS. Fecal calprotectin as a correlative marker in clinical severity of infectious diarrhea and usefulness in evaluating bacterial or viral pathogens in children. *J Pediatr Gastroenterol Nutr* 2012; **55**: 541-547 [PMID: 22699836 DOI: 10.1097/MPG.0b013e318262a718]
- 21 Licata A, Randazzo C, Cappello M, Calvaruso V, Butera G, Florena AM, Peralta S, Cammà C, Craxi A. Fecal calprotectin in clinical practice: a noninvasive screening tool for patients with chronic diarrhea. *J Clin Gastroenterol* 2012; **46**: 504-508 [PMID: 22565607 DOI: 10.1097/MCG.0b013e318248f289]
- 22 Steinbakk M, Naess-Andresen CF, Lingaas E, Dale I, Brandtzaeg P, Fagerhol MK. Antimicrobial actions of calcium binding leucocyte LI protein, calprotectin. *Lancet* 1990; **336**: 763-765 [PMID: 1976144 DOI: 10.1016/0140-6736(90)93237-j]
- 23 Duman M, Gencpinar P, Biçmen M, Arslan N, Özden Ö, Üzümlü Ö, Çelik D, Saygıner AA, Gülay Z. Fecal

- calprotectin: can be used to distinguish between bacterial and viral gastroenteritis in children? *Am J Emerg Med* 2015; **33**: 1436-1439 [PMID: 26233616 DOI: 10.1016/j.ajem.2015.07.007]
- 24 **Limburg PJ**, Ahlquist DA, Sandborn WJ, Mahoney DW, Devens ME, Harrington JJ, Zinsmeister AR. Fecal calprotectin levels predict colorectal inflammation among patients with chronic diarrhea referred for colonoscopy. *Am J Gastroenterol* 2000; **95**: 2831-2837 [PMID: 11051356 DOI: 10.1111/j.1572-0241.2000.03194.x]
 - 25 **Sutherland AD**, Gearry RB, Frizelle FA. Review of fecal biomarkers in inflammatory bowel disease. *Dis Colon Rectum* 2008; **51**: 1283-1291 [PMID: 18543035 DOI: 10.1007/s10350-008-9310-8]
 - 26 **Tibble J**, Teahon K, Thjodleifsson B, Roseth A, Sigthorsson G, Bridger S, Foster R, Sherwood R, Fagerhol M, Bjarnason I. A simple method for assessing intestinal inflammation in Crohn's disease. *Gut* 2000; **47**: 506-513 [PMID: 10986210 DOI: 10.1136/gut.47.4.506]
 - 27 **Schröder O**, Naumann M, Shastri Y, Povse N, Stein J. Prospective evaluation of faecal neutrophil-derived proteins in identifying intestinal inflammation: combination of parameters does not improve diagnostic accuracy of calprotectin. *Aliment Pharmacol Ther* 2007; **26**: 1035-1042 [PMID: 17877510 DOI: 10.1111/j.1365-2036.2007.03457.x]
 - 28 **Mindemark M**, Larsson A. Ruling out IBD: estimation of the possible economic effects of pre-endoscopic screening with F-calprotectin. *Clin Biochem* 2012; **45**: 552-555 [PMID: 22056737 DOI: 10.1016/j.clinbiochem.2011.10.015]
 - 29 **Ternhag A**, Asikainen T, Giesecke J, Ekdahl K. A meta-analysis on the effects of antibiotic treatment on duration of symptoms caused by infection with *Campylobacter* species. *Clin Infect Dis* 2007; **44**: 696-700 [PMID: 17278062 DOI: 10.1086/509924]
 - 30 **Shastri YM**, Bergis D, Povse N, Schäfer V, Shastri S, Weindel M, Ackermann H, Stein J. Prospective multicenter study evaluating fecal calprotectin in adult acute bacterial diarrhea. *Am J Med* 2008; **121**: 1099-1106 [PMID: 19028207 DOI: 10.1016/j.amjmed.2008.06.034]



Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

Telephone: +1-925-3991568

E-mail: bpgoffice@wjgnet.com

Help Desk: <https://www.f6publishing.com/helpdesk>

<https://www.wjgnet.com>

