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First Report of Sepsis Caused by *Rhodococcus corynebacterioides* in a Patient with Myelodysplastic Syndrome

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We report a case of sepsis caused by *Rhodococcus corynebacterioides*, identified using 16S rRNA gene sequencing, in a myelodysplastic syndrome patient who had undergone hematopoietic stem cell transplantation. This is the first report of *R. corynebacterioides* infection in a human.

CASE REPORT

A 64-year-old man was hospitalized because of a high fever. Three years earlier, he was diagnosed with myelodysplastic syndrome and underwent allogeneic hematopoietic stem cell transplantation. Since that time, he had been taking low-dose prednisolone to control chronic graft-versus-host disease. One year posttransplant, he experienced a cerebral hemorrhage, which caused left hemiplegia. Due to repeated aspiration pneumonia, a central venous catheter and port system were implanted in his chest wall to administer antibiotics and for nutritional support. The cause of the high fever at presentation was diagnosed as a bloodstream infection caused by *Candida albicans*. Removal of the catheter system and administration of micafungin resulted in the patient's recovery; the elevated serum β-D-glucan level returned to below the detectable limit.

Two weeks later, the patient again developed a fever (39.7°C). His white blood cell count was 2.1×10^9 cells/liter, and his C-reactive protein (CRP) level was 159 mg/liter. Blood cultures were collected, and empirical treatment with cefepime (1 g twice daily) was initiated. After 3 days of incubation, Gram-positive rods were detected in the blood cultures, which were unidentifiable by routine microbiological examinations. *Pseudomonas aeruginosa* was detected in his sputum, but this bacterium was not thought to be the causative agent of his sepsis, because he showed no findings suggestive of pneumonia and because the bacterium had been repeatedly detected before. Following the initiation of antibiotic treatment with cefepime, the patient became afebrile, his CRP level decreased to 47 mg/liter, and repeated blood cultures were negative. However, 18 days later, the high fever (39.2°C) recurred, and CRP level was again elevated to 106 mg/liter. Blood cultures again showed Gram-positive rods. Treatment was changed to cefozopran (2 g twice daily). However, his condition deteriorated, and he died 9 days later.

Microbiological data. The blood cultures were positive for Gram-positive rods in aerobic bottles of the Bactec 9240 system (BD, Franklin Lakes, NJ) twice, as described above, after 52 h and 88 h of incubation, respectively. Gram staining of the specimens revealed long and slightly bent Gram-positive rods in pairs, V forms, and palisade arrangements (Fig. 1A). The specimens from the bottles were plated onto Trypticase soy agar II with 5% sheep blood (BD) and incubated at 35°C in air supplemented with 5% CO_2. After 72 h, odorless, orange-colored, smooth, and nonhemolytic colonies, 4 mm in diameter, were observed (Fig. 1B). The colonies were positive for the catalase test and negative for the oxidase test. The RapID CB Plus system (Remel Inc., Lenexa) was used for identification. The isolates were identified as *Turicella otitidis* (microcode 0007511; probability level, 98.29%). However, the colonial features described above were clearly different from those of *T. otitidis*, colonies of which are whitish, convex, and creamy with entire edges (7). Therefore, we performed molecular identification by PCR amplification and sequencing analysis of the 16S rRNA gene using DNA extracted from the isolates. The universal primers 8UA (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1485B (5′-ACGGGCGGTGTGTRC-3′) were used as described previously (11). We performed sequencing analysis using a GenBank BLAST search and sequence editing and phylogenetic analysis using CLUSTAL W (neighbor-joining method) with

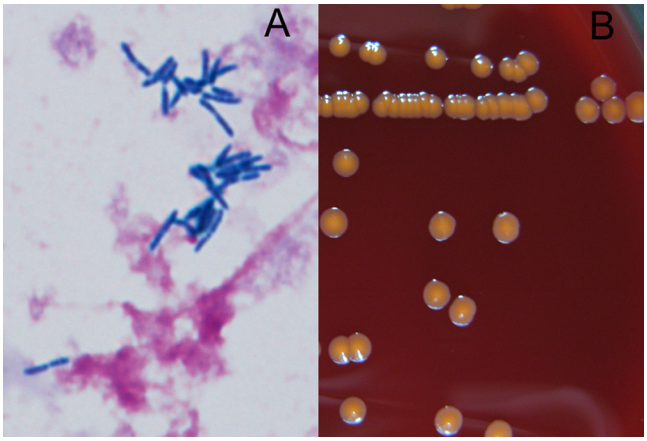

FIG 1 (A) Gram staining of *Rhodococcus corynebacterioides* isolated from blood cultures (oil immersion, ×1,000); (B) colonial appearance of *R. corynebacterioides* on a sheep blood agar plate after 72 h of incubation in air supplemented with 5% CO_2.

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TABLE 1 MICs for *Rhodococcus corynebacterioides* isolated from the present patient

Antimicrobial agent	MIC (μg/ml)
Benzylpenicillin	0.5
Ampicillin	1
Ampicillin-sulbactam	2
Cefazolin	1
Ceftriaxone	>2
Cefepime	1
Imipenem	≤0.06
Meropenem	0.5
Clarithromycin	≤0.12
Azithromycin	0.25
Clindamycin	0.5
Minocycline	≤0.12
Levofloxacin	≤1
Moxifloxacin	≤0.5
Vancomycin	0.5
Trimethoprim-sulfamethoxazole	>2/38

Treeview. The sequence of the 16S rRNA gene (GenBank accession number AB685427) was 99.86% identical (1,440 bp over the entire 1,442-bp fragment) with that of the type strain of *Rhodococcus corynebacterioides* (DSM 20151; accession number NR 041873). Based on this result, we identified the isolate as *R. corynebacterioides*. The features of the isolate were consistent with the reported features of *R. corynebacterioides*, such as the rods in V forms and palisade arrangement and orange-colored colonies (12). The antibiotic susceptibility of the bacterium was determined by the broth microdilution method approved by the Clinical and Laboratory Standards Institute (4) using a commercially prepared microtiter plate containing a series of lyophilized antimicrobials (DP34; Eiken Chemicals, Japan). MICs are shown in Table 1.

Rhodococcus, which belongs to the family *Nocardiaceae*, is a genus of aerobic, nonmotile, non-spore-forming, Gram-positive bacteria. The morphology can range from coccoid to bacillary depending on species and specimen type. Colonies are salmon-pink to red colored and teardrop shaped or coalescent mucoid (3, 5, 13). There are 34 named species in the genus. Most of these bacteria are nonpathogenic and found in a broad range of environments, such as soil, groundwater, animal dung, and plants. The most common pathogenic species in the genus is *Rhodococcus equi*. This bacterium is known to be a pathogen that causes pulmonary abscesses in horses. Recently, *R. equi* infection in immunocompromised patients has been reported (5). So far, 30 cases of *R. equi* infection in transplant patients have been described, and the clinical diagnosis in 24 of these 30 cases was pneumonia or a lung abscess (13). Besides *R. equi* infection, a case with *Rhodococcus erythropolis* causing bloodstream infection (2) and *Rhodococcus rhodochrous* infection causing a corneal ulcer have been reported (3, 8).

R. corynebacterioides was originally named *Corynebacterium rubrum* (6). Later, it was reclassified as *Nocardia corynebacterioides* based on its physiologic, chemical, and ultrastructural characteristics (12). Recently, the bacterium was again reclassified as *R. corynebacterioides* based on phylogenetic data from 16S rRNA gene sequencing and chemotaxonomic data (14). The colonial morphology and Gram stain findings of *R. corynebacterioides* are similar to those of other species of the genus *Rhodococcus*. It has been reported that *R. corynebacterioides* can be distinguished from other species by its physiologic characteristics, such as assimilation of various sugars as carbon sources and utilization of various amino acids as simultaneous carbon and nitrogen sources (14). However, these characteristics cannot be examined in routine hospital laboratories, and so 16S rRNA gene sequencing is generally needed to positively identify *R. corynebacterioides*.

We believe that *R. corynebacterioides* was the cause of the high fever in the present patient, because the bacterium was detected in blood cultures twice at the same time that he developed a high fever and CRP levels increased. Because we had not seen orange-colored colonies like this before and because we thought that the result from the identification kit was incorrect, sequencing analysis of the 16S rRNA gene was performed, leading to the positive identification of the isolate.

We were unable to specify the cause underlying his sepsis, as he had no active lesions in the lungs, eyes, or skin. We were also unable to specify the source of the infection, because he was not exposed to farming environments, to which the patients with *R. equi* infection were often exposed (13). Recently, it was reported that *R. corynebacterioides* was recovered from the mouth of healthy volunteers (9). This may be suggestive of a possible source for *R. corynebacterioides* in immunocompromised patients.

Antimicrobials such as imipenem, vancomycin, and quinolones are recommended for the treatment of *R. equi* infection (5, 13). The MICs for *R. corynebacterioides* observed in this case were almost identical to those reported for *Rhodococcus* species (1, 2, 10). In this patient, cefepime, which seemed susceptible in the susceptibility testing, was administered; however, this treatment was unsuccessful.

To the best of our knowledge, this is the first reported case of *R. corynebacterioides* infection in a human. Because this species is not well known and is difficult to identify, it may previously have been overlooked or misidentified. Therefore, molecular identification by 16S rRNA gene sequencing is useful for the positive identification of *R. corynebacterioides* infection. To clarify the clinical features of *R. corynebacterioides* infection and antimicrobial susceptibility patterns, the accumulation of more cases is required.

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