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The First Case of *Ochrobactrum pseudogrignonense* Bacteremia in Korea

Hae Weon Cho, M.D.[1], Jung-Hyun Byun, M.D.[2], Daewon Kim, M.D.[1], Hyukmin Lee, M.D.[1], Dongeun Yong, M.D.[1], and Kyung Won Lee, M.D.[1]

[1]Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea; [2]Department of Laboratory Medicine, Gyeongsang National University Hospital Gyeongsang National University College of Medicine, Jinju, Korea

Dear Editor,

Ochrobactrum pseudogrignonense is a gram-negative, non-spore-forming, aerobic bacillus, rarely encountered in clinical specimens. It was originally isolated in Scandinavia from the blood of a 28-year-old man in 1992 and the ear of a newborn in 2000 [1]. Since then, *O. pseudogrignonense* has been isolated from environmental sources [2-4]; however, its pathogenicity in humans has not been explored extensively. We report the first case of *O. pseudogrignonense* bacteremia in Korea—and only the third in the world [1], to our knowledge—and species identification was verified by 16S rRNA and *recA* gene sequencing. This study was approved by the Institutional Review Board (IRB) of Yonsei University of Medicine, Seoul, Korea (2019-1369). This is a retrospective report and hence IRB approval was obtained without consent forms.

A 44-year-old man with hypertension, diabetes mellitus, and dilated cardiomyopathy presented with aggravated dyspnea and falling blood pressure and was admitted to a hospital in March 2018. After three days, the patient received extracorporeal membrane oxygenation (ECMO) and was referred to a tertiary-care hospital in Seoul for heart transplantation. After transfer, the patient's vital signs were as follows: blood pressure, 110/87 mm Hg; pulse rate, 120/minutes; respiration rate, 16/minutes; and body temperature, 36.5°C. The initial laboratory results were as follows: hemoglobin, 175 g/L; white blood cell (WBC) count, 28.5×10^9/L; and platelet count, 118×10^9/L. The patient was initially placed on a vancomycin and piperacillin-tazobactam regimen. Although the patient had no fever one day after admission, his serum C-reactive protein level was 514.3 nmol/L, WBC count was 42.6×10^9/L, and delta neutrophil index was 12.4%, suggesting an increased immature granulocyte fraction [5]. As fever may not be apparent in ECMO patients with bacteremia [6] and considering the deteriorated laboratory results, the antibiotic regimen was changed from piperacillin-tazobactam to meropenem.

One set of aerobic and anaerobic blood cultures from the central venous line and two sets from different peripheral venipunctures were collected before initiation of antibiotic therapy and incubated for 36 hours in the BACT/ALERT 3D blood culture system (bioMérieux, Durham, NC, USA). All three aerobic vials demonstrated bacterial growth; they formed beige, non-hemolytic, distinct small circular colonies on blood and MacConkey agar plates within 24 hours of incubation at 35°C (Fig. 1A & B). Gram-staining indicated gram-negative, rod-shaped bacteria without spores (Fig. 1C). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker

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Corresponding author: Dongeun Yong, M.D.
Department of Laboratory Medicine, Research Institute of Bacterial Resistance, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel: +82-2-2228-2442, Fax: +82-2-364-1583, E-mail: deyong@yuhs.ac

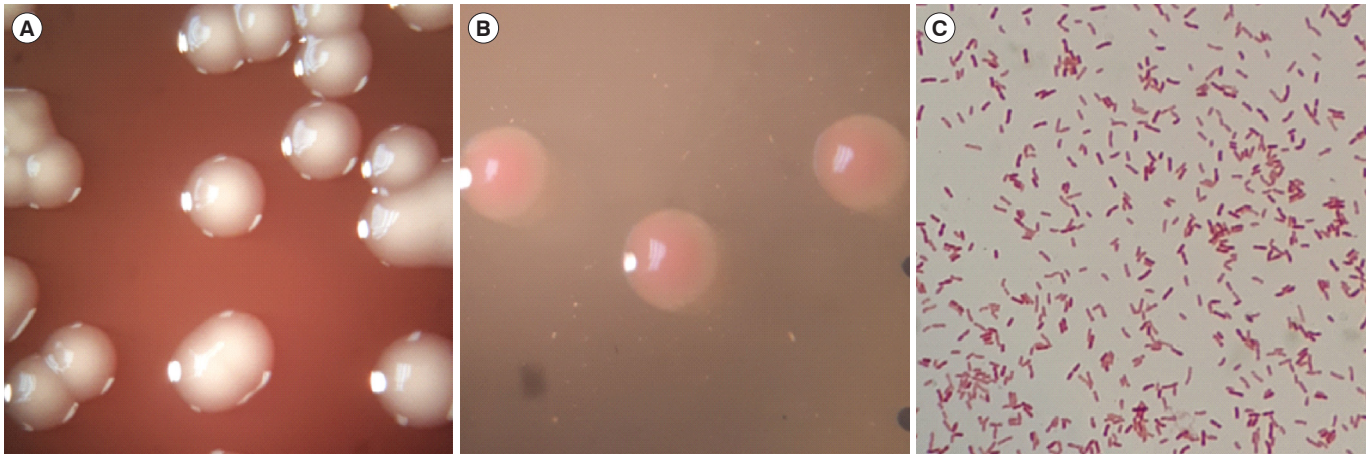

Fig. 1. Colony and microscopic morphology of the *O. pseudogrignonense* isolate. (A) Colonies on blood agar (10×) and (B) MacConkey agar (10×) plates. (C) Gram staining of the isolate (1,000×).

Daltonics, Bremen, Germany) of the isolate revealed signals characteristic of *O. gallinifaecis* with a score of 1.81; however, both the VITEK MS and VITEK2 systems (bioMérieux, Marcy l'Etoile, France) failed to identify the isolate.

To characterize the bacterium, molecular identification was performed by DNA amplification and sequencing of 16S rRNA [7]. Based on the EzTaxon database (http://www.ezbiocloud.net), both 373 and 1,374 bp 16S rRNA sequences of the isolate shared 100% sequence identity with those of *O. pseudogrignonense* (GenBank accession number AM422371) and 99.3% with those of *Ochrobactrum thiophenivorans* (GenBank number AM490617). Based on the Ribosomal Database Project (https://rdp.cme.msu.edu/), the isolate had a sequence match score of 1.0 for *O. pseudogrignonense* and 0.96 for *O. thiophenivorans*.

As 16S rRNA did not provide the resolution to distinguish the species [7], alternative analysis using the *recA* gene was performed [8, 9]. We constructed primer pairs for partial sequencing *O. pseudogrignonense* (GenBank number AM422877.1) and *O. thiophenivorans* (GenBank number KF866345) *recA: recA-O.pseudo-f* (5′-TTCGGGTAAAACCACTCTCG-3′)/*recA-O. pseudo-r* (5′-ATGTCGAACTCGACCTGCTT-3′) and *recA-O.thio-f* (5′-GCAAGGGCTCAATCATGC-3′)/*recA-O.thio-r* (5′-AATCACCCATTTCACCTTCG-3′). The isolate was amplified successfully only with the *O. pseudogrignonense recA* primer pair (510 bp). This PCR product shared 100% sequence identity with the *recA* gene of *O. pseudogrignonense*, as determined using the GenBank alignment search tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi).

We performed antimicrobial susceptibility testing (AST) of the isolate using the VITEK2 system with the AST-N225 card, which was interpreted based on the breakpoints for other non-*Enterobacteriaceae* [10]. The results showed that the isolate was susceptible to meropenem and resistant to piperacillin-tazobactam (Table 1). Consistent with the AST results, two days after meropenem treatment, the patient's WBC count decreased to 16.0 $\times 10^9$/L, and blood culture results were negative for five days. The patient recovered without any evident sequelae.

Table 1. Antimicrobial susceptibilities of the *O. pseudogrignonense* isolate

Antimicrobial agents	Susceptibility	MIC (μg/mL)
Amikacin	S	8
Ampicillin/Sulbactam	S	8/4
Aztreonam	R	≥64
Ceftazidime	S	8
Ciprofloxacin	S	≤0.25
Colistin	S	≤0.5
Cefepime	S	≤1
Cefotaxime	S	4
Gentamicin	S	≤1
Imipenem	S	0.5
Meropenem	S	1
Minocycline	S	≤1
Piperacillin	R	≥128
Piperacillin-Tazobactam	R	≥128/4
Cotrimoxazole	S	≤20
Tigecycline	S	≤0.5

Abbreviations: MIC, minimum inhibitory concentration; S, susceptible; R, resistant.

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Ochrobactrum species are emerging pathogens with low virulence that can cause severe systemic infections not only in patients with underlying diseases but also in immunocompetent patients [8, 9]. This is the first case of human infection with *O. pseudogrignonense* in Korea. As the isolate showed resistance to piperacillin-tazobactam, accurate identification and AST were critical for timely and appropriate treatment; however, it was difficult to identify the isolate using commercial bacterial identification kits and MALDI-TOF MS. Owing to the high similarity of 16S rRNA sequences among *Ochrobactrum* species, as well as *Brucella* species [8, 9], additional sequencing, such as of the *recA* gene, is needed for accurate species-level identification.

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AUTHOR CONTRIBUTIONS

HWC and DY conceptualized and designed the study; searched the scientific literature; collected, analyzed, and interpreted the data; and wrote and revised the report. JHB, DK, HL, and KWL designed the study and revised the report. DY and KWL supervised the study and gave administrative, technical, and material support and secured the funding.

CONFLICTS OF INTEREST

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

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ORCID

Hae Weon Cho https://orcid.org/0000-0002-3017-5026
Jung-Hyun Byun https://orcid.org/0000-0001-5909-5807
Daewon Kim https://orcid.org/0000-0002-3487-5943
Hyukmin Lee https://orcid.org/0000-0002-8523-4126
Dongeun Yong https://orcid.org/0000-0002-1225-8477
Kyungwon Lee https://orcid.org/0000-0003-3788-2134

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