Letter to the Editor
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Two Cases of *Clostridium tertium* Infection and Successful Identification of the Organism by Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry Analysis

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Dear Editor,

Clostridium tertium is an endospore-forming anaerobic gram–positive bacillus. This organism is aerotolerant and is easily decolorized in Gram-stained smears, often leading to mistaken identification as a gram-negative organism. The major risk factors for *C. tertium* bacteremia are hematological disease, intestinal mucosal injury, and history of exposure to β-lactam antibiotics (such as the third and fourth generation cephalosporins) where fever and leukopenia are often seen in patients [1]. We report two cases of *C. tertium* isolated from blood culture, one of which was successfully identified by using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis and subsequent 16S ribosomal DNA sequencing.

Case 1. A man in his 50s collapsed at home, became hypothermic with shock, and was hospitalized. On day 2 after hospitalization, two sets of blood cultures were taken, and antimicrobial treatment began with tazobactam/piperacillin (TAZ/PIPC) (2.25*g*×4/day). On day 3, he died and an aerobic isolate from the blood cultures became positive (BacT/Alert3D; bioMérieux, Tokyo, Japan) where gram-negative bacilli were recovered and sub-cultured aerobically at 35°C for 24 hr (Fig. 1A). Shiny colonies (about 1 mm in diameter) grew on blood agar plates, but not on BTB agar plates. Further, anaerobical culture of blood on Brucella HK agar plates at 35°C for 48 hr yielded gray colonies (about 3 mm in diameter). Gram stain showed spore-forming gram-variable bacilli (Fig. 1B). We therefore suspected *Clostridium* species. The organism was identified as *C. tertium* on the basis of the results of an automated identification apparatus (VITEK2 Compact; Sysmex bioMérieux), an identification kit (Rap ID ANAII; Amuko, Tokyo, Japan), and negative catalase test results. Later examination by MALDI-TOF MS-Biotyper (Bruker Daltonics, Yokohama, Japan) confirmed the organism as *C. tertium* with an identification log score 2.030 (2.000-2.299: secure genus identification and probable species identification). Antibiotic susceptibility showed that the minimum inhibitory concentration (MIC) value against *C. tertium* was 8 μg/mL for TAZ/PIPC, 16 μg/mL for cefotaxime (CTX), and 1 μg/mL for metronidazole.

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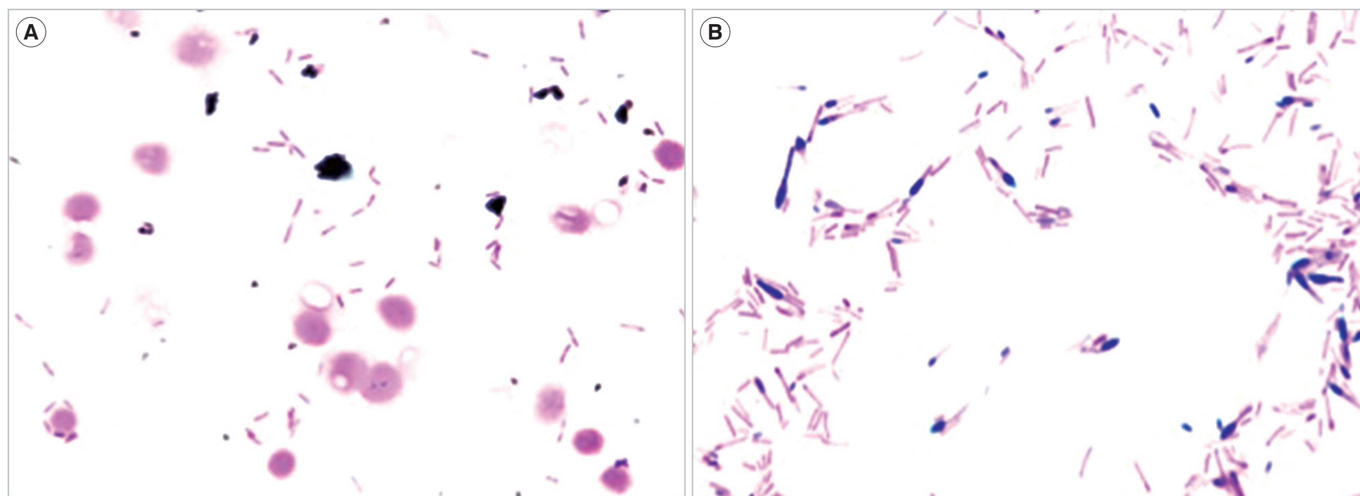

Fig. 1. Light microscope observation of Gram stain preparations. (A) Gram stain of aerobic blood cultures showed Gram-negative bacilli (×1,000). (B) Gram stain of colonies on Brucella HK agar plates showed an oval spore on the end of gram-variable bacilli (×1,000).

Case 2. A man in his 60s had been diagnosed as having acute myelogenous leukemia and hospitalized for chemotherapy. Two days after beginning chemotherapy, he presented with fever (38.0°C) and his leukocyte count declined to 1.3×10^9/L. We began an antimicrobial treatment with cefepime (CFPM) (1*g*×4/day) which proved ineffective. Three blood cultures gave negative results. On day 14 after the start of the chemotherapy, two sets of blood cultures were taken; and the antimicrobial therapy was changed to meropenem (MEPM) (1*g*×3/day). On day 16, one of the two sets of aerobic blood cultures grew Gram-variable bacilli similar to Case 1, leading us again to suspect a *Clostridium* species. Antibiotic susceptibility tests showed MICs for CTX at ≧32 µg/mL, ceftriaxone at ≧32 µg/mL, cefozopran at 16 µg/mL, MEPM at ≦0.25 µg/mL, and vancomycin (VCM) at ≦1 µg/mL. The antimicrobial treatment regimen was then supplemented with VCM (1*g*×3/day). Blood was aerobically cultured and colonies similar to Case 1 grew on blood, chocolate, and Brucella HK agar plates but not on BTB agar plates. VITEK2 compact and Rap ID ANA II analyses reported the presence of the test strain as *C. clostridioforme*; however, we doubted that report for several reasons. *C. clostridioforme* is anaerobic, so it can be mistaken for a gram-negative bacillus similar to *C. tertium*. Further, *C. clostridioforme* is usually susceptible to β-lactam antibiotics. The clinical strain formed endospores in anaerobic culture while *C. clostridioforme* does not form endospores but rather a so-called 'football-form' in Gram stains [1].

Consequently, we performed further identification using MALDI-TOF MS-Biotyper (Bruker Daltonics). That analysis reported the clinical strain as *C. tertium* with an identification log score of 2.030. Sequencing of the 16S rDNA gene showed complete identity to *C. tertium*. *C. tertium* was considered a pathogenic bacterium in the case because it had been isolated from blood culture and because of the neutropenia. *C. tertium* is resistant to the third and fourth generation cephalosporins and aminoglycosides, and VCM and/or imipenem are often used therapeutically to treat *C. tertium* infection [2, 3]. MEPM and VCM were administered for seven days and the patient survived.

Given the difficulties in differentiating between the several *Clostridium* species, our work underscores the necessity of using a variety of techniques to assist clinical identification. Our study suggests identification of bacterial species by combining MALDI-TOF MS with 16S rRNA gene sequencing provides a reliable means to obtain rapid identification of pathogens in the clinical setting, removing the ambiguity around poorly differentiable strains such as those of *Clostridium* species.

Authors' Disclosures of Potential Conflicts of Interest

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

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