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CASE REPORT

First Case Report of *Campylobacter volucris* Bacteremia in an Immunocompromised Patient

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We report a case of *Campylobacter volucris* bacteremia in an immunocompromised patient with polycythemia vera and alcoholic liver cirrhosis. To our knowledge, this is the first case report in which this organism has been isolated from a human clinical specimen.

CASE REPORT

A 75-year-old male patient visited the Chung-Ang University Hospital with a chief complaint of abdominal discomfort due to accumulation of ascites fluid. The abdomen was diffusely distended without tenderness or rebound tenderness. The patient had a medical history of polycythemia vera with splenomegaly and alcoholic liver cirrhosis classified as Child-Pugh score B. The patient was taking medication for liver cirrhosis, including diuretics and ursodeoxycholic acid but was receiving no treatment for polycythemia vera. He had no travel history within 1 year and was not currently employed. Laboratory results revealed anemia (hemoglobin level of 5.0 g/dl), and vital signs were stable. The patient was admitted to the gastrointestinal department for management of abdominal distension, and a paracentesis was performed.

On the next day, the patient complained (subjectively) of feeling febrile, and at that time the vital signs were as follows: body temperature, 38.3°C; pulse rate, 86/min; respiration rate, 20 breaths/min; and blood pressure, 100/60 mm Hg. A complete blood count (CBC) at the time of the fever revealed a white blood cell (WBC) count of 4,670/μl, hemoglobin level of 6.8g/dl, and platelet count of 106,000/μl. A peripheral blood smear showed a left shift in the maturation of WBCs. Chemistry results showed increased values of total bilirubin/direct bilirubin of 4.3/2.1 mg/dl, lactate dehydrogenase of 382 IU/liter, and blood urea nitrogen/creatinine of 77/1.81 mg/dl and decreased values of total protein/albumin of 4.9/2.7 g/dl. The estimated glomerular filtration rate was decreased to 36.83 ml/min, and the C-reactive protein level was 51.3 mg/liter. Ascites analysis results revealed that specific gravity, pH, red blood cell (RBC), and WBC counts were within normal ranges, and no bacteria were observed on a Gram stain. Urinalysis results showed an increased WBC count (>100/high-power field) with proteinuria (1+), hematuria (1+), and nitrites (+). Therefore, the patient was diagnosed with acute kidney injury due to a urinary tract infection, and empirical treatment with ceftriaxone (1 g/day) was administered intravenously. To determine the source of fever and the causative pathogen, urine, ascites, and blood cultures were performed. Ascites culture resulted in no growth for any microorganisms after 72 h of incubation. Urine culture resulted in the growth of an extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolate (>100,000 CFU/ml) after 24 h of incubation. The clinician changed the antibiotic to meropenem (1 g/day).

Blood culture results were positive for growth after 72 h of incubation in 35°C by the BacT/Alert 3D blood culture system (bioMérieux, Inc., Durham, NC). Positive signals were detected from both sets of anaerobic (BacT/Alert FA Plus; bioMérieux, Inc.) and aerobic (BacT/Alert Standard Anaerobic; bioMérieux, Inc.) bottles. Direct Gram stain of positive blood culture broth media revealed visible Gram-negative bacilli. To isolate and identify this slowly growing Gram-negative bacillus, a subculture of the positive blood culture bottle on a blood agar plate (BAP) was performed and yielded growth after 72 h of incubation at 42°C under microaerobic conditions with 10% carbon dioxide and anaerobic conditions produced using GasPaK EZ gas-generating pouch systems (Bruker Daltonics, Franklin Lakes, NJ). There was no growth at 15°C or 37°C under either the microaerobic or anaerobic conditions. Colonies on BAP were gray and 1 mm in size. A Gram stain using carbol-fuchsin as a counterstaining reagent showed a slightly curved Gram-negative bacillus. The bacillus was oxidase positive, catalase positive, urease negative, and pyruvate negative. Vitek 2 (bioMérieux, Inc.) identified the microorganism as *Campylobacter coli*; however, the identification probability was too low (90%) to consider these results the correct identification. Therefore, PCR amplification and sequencing analysis were performed to obtain accurate identification. The target amplified regions or genes were 16S rRNA, *gyrB*, and *hsp60*. For 16S RNA sequencing, primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) were used (1). We analyzed the sequence data using a GenBank BLAST search. However, because BLAST results showed identities greater than 99% for several *Campylobacter* species, it was impossible to assign this microorganism to a species level with the 1,172-bp 16S rRNA gene amplification product. For *gyrB* gene amplification, primers JCL forward (5′-GHCAAGAATTTTCAGAAGGWAAA

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GT-3′) and JCL reverse (5′-GGATCTTTACTTTGACAATCAGC TA-3′) were used as described previously (2). Using a BLAST search, the sequence of *gyrB* was 99% (702/711 positions) identical to that of *Campylobacter volucris* strain LMG 24379 (GenBank accession no. CP007774.1). Identities to any other species of *Campylobacter* were below 93%. For *hsp60* gene amplification, primers H279 and H280, with the nucleotide sequences 5′-GAATTCGAIIIIGCIGGI GA(TC)GGIACIACIAC-3′ and 5′-CGCGGGATCC(TC)(TG)I (TC)(TG)ITCICC(AG)(AAICCIGGIGC(TC)TT-3′, respectively, were used as described previously (3–5). The identity of *hsp60* sequence was also 99% (548/556 positions) for only the *C. volucris* strain LMG 24379. The other *Campylobacter* species showed no higher than 94% identities. Therefore, we identified the isolates as *C. volucris* based on the results of sequencing analysis targeting three different genes. In addition to sequence analysis, we analyzed the isolates using two matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) systems, the Vitek MS system (bioMérieux) and a Bruker Biotyper (Bruker Daltonics). However, *C. volucris* was not included in the databases of the two MALDI-TOF analyzers, and both analyzers identified the isolates as *Campylobacter lari* with a high probability of identification (99.9% confidence value with the Vitek MS and a 2.27 score value for the Bruker Biotyper). For antimicrobial susceptibility tests, we followed the Clinical and Laboratory Standards Institute (CLSI) guidelines targeting *Campylobacter jejuni* and *C. coli* (6). The disk diffusion test was performed, and a 0.5 McFarland standard value of the isolate was inoculated onto Mueller-Hinton agar with 5% sheep blood. Disks with 15 μg erythromycin and 5 μg ciprofloxacin were used as indicators of macrolide and quinolone resistance, respectively. After incubation under microaerobic conditions (10% of CO_2), isolates were shown to be "susceptible" to both agents and had zone diameters of 31 mm and 11 mm, respectively. (The zone diameter interpretive criterion was 6 mm for both agents according to the guidelines.)

After 3 days of meropenem administration, no microorganisms were cultured from follow-up blood and urine cultures. Abdominal distension improved after five paracentesis procedures, and CBC profiles normalized after several transfusions. Vital signs, including body temperature, stabilized, and the general condition of the patient improved. The patient was discharged on the seventh day after admission.

C. volucris is a novel species of the genus *Campylobacter* first isolated from rectal swab specimens from black-headed gulls (*Larus ridibundus*) in 2010 in Sweden (5). To the best of our knowledge, this is the first case report of the isolation of the organism from human blood as well as from clinical specimens. *C. volucris* is known to be Gram negative and non-spore forming and has a curved-rod morphology. The bacillus produces oxidase and catalase and reduces nitrate and selenite. Indoxyl acetate and hippurate are not hydrolyzed. Hydrogen sulfide is not produced in triple-sugar iron agar. Strains grow on blood agar at 37 or 42°C under microaerobic and anaerobic conditions but not under aerobic conditions (5).

Campylobacter spp. grow slowly, and this factor could be one of the causes of false-negative results for isolation of causative pathogens; the isolates in our report grew after 72 h of incubation, not after 24 or 48 h. The colonies were gray, watery, and very small, approximately 1 mm in width. In a conventional Gram stain, the bacillus morphology was too faint to observe. Gram stain using carbol-fuchsin counterstaining was helpful to observe the bacillus morphology accurately. Therefore, sufficient incubation time, careful observation of small colonies, and special Gram stain techniques are crucial for the isolation and accurate identification of *Campylobacter* spp.

For the molecular species identification of the *Campylobacter* strain, 16S rRNA sequencing was problematic because most *Campylobacter* species share an approximate 99% identity with each other. Therefore, CLSI guidelines suggest performing sequence analyses targeted to alternative genes, such as *hsp60*, 23S rRNA, *gyrA*, or *gyrB* (1). Given these facts, we were not able to discriminate the isolates accurately based on 16S rRNA sequencing. We selected the *hsp60* gene because a previous article that discovered *C. volucris* targeted this gene. We additionally performed *gyrB* sequencing to support the results of *hsp60* gene sequencing. With these genes, we successfully identified the isolates as *C. volucris*.

The phylogenetic position of *C. volucris* was originally determined by 16S rRNA and *hsp60* gene sequences (5). These data revealed that the nearest phylogenetic neighbors were *C. lari* subsp. *concheus*, *C. lari* subsp. *lari*, *C. jejuni*, *C. coli*, *Campylobacter insulaenigrae*, *Campylobacter peloridis*, and *Campylobacter subantarcticus*. Later, based on these data, *C. volucris* was included in the *C. lari* group with other neighbor species (*C. lari*, *C. insulaenigrae*, *C. subantarcticus*, and *C. peloridis*) (7). In this case, both MALDI-TOF analyzers misidentified *C. volucris* as *C. lari* with a high probability of identification. Considering the databases of both MALDI-TOF analyzers did not contain any other species of the *C. lari* group except *C. lari*, the mass spectrum of *C. volucris* may be almost identical to that of *C. lari*. Further database development and accumulation are needed for accurate identification of *Campylobacter* spp. with MALDI-TOF analyzers.

Campylobacter bacteremia is mainly caused by *C. jejuni*, *C. fetus*, or *C. coli*, especially in immunocompromised patients (8–13). However, *C. lari* also has been occasionally associated with human illnesses, including bacteremia in immunocompromised patients (14–16). Other members of the *C. lari* group are also, but more infrequently, isolated from human clinical samples. *C. insulaenigrae* was reported as a cause of enteritis and septicemia in humans (17), and *C. peloridis* was identified in human feces and dialysis fluid (18).

For *Campylobacter* infection, erythromycin is considered a primary antibiotic of choice for successful treatment, while ciprofloxacin is also an acceptable alternative (19, 20). However, strains that are resistant to macrolide or quinolone agents have emerged and are currently becoming more widespread (21, 22). Therefore, precautions against promoting resistance are needed, especially when the condition of the patient is worsening despite the use of these agents. CLSI guidelines recommend that primary antimicrobial susceptibility testing should be performed with these two reagents (6). Our isolates showed "susceptible" results for both agents; however, clinicians made the decision to use meropenem to cover the pathogen of the concomitant urinary tract infection, *K. pneumoniae*, which was found to be producing extended-spectrum β-lactamases. *Campylobacter* is also known to be susceptible to carbapenem agents (21) as patients were cured with meropenem and discharged with an improved general condition.

In conclusion, this is the first report of *C. volucris* bacteremia in

an immunocompromised patient, and this case report is valuable for its novelty. Further studies are required to determine the pathogenicity and clinical spectrum of *C. volucris* bacteremia as well as those of other *Campylobacter* spp. of the *C. lari* group.

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