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Flavobacterium ceti From Blood Samples of a Korean Patient With Alcoholic Liver Cirrhosis

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Dear Editor

Flavobacterium species are nonfastidious, oxidase-positive, glucose nonfermenting, gram-negative rods. Few *Flavobacterium* species have been extensively studied [1]. *Flavobacterium ceti* was first isolated from the lung and liver of two beaked whales, as described in 2007 by Vela *et al*. [2]. *Flavobacterium* species are mainly found in aquatic animals and are rarely isolated from human clinical specimens. We present a case in which *F. ceti* was identified in the blood of a patient admitted to Seoul Metropolitan Government-Seoul National University Boramae Medical Center, Seoul, Korea.

A 43-yr-old man with alcoholic liver cirrhosis presented with mild fever and abdominal distension. Laboratory investigation revealed mild leukocytosis with neutrophilia (10.34×10^9/L; neutrophils, 83%) and hepatic dysfunction. Ascitic fluid showed leukocyte counts $>1\times10^9$ cells/L (neutrophils, 62%), whereas the serum ascites albumin gradient was >1.1 g/dL, total protein was 0.8 g/dL, glucose was 107 mg/dL, and lactate dehydrogenase was 97 U/L. Blood culture was drawn from two separate sites on the day of admission, but ascites cultures were not performed. Intravenous antibiotics were administered from the day of admission: one day of cefotaxime followed by ceftriaxone for the next 20 days. Follow-up blood cultures on day 4, 11, and 14 were all negative. Ascitic cultures performed after 14 days of antibiotics revealed no organisms. Signs of inflammation were gradually resolved, and the patient was discharged 25 days after admission.

After two days of incubation, bacterial growth was detected in aerobic culture bottles of two separate blood cultures. The next day, circular, orange-colored, non-hemolytic colonies were observed on the blood agar plates (Fig. 1A), and gram stain smear revealed gram-negative rods (Fig. 1B). No colonies were observed on MacConkey agar plates. Vitek2 GN system (bioMérieux, Marcy l'Etoile, France) identified the isolated organism as *Brevundimonas diminuta/vesicularis* with 95% probability. The contraindicating typical biopattern was phosphatase negative, enzyme activity that is almost always observed in *Brevundimonas* [3]. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; BrukerDaltonik GmbH, Bremen, Germany) revealed the most matched pattern as *Acinetobacter lwoffii*, but showed no reliability with a score of 1.191 (Biotyper Version 3.1, Bremen, Germany). To confirm the identification, 16S rRNA sequence was analyzed by using polymerase chain reaction with 27F and 1492R primers and sequencing with 518F and 801R primers in order to span all bacteria [4]. Sequencing reactions were performed by using a DNA Engine Tetrad 2 Peltier Thermal Cycler (BioRad, Hercules, CA, USA)

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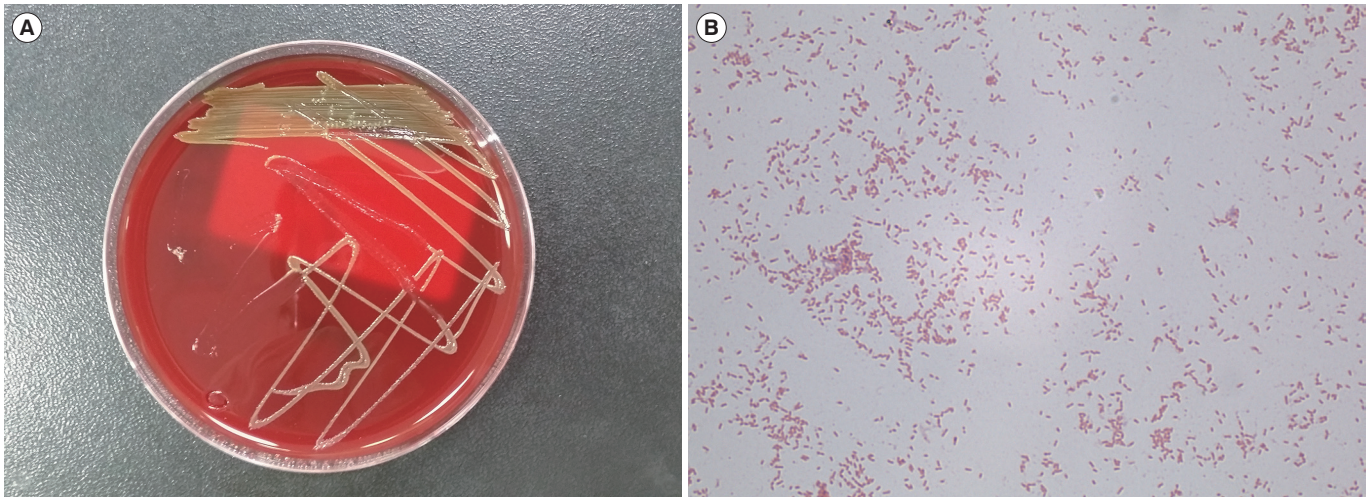

Fig. 1. Colony morphology and microscopic characteristics of *Flavobacterium ceti*. (A) Orange-colored, non-hemolytic colonies were observed after two days of aerobic culture on a blood agar plate. (B) Gram-negative rods from positive aerobic blood culture smear preparations (Gram stain, ×1,000).

with a ABI BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and were analyzed by using electrophoresis in an ABI 3730xl DNA Analyzer (Applied Biosystems) with ChromasPro 1.7.6 (Technelysium Pty Ltd, South Brisbane, Australia). The 1,367 bp of the 16S rRNA gene sequence shared 99% identity with the GenBank sequence NR_042540 (*F. ceti* strain 454-2).

Flavobacterium species are well-known pathogens of bacterial cold-water disease, with some causing opportunistic infections. There have been some reports of human infections due to pathogens that were previously known as *Flavobacterium* but are currently classified under other genera such as *Chryseobacterium* [5] and *Myroides* [6]. Thus, known human infections from current *Flavobacterium* are limited, with only a few reports of different species [7, 8]. Mosayebi *et al*. [9] reported a *Flavobacterium* sepsis outbreak due to contaminated distilled water in a neonatal intensive care unit. To our knowledge, our report discusses the first case of *F. ceti* infection in human. Physical examination and ascites lab findings were consistent with spontaneous bacterial peritonitis. *F. ceti* was considered the causative pathogen because it was isolated from two separate blood cultures collected on the day of admission and the cause of bacteremia was presumed to be peritonitis although ascitic culture was not performed. Clinical manifestations were relatively mild, with good outcomes. Identification of gram-negative non-fermenting rods is quite challenging, and commercial systems often provide unreliable results. Molecular sequencing is one of the most accurate ways characterizing these bacteria to the species level. In this case, conventional methods and MALDI-TOF MS were insufficient in identifying *Flavobacterium*. The MALDI database contained a limited number of *Flavobacterium* species, and *F. ceti* was not included. 16S rRNA sequence analysis successfully confirmed the isolate as *F. ceti*.

Here, we report the first probable human case of *F. ceti* detected in the blood of a patient with liver cirrhosis. *Flavobacterium* may cause an opportunistic infection in humans, although its significance remains unclear owing to the limited number of human infections reported. Molecular sequencing is currently the most reliable method for identifying this unusual pathogen.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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