

Campylobacter jejuni Bacteremia in a Healthy Child

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Campylobacter jejuni is one of the important bacterial pathogens causing entero-invasive diarrhea; however, *C. jejuni* infection is rarely complicated by bacteremia or extra-intestinal localization. In the domestic literature, the majority of the relevant reports have focused on *Campylobacter fetus*, which causes bacteremia more frequently than enteritis, but there are no reports of *C. jejuni* bacteremia in Korea. We present the case of a 13-year-old girl who presented with abdominal pain. Blood cultures revealed curved Gram-negative bacilli and small, mucoid, gray colonies on blood agar plates at 37°C. Biochemical tests

showed oxidase-positive colonies. To confirm the species, 16S rRNA sequence analysis was performed. The isolate exhibited 99.7% homology to *C. jejuni* subsp. *jejuni*. The patient was treated with third-generation cephalosporin and aminoglycoside and had negative blood cultures after three days of treatment. She fully recovered within four days with no complications. (Korean J Clin Microbiol 2011;14:110-114)

Key Words: *Campylobacter jejuni*, Bacteremia, Blood culture, 16s rRNA sequence analysis

INTRODUCTION

Campylobacter is a gram-negative, motile, comma-shaped and an important pathogen that causes disease [1]. *Campylobacter* infections are the main bacterial cause of entero-invasive diarrhea, but *Campylobacter* bacteremia is uncommon. Compared to *Salmonella* infections, *C. jejuni* bacteremia is quite rare and only occurs in <1% of *C. jejuni* infections and is rarely complicated by extra-intestinal localization. In contrast to *C. jejuni*, *C. fetus* is usually isolated from blood samples and is less frequently associated with enteritis [2].

Bacteremia with *Campylobacter* species has mainly been reported in the elderly, in patients with human immunodeficiency virus (HIV), and in other immunocompromised patients [3,4]. Domestic cases of *C. fetus* causing bacteremia and sepsis have been reported in >50 cases [5], but no domestic cases of *C. jejuni* as a cause of bacteremia have been reported.

In the current case, curved gram negative bacilli were isolated from blood cultures of a pediatric patient with abdominal pain. Based on the specific biochemical analysis and 16S rRNA sequence analysis, we reported the isolate as *C. jejuni*.

CASE REPORT

A 13-year-old female patient visited the emergency department with a chief complaint of abdominal pain. The patient was a student with a nonspecific medical history and no travel history. She experienced diffuse abdominal pain for 1 day and the pain localized to the right lower quadrant, she visited a local clinic and then the Kyung Hee University medical center emergency department. She did not have diarrhea. Her vital signs at the time of visit were as follows: temperature, 37.7°C; pulse, 88 per minute; respiration, 20 times per minute; and blood pressure, 120/80 mmHg. The abdomen was diffusely distended, with tenderness and rebound tenderness. The patient was admitted to the pediatric department with a diagnosis of infectious colitis.

1. Laboratory results

The complete blood count at the time of the emergency department visit included hemoglobin of 13.3 g/dL, white blood cell (WBC) count of 14,150/ μ L, and platelet count of 230,000/ μ L. Chemistry results were AST/ALT 17/7 IU/L, Alkaline phosphatase 114 IU/L, total bilirubin 0.76 mg/dL, Protein/albumin 7.5/4.6 g/dL, and BUN/creatinine 13/0.6 mg/dL showing a result within the normal range, and C-reactive protein (CRP) level was increased to 4.41 mg/dL (reference range <0.5 mg/dL). Urinalysis showed hematuria and coagulation test results were slightly increased with a PT of 14.9 seconds

Received 21 March, 2011, Revised 31 May, 2011
Accepted 3 August, 2011

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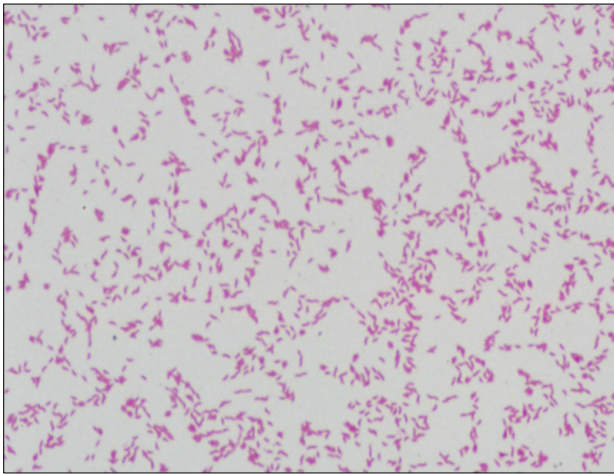


Fig. 1. Microscopic findings of *Campylobacter jejuni* isolated from the blood culture showing slightly curved gram-negative bacilli (Gram stain, $\times 1,000$).

(reference range 12.5-14.7 seconds) and a normal aPTT of 40.5 seconds (reference range 29-43 seconds). The chest radiography findings were unremarkable. The abdominal CT and ultrasonographic findings performed on the same day were unremarkable with the exception of mesenteric lymphadenitis. The stool WBC examination was positive.

2. Microbiologic procedure

One set blood culture was performed in emergency department, and results were found using an automated blood cultivator BACTEC9240 system (BD, Sparks, MD, USA), and showed bacterial growth from one aerobic culture bottle (BD BACTEC PEDS PLUS /F culture vial) obtained at the time of the emergency department visit after 21 hours. Subculture on blood agar and MacConkey medium for 24 hours at 36°C and 42°C in 5% CO₂ showed gray or colorless colonies, 1 mm in size. The gram stain results revealed curved gram-negative bacilli (Fig. 1). The bacillus was oxidase-positive, catalase-positive, and proliferated at 36°C, but not at 25°C. The bacillus was susceptible to nalidixic acid (30 μ g; Oxoid, Basingstoke, Hampshire, England) and resistant to cephalothin (30 μ g; Oxoid). The other phenotype results are listed in Table 1. Curved bacteria on the initial gram stain were suspicious for *Campylobacter*, and the biochemical tests were inadequate to identify a specific strain, therefore 16S rRNA sequence analysis was performed. The urine culture results were negative and the stool culture showed no growth for *Salmonella*, *Shigella*, and *Vibrio* strains. The stool culture for *Campylobacter* was not performed.

Table 1. Phenotypic characteristics of *Campylobacter jejuni* isolated from the patient

Test		Result
Biochemical tests	Oxidase	Positive
	Catalase	Positive
	Urease	Negative
	Hippurate hydrolysis	Positive
	H ₂ S on TSI*	Negative
Antibiotic susceptibility	Nalidixic acid (30 μ g)	Susceptible
	Cephalothin (30 μ g)	Resistant
Growth temperature	25°C	No growth
	36°C	Growth
	42°C	Growth
Growth in MacConkey		Growth

*Triple sugar iron agar.

3. 16S rRNA sequence analysis

16S rRNA sequence analysis was requested to Macrogen (Seoul, South Korea) and the methods used are described as below. A colony was selected after proliferation for 48 hours at 36°C in the media and dissolved into sterilized physiologic saline solution. After centrifugation, the precipitate was processed using InstaGene Matrix (Bio-Rad, Hercules, CA, USA) to extract the DNA. The primers 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3' and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3' were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 μ L reaction mixture by using a *EF-Taq* (SolGent, Korea) as follows: activation of Taq polymerase at 95°C for 2 minutes, 35 cycles of 95°C for 1 minutes, 55°C, and 72°C for 1 minutes each were performed, finishing with a 10-minute step at 72°C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95°C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). To compare the 16S rRNA sequence analysis result for similarities, the GenBank database from the National Center for Biotechnology Information (NCBI) was searched. The bacteria separated from this case had a 99.7% similarity to *Campylobacter jejuni* subsp. *Jejuni* NTCT11168 genome sequence (GenBank accession number AL111 168) [6].

4. Progress

The patient was initially administered cefnidir, ampicillin/sulbactam, gentamicin resulting in a normalized body temperature after 2 days and no *Campylobacter* was cultured from the blood culture after 2 days of treatment. The condition of the patient improved and she was discharged on the 5 days after admission.

DISCUSSION

Curved or spiral, gram-negative bacilli are seldom observed from blood culture. Therefore, a careful approach is needed for bacterial identification. In doing so, the type of the sample and the culture condition needs to be considered first, biochemical tests, antigen tests, or molecular biological methods should be implemented depending on the need after carefully observing the shape of the gram stain [7]. The gram-negative bacilli are difficult to distinguish owing to morphologic similarities include *Arcobacter*, *Campylobacter*, and *Helicobacter* species. *Helicobacter* species have been reported to cause bacteremia for immunocompromised patients for certain species, such as *H. cinaedi* or *H. fennelliae* [8-10], but most *Helicobacter* species are excluded from the automatic blood culture system because biochemical method is used to differentiate *Arcobacter* and *Campylobacter* species.

Cases of *Campylobacter* bacteremia are seldom reported because most of the patients are immunosuppressed or have an underlying disease [2]. Pacanowski et al. [3] reported that 63% of patients with *Campylobacter* bacteremia had the following co-existing medical conditions: cancer; neutropenia; chemotherapy; liver disease; HIV infection; steroid therapy; diabetes; splenectomy; organ transplantation; and autoimmune disease. Contrary to the above cases, Fernández-Cruz et al. [4] reported that only 4 of 63 patients with *Campylobacter* bacteremia were healthy and free from underlying medical conditions. These studies also demonstrated that *C. fetus* is a more common caused *Campylobacter* bacteremia than *C. jejuni*. Nevertheless, *C. jejuni* bacteremia occurs in healthy patients, albeit rarely [11-16].

There are several reports of patients with *Campylobacter* infections and diarrhea progressing to bacteremia, and *C. jejuni* bacteremia in pediatric patients is mostly confined to malnourished children in developing countries [11-14]. Also, there have been some cases of *C. jejuni* bacteremia in immunocompetent patients [15,16].

Campylobacter bacteremia has three distinctive clinical findings. First, transient bacteremia may occur in a healthy or immunocompromised host following acute *Campylobacter* enteritis, in which the patient's condition may improve without specific treatment. Second, sustained bacteremia may occur in patients infected with resistant strains. In this case, treatment with appropriate antibiotics can achieve favorable results. Third, sustained bacteremia may occur in an immunocompromised host in which repetitive bacteremia requires continued antimicrobial therapy, and is accompanied by relapsing fever, endocarditis, and meningitis [2]. With respect to the patient described herein, the blood cultures were obtained 2 days after hospitalization and showed no growth results. The fact that improvement of symptoms was accomplished by 3rd generation cephalosporin whereas general treatment of *Campylobacter* requires fluoroquinolone and macrolide suggests transient bacteremia (the first case listed above).

The patient did not provide a stool culture for *Campylobacter*. Thus, it would be difficult to attribute the positive WBC results in the patient's stool to acute enteritis caused by *Campylobacter* species. However, the possibility of *C. jejuni* invasion into the blood stream cannot be excluded as *C. jejuni* enteritis can cause damage to the mucosa of the gastrointestinal tract, being responsible for the complaint of abdominal pain and WBC observed in stool.

Most domestic microbiology laboratories perform stool culture for only *Salmonella*, *Shigella*, *Yersinia* and *Vibrio* in the evaluation of diarrhea. But according to the 2008 Laboratory Surveillance System by Korea Center for Disease Control & Prevention and report by Cho et al., *Campylobacter* is included as a common pathogen causing diarrhea [17,18]. This case also did not perform tests for *Campylobacter* from stool culture therefore caused a confusion in translating blood culture results which implicates the needs of *Campylobacter* analysis when a stool culture is done. In addition, 16S rRNA sequencing and phenotype analysis could be of use to increase the sensitivity of the reported diagnosis with *C. jejuni* in which bacteremia is uncommon.

Thus, it appears that the current case involved a transient *C. jejuni* bacteremia using biochemical and 16S rRNA sequencing on the bacteria isolated from the blood cultures in a healthy child. To our knowledge, this is the first case of *C. jejuni* subsp. *jejuni* bacteremia in Korea.

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=국문초록=

건강한 어린이에서의 캄필로박터 균혈증

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*Campylobacter jejuni*는 위장관질환을 일으키는 중요한 원인균이지만 균혈증이나 장외증상을 일으키는 것은 드물게 알려져 있다. 국내에서 보고된 캄필로박터 감염증은 위장관염보다는 균혈증을 더 많이 일으키는 *Campylobacter fetus*에 초점이 맞춰져 있어 상대적으로 *C. jejuni* 균혈증에 대한 증례보고는 없는 상태다. 저자들은 복통을 주소로 내원한 13세 여자 환자에서 혈액배양검사상 그람음성간균이 나왔고 생화학적 검사에서 oxidase 양성인 집락을 보였다. 확진하기 위해 16s rRNA 염기서열 분석을 시행하였고 99.7%의 유사도를 보였다. 본 환자는 3세대 cephalosporin과 aminoglycoside를 이용하여 치료하였고, 치료 후 3일 뒤 시행한 혈액배양검사상 음성을 보였으며 4일 뒤 회복되어 퇴원하였다. [대한임상미생물 학회지 2011;14:110-114]

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