International Journal of Medical and Pharmaceutical Case Reports
4(3): 77-81, 2015; Article no.IJMPCR.2015.065
ISSN: 2394-109X

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Colonoscopy-induced Enteropathogenic *Escherichia coli* Bacteremia Diagnosed by Serum Polymerase Chain Reaction Method

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Authors' contributions

This work was carried out in collaboration between all authors. Author MM designed, analyzed, interpreted and prepared the draft of the manuscript. Author TO helped the microbiological analysis and preparation of the draft of the manuscript. Authors TA, HG and MO helped the designation, interpretation and preparation of the draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJMPCR/2015/18449
Editor(s):
(1) Daniel Laubitz, Steele Children's Research Center, Dept. of Pediatrics /Gastroenterology and Nutrition, Arizona Health Sciences Center, University of Arizona, Tucson, USA.
Reviewers:
(1) Anonymous, University of Londrina, Brazil.
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(3) Carla Fontana, Tor Vergata Univertity of Rome, Rome.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1101&id=38&aid=9678

Case Study

Received 22nd April 2015
Accepted 29th May 2015
Published 9th June 2015

ABSTRACT

We report the case of colonoscopy induced enteropathogenic *Escherichia coli* (EPEC) bacteremia by bacterial translocation. A 58-year old woman who complained of abdominal pain was performed colonoscopy. Intestinal mucosal culture by biopsy showed EPEC O18. She developed a high grade fever after colonoscopy. Although blood culture did not detect any bacteria, *eaeA* gene of EPEC was detected from patients' serum by polymerase chain reaction (PCR). Though colonoscopy was

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performed again after receiving antibiotics, high grade fever was not seen. EPEC was not cultured from mucosa nor *eaeA* gene was not detected from sera. Serum PCR method is useful for detection of bacteremia.

Keywords: Enteropathogenic Escherichia coli; bacterial translocation; colonoscopy; eaeA; polymerase chain reaction.

1. INTRODUCTION

Enteropathogenic *Escherichia coli* (EPEC) is an important etiological pathogen of infant diarrhea [1]. Serogroup classification based on the O antigen is widely used for EPEC. The O antigen is one part of lipopolysaccharide (LPS), also known as endotoxin, and usually consists of 10 – 15 repeating units containing two to seven sugar residues [2]. Colonoscopy is a widely used diagnostic and therapeutic procedure for gastrointestinal disorders, and it is only rarely associated with transient bacteremia [3]. Here, we report a case of post-colonoscopy bacteremia accompanied by transient high-grade fever caused by EPEC.

2. PRESENTATION OF CASE

A 58-year-old woman complained of high fever and low right abdominal pain and was admitted to Nagoya University Hospital. She underwent double-balloon intestinal colonoscopy for surveillance of the small intestine. The colonoscopic findings revealed an edematous stenotic lesion of the terminal ileum. From the results of the bacterial culture of intestinal mucosa, she was diagnosed as intestinal tuberculosis. In addition, both stool and intestinal mucosal cultures revealed the presence of EPEC O18. Blood culture showed no bacteria. Although the patient did not experience frequent episodes of watery diarrhea she had a history of these symptoms prior to the treatment with anti-tuberculosis drugs including isoniazid, rifampicin, pyrazinamide, and ethambutol. Following this treatment, her temperature decreased, her abdominal symptoms improved, and she was discharged. Though she had not complained of any symptoms for several months, she was re-hospitalized because of abdominal pain. Upon hospitalization, physical examination revealed almost normal vital signs without mild abdominal pain. Laboratory tests demonstrated mild anemia with a hemoglobin value of 11.6 g/L and a normal white blood cell count. The serum levels of electrolytes, liver enzymes, and creatinine were within the normal ranges. She did not have high-grade fever at the time of rehospitalization. She was treated with an intravenous drip injection for 3 days, and her symptoms, including abdominal pain, were improved. Taking into consideration her intestinal status, we performed a repeat colonoscopy, which revealed an improvement in the intestinal edema and stenosis. Although tuberculosis was not detected by intestinal mucosal culture, the presence of EPEC O18 was detected. Following colonoscopy, the patient developed a temperature of 39.2ºC. She experienced marked signs of fatigue and malaise, but the other findings of physical examination were unremarkable. The total white blood cell count was 5500 cells/mm^3 with 20.8% lymphocytes and 74.8% neutrophils. No bacteria were detected in blood culture. Under informed consent of patient, the investigation of *eaeA* gene of EPEC DNA was performed in the patient's serum after colonoscopy by PCR (Fig. 1). Briefly, the DNA from the patient's serum and EPEC 2348/69, an *eaeA*-positive strain, were extracted by using the QIAGEN DNA extraction kit (QIAGEN, Hilder, Germany). The *eaeA* gene was amplified by using two PCR primers, namely, eaek1 (5′-GCTTAGTGCTGGTTTAGGAT-3′) and eaek4 (5′-TCGCCGTTCAGAGATCGC-3′), and PCR products (approximately 600 bp in size) were subsequently confirmed as *eaeA* gene by sequence analysis (data not shown) [4]. The *eaeA* gene was not detected in the patient's serum on rehospitalization. We had performed this PCR assay at least three times and confirmed the results. Prophylactic antibiotic therapy was not administered because the blood culture was negative at this time. After 2 days, the patient's temperature decreased and was within the normal limit. She was discharged after 1 week. She continued to out-patient for follow-up examination every two weeks. As EPEC O18 was isolated from her feces, she was administered oral levofloxacin for 1 week before her intestinal status was reevaluated. The colonoscopy findings revealed complete elimination of the edema and further improvement of the stenosis. After the colonoscopy, the fever did not recur. Stool culture no longer demonstrated EPEC, and the *eaeA* gene was not detected in the patient's serum (Fig. 1). The patient has been followed up for 12 months with no recurrence of fever.

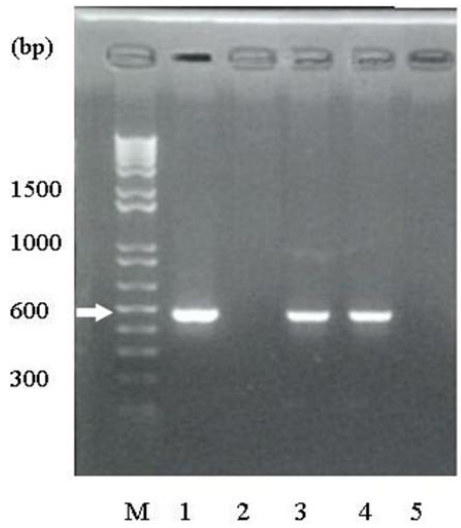

Fig. 1. PCR detection of the eaeA gene in EPEC O18

Agarose gel electrophoresis showing the PCR fragments of the eaeA gene.
M markers; (1), E2348/69; (2), negative; (3), serum before antibiotic treatment; (4), O18; and (5), serum after eradication. Arrow indicates the PCR products of *eaeA* gene

3. DISCUSSION

This case report represents atypical EPEC bacteremia after colonoscopy.

E. coli is a clonal species and includes both commensals and pathogens, which are normally, identified by the combination of their O and H (and occasionally K) antigens [2]. O antigens are present in all *E. coli* strains, and at least 166 O antigen forms have been recognized in *E. coli*; of these, only a few are commonly found in pathogenic strains [5]. In contrast to EHEC O157, EPEC is also found in healthy carriers and is believed to possess low virulence; therefore, physicians tend to neglect this bacterium. This is the one of the reasons why the relationship between virulence and serogroup has not been elucidated thus far.

The *eaeA (E. coli* attaching and effacing (AE)) gene is known to be a major genetic factor for the virulence of EPEC. However, *eaeA* detection is not widely used clinically in order to classify virulence factors. This gene encodes the 94-kD outer membrane protein initimin that is necessary for AE lesion formation but not for adherence per serum [6]. An *eaeA* mutant strain of EPEC is unable to induce AE lesions in cultured cells. In an experimental human model of EPEC infection, this *eaeA* mutant strain demonstrated reduced virulence [7]. This case report suggests that an EPEC strain with a virulence factor caused high fever through transient bacteremia. Although no bacteria were cultured from the blood sample, sampling errors could not be excluded since the *eaeA* gene was detected in the patient's serum. In the sera of healthy carriers without EPEC, this gene is usually not detected. In our case, after the eradication of EPEC, we were unable to detect the *eaeA* gene in the patient's serum by PCR. The fact that high fever did not recur following colonoscopy after EPEC eradication may also support the possibility of EPEC bacteremia.

E. coli is one of the most frequent isolates from patients with sepsis [8]. However, most often sepsis caused by *E. coli* occurs due to urinary tract infection and cholangitis. Some cases of sepsis occur as a result of manipulation such as urinary catheterization. Simple intestinal *E. coli* infection rarely causes sepsis [9,10].

Although colonoscopy is a widely used procedure, the subsequent bacteremia is quite rare [3]. EPEC was present in the intestine in this patient, and it is suggested that the portal of entry might have been the inflamed intestines, from where the EPEC reached the systemic circulation due to the endoscopic air pressure [11]. Ileal inflammation may be another reason underlying easy intestinal bacterial translocation.

Blood culture is widely used to diagnose bacteremia. However, in many clinical situations, the bacterial yield from blood culture is low; positive cultures are obtained from fewer than 30% of all cases. Some patients with false-negative blood cultures may have undergone prior antibiotic treatment or had not yet developed bacteremia at the time of blood collection. In previous report, the sequence of the 16S rRNA gene has been used to detect and identify bacterial infection in clinical practice [12], but many of the PCR methods in use are unable to further differentiate between different bacterial species.

Bacterial pathogens pose a significant threat to human health. Consequently, considerable effort has been devoted to the development of rapid, sensitive, and specific assays for the detection of these organisms. PCR is the most important molecular tool because of its potential to detect less than 3 copies of the DNA of a specific

bacterium [12]. Conventional PCR assays incorporate a pair of oligonucleotide primers in order to amplify a specific gene that is then detected using agarose gel electrophoresis combined with an intercalating dye. Blood specimens from 172 cases of suspected bacteremia were separately used for bacterial culture and detection of bacterial 16S rRNA. These results demonstrated that the sensitivity of PCR in the detection of bacterial sepsis was higher than that of traditional blood culture. This could be due to the fact that PCR amplification allows for the detection of fewer bacteria than the lower limit of blood culture, particularly in patients who have been treated with antibiotics [13].

In this study, we have demonstrated that molecular methods can potentially be used as rapid alternatives for the detection of bacteremia. In our case, we selected the PCR method involving the *eaeA* gene instead of the 16S rRNA gene and successfully detected EPEC. The reasons for this are that the *eaeA* gene encodes a virulence factor of EPEC and that EPEC cannot be distinguished from other bacteria by mere 16S rRNA PCR without sequencing.

Although the protocols for both the prevention and detection of possible bacteremic complications have not been addressed, the *eaeA* gene PCR method is potentially useful for the detection of virulent EPEC bacteremia, and a prospective study investigating this problem is required.

4. CONCLUSION

We suggested that colonoscopy induced EPEC bacteremia by bacterial translocation. Although EPEC is not frequently recovered from human clinical specimens, it can be considered as an authentic cause of bacteremia. Proper molecular biological detection methods including serum PCR may facilitate correct bacterial diagnosis.

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication of this case report and accompanying images'.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENTS

We would thank Dr. J. B. Kaper for providing the EPEC E2348/69 strain. We would also thank the members of gastroenterology and bacteriology in Nagoya University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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