The First Case of *Azorhizobium caulinodans* Bacteremia in a Patient with Leukemia

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Dear Editor,

Azorhizobium spp. are gram-negative, rod-shaped, obligately aerobic, motile soil bacteria [1]. They are free-living microorganisms that can fix nitrogen in symbiosis with plants of the genus *Sesbania* [2]. Infection by *Azorhizobium* spp. in humans has not been reported yet. We report the first case of bacteremia caused by *Azorhizobium caulinodans* in a patient with AML. The Institutional Review Board of Seoul National University Hospital, Seoul, Korea approved this study (2103-179-1207) and waived the need for informed consent.

An 85-year-old male patient undergoing induction chemotherapy for AML diagnosed in July 2020 was hospitalized for additional chemotherapy in September 2020. He was in a nursing hospital before being admitted. From day 2 of hospitalization, decitabine was administered for five days. On day 2, his temperature was 37.2°C, his heart rate was 93 beats/min, and his blood pressure reached 91/60 mmHg. A chest computed tomography scan showed destruction of the right lung after pulmonary tuberculosis and pneumonia with pleural effusion in the left lung. Hematological testing revealed a leukocyte count of 8.30×10^9/L; the neutrophil count was 2.39×10^9/L with a left shift and blasts (5%). The C-reactive protein (CRP) level was elevated to 82.6 mg/L. Two pairs of peripheral blood cultures and one pair of central venous catheter (CVC) blood cultures were collected. The patient received empirical antibiotic treatment intravenously (4.5 g piperacillin/tazobactam at 6-hour intervals).

The three pairs of blood cultures were inoculated into aerobic and anaerobic blood-culture bottles and incubated in the BacT/ALERT VIRTUO system (bioMérieux, Durham, NC, USA). Two aerobic peripheral blood cultures revealed gram-negative rods after 50 hours of incubation. Colonies on a blood agar plate appeared within 24 hours of incubation at 35°C and were pinpoint, round, and cream-colored (Fig. 1A). Using a GN ID card of the VITEK 2 system (bioMérieux), the gram-negative rods were identified as *Bordetella bronchiseptica*, with a good confidence level (91%). The bacteria could not be identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a MALDI Biotyper (Bruker Daltonics, Bremen, Germany).

For accurate identification, 16S rRNA gene sequencing was performed using 27F/1492R PCR primers and 785F/907R sequencing primers [3]. The 16S rRNA sequences were searched in the GenBank database (http://www.ncbi.nlm.nih.gov/blast/) and interpreted according to the CLSI guidelines [3]. The results revealed 99.86% (1,396/1,398) identity with *A. caulinodans* (GenBank, NR_074185.1/NR_113675.1) and 97.93% (1,372/1,401) identity with *Xanthobacter flavus* (GenBank, NR_113665.1). Phylogenetic analysis using MEGA X (http://www.megasoftware.net)
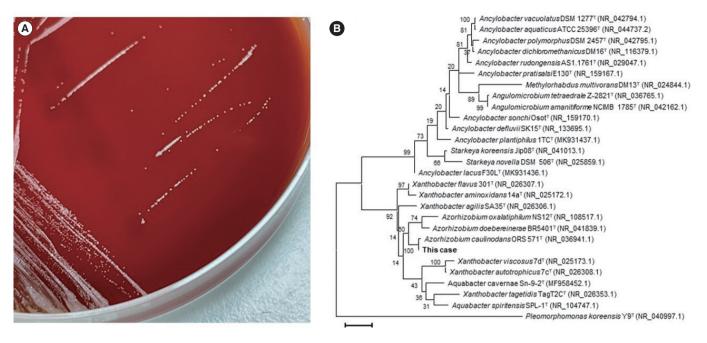
Fig. 1. Colony morphology of the *Azorhizobium caulinodans* isolate and a phylogenetic tree based on 16S rRNA gene sequences. (A) Round, cream-colored pinpoint colonies on a blood agar plate. (B) Phylogenetic tree constructed by the maximum-likelihood method using the model GTR+I+G and *Pleomorphomonas koreensis* Y9^T (NR_040997.1) as the subgroup. Bootstrap values are expressed as percentages of 1,000 replications, and the scale bar indicates the estimated number of substitutions per base. GenBank accession numbers are indicated within parentheses.

Table 1. Antimicrobial susceptibility of the *Azorhizobium caulinodans* clinical blood-culture isolate as determined using Etest

Antimicrobial agent	MIC (μg/mL)	Interpretation
Piperacillin/tazobactam	≥256/4	R
Cefotaxime	≥32	I/R
Ceftriaxone	≥32	I/R
Ceftazidime	≥256	R
Imipenem	≥32	R
Meropenem	≥32	R
Gentamicin	0.5	S
Ciprofloxacin	1.5	I
Levofloxacin	1	S
Moxifloxacin	0.19	NA
Clindamycin	16	NA
Trimethoprim/sulfamethoxazole	0.012/0.228	S

Abbreviations: MIC, minimal inhibitory concentration; R, resistant; I, intermediate; S, susceptible; NA, not available.

identified the isolate as *A. caulinodans* (Fig. 1B). Antimicrobial susceptibility tests (ASTs) were performed by measuring minimal inhibitory concentrations (MICs) using Etest (bioMérieux, Marcy l'Étoile, France). Etest strips were placed on Mueller–Hinton agar and read after 48 hours. AST results, interpreted according to other non-*Enterobacterales* per the CLSI guidelines, are presented in Table 1 [4].

On day 6, two pairs of CVCs and two pairs of peripheral blood follow-up blood cultures were prepared. *A. caulinodans* was detected in the two pairs of CVC blood cultures and confirmed using 16S rRNA gene sequencing. On day 8, he developed neutropenia. On day 10, his CRP level decreased to 20.0 mg/L, and the pleural effusion was drained by ultrasound-guided aspiration; however, no microorganisms were recovered from the pleural fluid culture. Follow-up CVC and peripheral blood cultures on days 10 and 15 were negative. After resolving the chemotherapy-caused neutropenia, he was discharged on day 20. Piperacillin/tazobactam was administered until discharge.

Azorhizobium spp. belong to rhizobia, a group of soil bacteria that form nodules on the roots of legumes [5]. Among them, the *Burkholderia cepacia* complex is a major human pathogen [5], and *Agrobacterium* (formerly *Rhizobium*) *pusense* infections are increasing [6].

During decitabine induction in elderly AML patients, neutropenic fever and infection are major adverse events [7]. Because the patient was previously admitted to a nursing hospital, there was no risk of soil exposure. He appeared to have developed the bacteremia owing to decreased immunity caused by AML.

The isolate showed high MICs for β-lactam antibiotics and positive for β-lactamase on a Cefinase test (Becton Dickinson, Sparks, MD, USA), possibly attributable to a class A β-lactamase gene (*penA*) [8]. However, the bacteremia was resolved with piperacillin/tazobactam, to which the isolate was resistant. Identification and ASTs were conducted after discharge, and thus, the antibiotics could not be changed during hospitalization. The bacteremia probably improved due to an increase in neutrophils or *in vivo* piperacillin/tazobactam activity.

The isolate was misidentified by VITEK 2 and failed to be identified by MALDI-TOF MS. The identification of root nodule bacteria by MALDI-TOF MS has been reported [9]. By analyzing the mass-to-charge ratio of the isolate using Ribopeaks, the database of which includes environmental bacteria, the isolate was accurately identified as *A. caulinodans* [10]. Unusual bacteria may be accurately identified by 16S rRNA sequencing and MALDI-TOF MS using a comprehensive environmental bacteria database.

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AUTHOR CONTRIBUTIONS

Research conception and design: Park JH. Data acquisition: Park JH, Kim TS, Park H. Data analysis and interpretation: Park JH. Figure preparation: Park JH. Drafting of the manuscript: Park JH. Revision of the manuscript: Park JH, Kim TS, Park H. Approval of the final manuscript: all authors.

CONFLICTS OF INTEREST

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

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