Letter to the Editor
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A Case of *Cruoricaptor ignavus* Isolated From the Blood of a Patient With Ewing Sarcoma

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Dear Editor,

Cruoricaptor ignavus, belonging to the family *Flavobacteriaceae*, is a gram-negative, non-motile, non-spore-forming coccoid- or coccobacilli-shaped bacterium first isolated from a human blood culture in 2012 and proposed as a novel genus and species [1]. With approval from Samsung Medical Center institutional review board (IRB; approval number: 2018-01-112), we report the second case worldwide of *C. ignavus*, isolated from the blood culture of a 16-year-old boy with Ewing sarcoma and identified by DNA target sequencing. The IRB waived the need for informed consent for this study.

The patient underwent wide excision of sarcoma and additional chemotherapy. On day 13 of chemotherapy, he developed fever with abdominal pain and visited the emergency room. His temperature was 38.3°C, blood pressure was 116/59 mmHg, pulse rate was 67 beats per minute, and respiratory rate was 18 breaths per minute. The C-reactive protein concentration was 146.7 nmol/L, and leukocyte count was 0.21×10^9/L with neutropenia (0.07×10^9/L). Blood, urine, and stool cultures were performed, and cefepime was administered. Positive growth was observed in one of two sets of blood cultures after two days of incubation. Microscopic examination revealed gram-variable cocci or coccobacilli (Fig. 1), which grew as tiny, yellowish colonies on blood agar plates (Fig. 2).

The microorganism could not be identified using the GP, GN, and NH cards of the VITEK 2 system (bioMérieux, Marcy l'Etoile, France) or by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with the Bruker MALDI Biotyper system (Bruker Daltonics GmbH, Leipzig, Germany). The VITEK MS system identified the organism as *Alloiococcus otitis* (99.9% confidence), but Gram staining and colony morphology showed that this identification was not accurate.

To identify the strain, we performed 16S ribosomal RNA (rRNA) target sequencing according to the CLSI guidelines [2]. Subregions of the 16S rRNA gene were amplified using the following primer pairs: forward, 4F: 5′-TTG GAG AGT TTG ATC CTG GCT C-3′ and reverse, 534R: 5′-TAC CGC GGC TGC TGG CAC-3′ and forward, 27F: 5′-AGA GTT TGA TCM TGG CTC AG-3′ and reverse, 801R: 5′-GGC GTG GAC TTC CAG GGT ATC T-3′ [2]. The amplified sequence was compared with the GenBank (National Center for Biotechnology Information) database, using the basic local alignment search tool (BLAST) algorithm. The 16S rRNA sequence of the isolate exhibited 99.72% (722/724 bp) similarity to *C. ignavus* (GenBank accession number NR_108875.1, Strain IMMIB L-12475^T). The second highest match was *Epilithonimonas xixisoli* with 87.07% (653/750 bp) similarity. When the sequence (724 bp) was submitted to the EzTaxon database v2.1 (http://www.ezbiocloud.net), the best-

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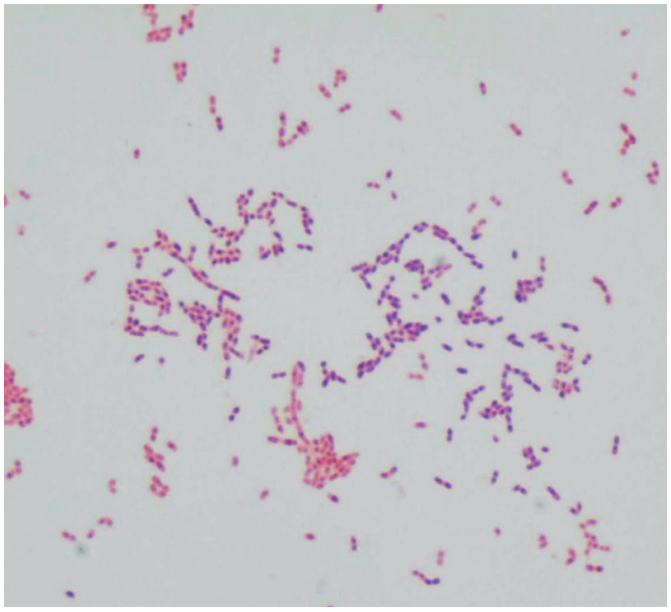

Fig. 1. Microscopic image of *Cruoricaptor ignavus*, showing gram-variable cocci or coccobacilli cells (Gram stain, ×1,000).

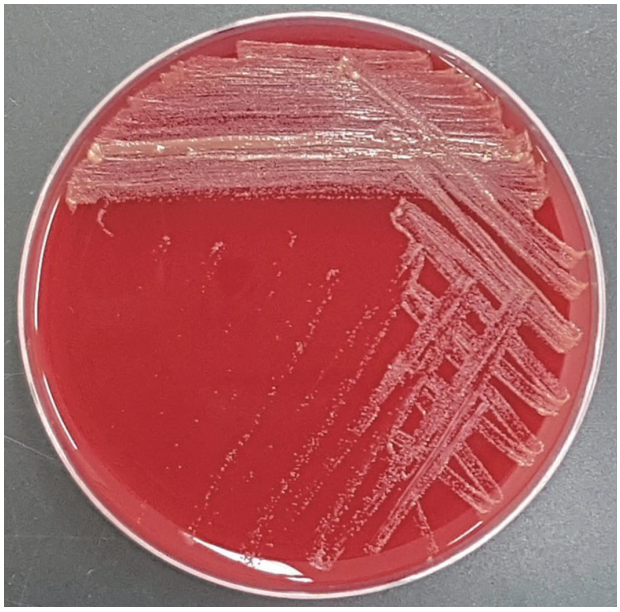

Fig. 2. Colony morphology of *Cruoricaptor ignavus* on blood agar plate.

matched strain was also *C. ignavus* (99.72%), which was accepted as a final confirmation.

C. ignavus has rarely been isolated in clinical microbiology laboratories. Therefore, its pathogenic role remains unclear. In this case, the patient became afebrile after six days of antibiotic treatment, and follow-up culture showed no microorganism growth. The patient was discharged on the ninth day after admission. We cannot exclude the possibility that the isolate did not represent a true pathogen. However, considering that *Flavobacteriaceae* are a group of commensal bacteria and opportunistic pathogens, isolation of this bacterium in immunocompromised hosts may also represent a real infection scenario.

This case indicates that laboratory staff should be aware that *C. ignavus* can be misidentified or unidentified by commonly used identification systems. As Gram staining of *C. ignavus* is polymorphic and its morphology can appear both coccoid and coccobacilli-shaped, it may be challenging to distinguish *C. ignavus* based on colony and microscopic examination. Moreover, this pathogen could not be identified using the VITEK 2 system, and the VITEK MS system misidentified it as *A. otitis*, causing confusion. In a previous study, *A. otitis* was misidentified using Bruker Biotyper MALDI-TOF MS [3]. Although MALDI-TOF MS is commonly used in routine diagnostic laboratories, identification of new isolates is possible only if the spectral database contains peptide mass fingerprints of the type strains of specific genera/species/subspecies/strains [4, 5]. *C. ignavus* was not included in the lists of identifiable species using the VITEK MS or Bruker Biotyper database.

To date, sequence-based typing remains the only available method for accurate identification of *C. ignavus*. Therefore, when an isolate is identified as *A. otitis* by MALDI-TOF MS and the microscopic morphology or biochemistry findings for that microorganism are inconsistent, confirmation via 16S rRNA-based gene sequencing is needed.

Authors' Disclosures of Potential Conflicts of Interest

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

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