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CASE REPORTS

First Case of Bloodstream Infection Caused by *Rhodococcus erythropolis*▽

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We describe the first case of bloodstream infection caused by *Rhodococcus erythropolis*. The identification was performed using 16S rRNA sequencing. This case illustrates that non-*equi* *Rhodococcus* infections may be underdiagnosed due to difficulties in identification in the routine clinical microbiology laboratory.

CASE REPORT

A 79-year-old man with esophageal cancer was admitted to the hospital for radiotherapy followed by chemotherapy. Parenteral nutrition was administered by a peripheral venous catheter. Three weeks after hospital admission, the patient developed a fever (38.4°C). History and physical examination did not reveal an obvious source of infection. The leukocyte count was 1,700/mm^3 with 1,300 neutrophils/mm^3. The C-reactive protein was elevated at 4.34 mg/dl. Chest radiographic findings were normal. Three sets of blood cultures were collected, and antibiotic treatment with intravenous cefoperazone-sulbactam (2 g twice daily) was initiated empirically. The blood cultures were incubated at 37°C in the Bact/Alert system (bioMérieux, Marcy l'Etoile, France) and became positive for a gram-positive rod on the fourth day of incubation. All three sets of blood cultures were positive. The fever gradually diminished under treatment with cefoperazone-sulbactam. Local redness at the peripheral venous catheter site was noted, and the catheter was removed.

Fever recurred, despite ongoing treatment with cefoperazone-sulbactam. Blood cultures collected 10 days after the initial sets were collected again grew a gram-positive rod, which appeared phenotypically identical to the earlier isolates. There was no evidence of endocarditis on transthoracic echocardiography. Computed tomography scans and ultrasonography of the proximal vein within which the catheter was inserted showed no evidence of thrombophlebitis. Treatment was then changed to a second-generation cephalosporin, cefotiam (1 g three times daily), and the peripheral venous catheter was again removed. No further fever was observed, and the patient was discharged from the hospital 1 month after the final set of blood cultures was collected.

All four isolates grew in the aerobic bottle of the Bact/Alert system. After 72 h of aerobic incubation at 37°C, smooth white colonies grew on 5% sheep blood agar. The API Coryne system (bioMérieux, Marcy l'Etoile, France) was used for the initial identification according to the manufacturer's instructions. The results were positive for pyrazinamidase, alkaline phosphatase, α-glucosidase, esculin, urease, and catalase and negative for nitrate, pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, gelatinase, and carbohydrate fermentation tests. The isolates gave a profile number of 2151004 and were identified as *Rhodococcus* spp. (98.8% identification; *T* index, 0.89).

To strengthen this biochemical identification, molecular identification was attempted by PCR amplification and sequencing analyses. A 1,450-bp sequence of the 16S rRNA gene was amplified with universal primers 8UA (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1485B (5′-ACGGGCGGTGTGTRC-3′), as described previously (14). Sequencing analysis was performed using a GenBank BLAST search. Sequence editing and phylogenetic analyses were performed with CLUSTAL W (neighbor-joining method) with Treeview. The sequence of the 16S rRNA gene (accession number AB499800) was 99.4% identical (1,441 bp over the entire 1,449-bp fragment) with that of *Rhodococcus erythropolis* strain DSM 43188^T (X80618). The 16S rRNA gene sequence of the isolate from our case differed by 55 bp (96.2% identical), 56 bp (96.1% identical), and 42 bp (97.0% identical) from that of *Rhodococcus equi* DSM 40307^T (X80614), *Rhodococcus rhodochrous* DSM 43241^T (X79288), and *Rhodococcus fascians* DSM 20669^T (X79186), respectively.

The MICs of the following antimicrobial agents were determined by the broth microdilution method according to the Clinical and Laboratory Standards Institute guidelines for aerobes (3): penicillin, 2 μg/ml; cefotiam, ≤1 μg/ml; cefoperazone-sulbactam, 4 μg/ml; vancomycin, 0.5 μg/ml; clindamycin, 1 μg/ml; tetracycline, 2 μg/ml; gentamicin, ≤1 μg/ml; and rifampin (rifampicin), ≤1 μg/ml.

Rhodococcus belongs to the order *Actinomycetales* and the family *Nocardiaceae* (1, 12). *Rhodococcus* are obligate aerobic,

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gram-positive bacilli and partially acid-fast like *Nocardia* because of their mycolic acid-containing cell wall. They have been isolated from a large variety of sources, including soils, rocks, ground water, seawater, plants, animals, and the guts of insects (1, 12). Rhodococci have broad catabolic diversity and unique enzymatic capabilities. Their ability to transform or degrade a wide range of chemicals raises the potential usefulness of rhodococci in environmental or industrial biotechnology (1, 5).

Although *Rhodococcus* species are generally considered to have low pathogenicity, they cause diseases in animals, plants, and humans. Since the first clinical case caused by *R. equi*, formerly *Corynebacterium equi*, was reported in 1967, the significance of *Rhodococcus* in humans, especially among immunocompromised hosts, is increasing. This is both in terms of the actual occurrence of infection and the extent to which it is recognized (1, 18). However, most *Rhodococcus* human infections have been caused by *R. equi*, and only a few cases were reported for other species, for example, *R. rhodochrous* and *R. fascians* (formerly *R. luteus*) (6, 9, 17).

R. erythropolis was originally called *Mycobacterium erythropolis* by Gray and Thornton in 1928, during an investigation of soil bacteria (11). "Erythropolis" is literally from the Greek meaning "red city," in reference to the orange and red colonies sometimes observed (7). After a period of being known as *Nocardia erythropolis*, the organism is now known as *R. erythropolis*. *R. erythropolis* is a bacterium that attracts some attention in the biotechnology field (5), because it is solvent tolerant. It contains a large set of enzymes, allowing it to carry out oxidations, dehydrogenations, epoxidations, hydrolysis, hydroxylations, dehalogenations, and desulfurizations. Thus, it may have considerable potential as a biocatalyst, especially in nonconventional systems. *R. erythropolis* may occur naturally in contaminated environments, thus allowing it to be used in the bioremediation of polluted sites, for example (5).

R. erythropolis is typically found in soil. It has been detected on the surface of the healthy human eye (in conjunctival samples) (10). There are no other reports of its presence at other sites (skin, fecal flora, etc.) of the normal human body. The typical colony of *R. erythropolis* has been described to be rough and orange to red colored on glucose yeast extract agar and Sauton's agar (7). However, these colonial features are not always observed. In *R. erythropolis*, the morphogenetic cycle is elementary branching-rod-coccus (13). *R. erythropolis* produces acid from glucose, glycerol, sorbitol, sucrose, and trehalose and not from adonitol, arabinose, cellobiose, galactose, glycogen, inulin, melezitose, rhamnose, and xylose (7). *R. erythropolis* differs from *R. equi* in its ability to grow on the following sole carbon sources: maltose, mannitol, glycerol, sorbitol, sodium adipate, sodium citrate, sodium gluconate, butan-2,3-diol, sebacic acid, citraconic acid, D-mandelic acid, and L-asparagine (7, 13). It is noteworthy that the colonial appearance of the genus *Rhodococcus* is quite variable and that phenotypic tests available in the clinical laboratory may be insufficient to differentiate the organism from other species. Therefore, it is impossible to obtain reliable species-level identification without resorting to molecular methods (4, 8).

To the best of our knowledge, four cases of human infection with *R. erythropolis* have been previously described, although not all have used molecular confirmation of species identification. Brown and Hendler reported peritonitis caused by *R. erythropolis* during continuous ambulatory peritoneal dialysis (2). Vernazza et al. reported a disseminated skin infection in a human immunodeficiency virus-infected patient, which was successfully treated with long-term amoxicillin (amoxicilline)-clavulanic acid therapy (16). von Below et al. identified *R. erythropolis* in chronic endophthalmitis after lens implantation (17). In this case, they needed to remove the implant and perform anterior vitrectomy plus the coadministration of cefotiam and amikacin. Finally, Osoagbaka from Nigeria described the isolation of *R. erythropolis* from sputum in a patient with pneumonia (15).

Thus, our case represents the first case of bloodstream infection with this organism. The source of infection is not entirely clear. Treatment with the second-generation cephalosporin cefotiam was successful. (This antibiotic is only available in Japan and some countries in Europe.) It is quite possible that the non-*equi Rhodococcus* infections are underdiagnosed due to difficulties in species identification and the delay in the growth of the organism. There is also a risk of the dismissal of these gram-positive bacilli as contaminating "diphtheroids." In our case, identification was confirmed by use of 16S rRNA sequencing, a useful molecular technique for speciation of the genus *Rhodococcus*. As the pool of immunosuppressed patients increases in the future, future cases of infection with non-*equi Rhodococcus* species such as *R. erythropolis* are expected.

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