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CASE REPORT

Bacteremia Caused by *Microbacterium binotii* in a Patient with Sickle Cell Anemia

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Microbacterium **species are non-spore-forming, Gram-positive rods rarely associated with human disease. In this report, we describe the first case of bacteremia caused by *Microbacterium binotii* in a patient with sickle cell anemia. The utility of using 16S rRNA gene sequence analysis along with phenotypic methods for identification is shown.**

CASE REPORT

A 28-year-old male presented to the emergency department (ED) complaining of fever, fatigue, nausea, and nasal congestion. The patient had a history of sickle cell anemia with recurrent pain crises and narcotic dependence. He reported no other localizing symptoms of infections, admitted no exposures to others who were ill, and had no indwelling lines or hardware.

On physical exam, the patient was febrile (38.4°C/101.2°F) with no other signs of sepsis. Hematological testing revealed leukocytosis, microcytic anemia, and mild thrombocytosis, which were relatively unchanged from prior labs. A urinalysis and chest X-ray were unremarkable. Two sets of blood cultures (BD Bactec; Becton, Dickinson and Co., Sparks, MD) were collected peripherally, and the patient was discharged from the ED with a presumed viral illness. Subsequently, both aerobic blood culture bottles grew small, Gram-positive rods after 43 h of incubation. The patient was asked to return to the hospital for a follow-up evaluation and treatment.

Upon readmission, the patient appeared nontoxic with a low-grade fever (38.0°C/100.4°F). Again, no localizing signs of infection were found, and repeat labs revealed near resolution of leukocytosis. Two sets of additional blood cultures were drawn prior to empirical administration of intravenous (i.v.) piperacillin-tazobactam (4,500 mg, once) and vancomycin (15 mg/kg of body weight, then 1,000 mg every 8 h). The patient rapidly defervesced following antibiotic therapy, with one repeat aerobic blood culture bottle growing Gram-positive rods after 67 h of incubation.

All three positive blood culture bottles produced nonhemolytic colonies about 1 mm in size after subculture to 5% sheep blood agar and overnight incubation at 37°C in ambient air. The isolates were catalase positive and oxidase negative and developed a light-yellow pigment that became more intense over time. Using the RapID CB Plus system (Remel, Lenexa, KS), the isolates produced a microcode of 3755513, which yielded a probability of 99.66% for *Oerskovia* spp. and 0.34% for *Microbacterium* spp. Consequently, the isolates were reported as "most closely resembles *Oerskovia* species." Antimicrobial MICs were determined using Etest strips (bioMérieux, Durham, NC) and Mueller-Hinton agar containing 5% sheep blood (Remel, Lenexa, KS). Interpretations were based on the criteria for coryneform bacteria found in the CLSI document M45-A2 (1). The isolate was susceptible to linezolid (0.5 μg/ml) and vancomycin (0.5 μg/ml) and nonsusceptible to daptomycin (8.0 μg/ml) (using Etest strips that contain a standard Ca^{2+} overlay equivalent to 40 μg/ml). Vancomycin was continued for 2 weeks, with repeat blood cultures drawn on day 5 of antibiotic therapy negative for growth. No obvious source for bacteremia was identified, and the patient recovered with no recurrence of the infection to date.

To determine the species identity of the organism, both genotypic and phenotypic methods were utilized. A partial sequence of the 16S rRNA gene (1,404 nucleotides [nt]) was obtained using previously described methods (2), and the sequence was deposited into the GenBank database (accession no. KF534779). The isolate was subsequently identified on the basis of 16S rRNA sequence comparison using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) (3). The analysis showed a 100% sequence similarity to the type strain of *Microbacterium binotii* (DSM 19164; GenBank accession no. EF567306) and 99.86% similarity with the type strain of *Microbacterium neimengense* (DSM 24985; GenBank accession no. JN408293). The Basic Local Alignment Search Tool was used to query the sequence (GenBank accession no. KF534779) against GenBank's curated 16S ribosomal sequence database, and 100% sequence similarity to the type strain of *M. binotii* (DSM 19164; GenBank accession no. NR_044290) was also demonstrated. Since no other genes were described in the literature that allow for differentiation between *M. binotii* and *M. neimengense* (4), phenotypic testing was utilized to further characterize the isolate.

The API 50 CHL medium was used to assess fermentation of various carbohydrates according to the manufacturer's instructions (bioMérieux). Additional phenotypic tests were carried out to determine motility in semisolid medium, gelatin hydrolysis within 7 days, growth at 37°C in tryptic soy broth (TSB) containing 5% and 6.5% NaCl (Remel, Lenexa, KS), indole production (Remel), growth in TSB at 37°C and 42°C, urease production (Remel), and H_2S production (Remel). Results obtained using the clinical isolate are displayed in Table 1 and are compared to previously published results (4–9) obtained using the five most

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TABLE 1 Phenotypic characteristics useful for differentiation of *Microbacterium* species as generated in this study (clinical isolate) and from previously published reports

Characteristic	Result[c]					
	Clinical isolate	*M. binotii* DSM 19164	*M. neimengense* DSM 24985	*M. resistens* DSM 11986	*M. thalassium* DSM 12511	*M. lemovicicum* DSM 25044
16S rRNA % similarity	NA	100	99.86	98.36	98.22	98.07
Pigment	Y	Y	Y	Y	Y	O
Catalase	+	+	−	+	ND	+
L-Arabinose[a]	+	+	−	ND	−	ND
D-Galactose[a]	+	+	−	ND	−	ND
D-Glucose[a]	+	+	+	+	+	−
D-Fructose[a]	+	+	−	ND	ND	ND
D-Mannitol[a]	+	+	−	−	ND	−
Sucrose[a]	+	+	+	+	−	−
Motility	−[b]	−	−	−	−	−
Gelatin hydrolysis	−	−	−	+	V	−
Growth in 5% NaCl	+	+	−	ND	+	−
Growth in 6.5% NaCl	−	−	−	ND	+	−
Growth at 37°C	+	+	+	+	−	+
Growth at 42°C	−	−	+	−	−	−
Urease production	−	−	ND	−	ND	−
H_2S production	−	−	ND	−	−	ND
Reference(s)	PR	5	4	6,7	8	9

[a] Acid from carbohydrate fermentation.
[b] In a semisolid medium consisting of 2.5% heart infusion broth, 0.2% KNO_3, 0.2% K_2HPO_4, 3% gelatin, and 1.5% agar (wt/vol).
[c] NA, not applicable; PR, present report; ND, not done; V, variable; Y, yellow; O, orange; −, negative reaction; +, positive reaction.

closely related species (based on 16S rRNA analysis). The combined results of phenotypic testing and 16S rRNA sequencing identified the clinical isolate as *M. binotii*.

Eighty-four species of *Microbacterium* have been described (List of Prokaryotic Names, www.bacterio.net, accessed 31 October 2013) which are typically associated with environmental sources such as soil, plants, water, industrial air systems, animal products, and insects. Accordingly, *M. binotii* has been previously identified as a component of the cellulolytic gut flora of beetle larvae (10), and *M. binotii* strains isolated from the tissue of the Jatropha plant were used to generate published 16S rRNA gene sequences (GenBank accession numbers JQ659823.1 and JQ659630.1). Of these species, only four have been described as pathogens of humans having been associated with a wide range of clinical specimens. Reported infections caused by *Microbacterium* species include endophthalmitis caused by a *Microbacterium* species that most closely resembled *Microbacterium laevaniformans* (11), catheter-related bacteremias caused by a motile *Microbacterium* sp., (12), isolates that most closely resembled *M. oxydans* and *Microbacterium trichothecenolyticum* (13), *Microbacterium paraoxydans* (14, 15), and an undefined *Microbacterium* species (16), interstitial pulmonary inflammation caused by *M. hydrocarbonoxydans* (17), skin and soft tissue infections caused by an undefined *Microbacterium* species (16), and peritonitis caused by an undefined *Microbacterium* species (18) and *M. paraoxydans* (19). *Microbacterium binotii* has been isolated from human clinical material at least 4 times previously (5, 20). Prior to validation of the species name, Gneiding et al. described isolation of *M. binotii* in Germany from a superficial wound on a 5-year-old male and from a bone infection of a 23-year-old male with no further clinical details published. In that study, 50 strains of yellow-pigmented, Gram-positive rods isolated from human specimens over the course of 5 years were differentiated to the species level with two isolates (4%) characterized as *M. binotii* (20). Moreover, Clermont et al. initially described the species of *M. binotii* using two strains originally isolated from human blood in France (5). No clinical information was provided regarding these isolates.

In the present case, *M. binotii* was repeatedly isolated in pure culture from aerobic blood culture bottles obtained from a febrile patient with sickle cell disease. At the onset of illness, the patient had no indwelling lines or device. Additionally, he had not received any i.v. medications or been hospitalized in the preceding 6 months, and his infection was unlikely to have been nosocomially acquired. While the source of this infection was unknown, recreational i.v. drug use could not be ruled out given the patient's history of opioid dependence. Additionally, the underlying sickle cell disease may have made the patient vulnerable to infection. Impaired splenic function, complement activation defects, zinc deficiencies, and various mechanical and genetic factors have all been linked to increased susceptibility to infection in sickle cell anemics (21). This increased susceptibility has been described for encapsulated and thus poorly opsonized bacteria, as well as *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and other Gram-negative organisms (21). There was no obvious link between *M. binotii* and the organism types that disproportionately affect individuals with sickle cell anemia.

Previously reported cases of bacteremia caused by *Microbacterium* species involved catheter-related infections (12–16). In 5 of the 6 published cases that provided information regarding treatment of patients, catheter removal was necessary for clearance of the infection (13–16). The antibiotics used for treatment included teicoplanin (400 mg/day for 10 days) (15), vancomycin (10 days) with ceftazidime (2 days) (16), ampicillin (100 mg/kg/day) (13, 14), and vancomycin alone (13). In the one case that did not require catheter removal, vancomycin (10 days) and ceftriaxone (1

day) were utilized for successful treatment (16). In the present case, empirical therapy was with intravenous piperacillin-tazobactam (4,500 mg i.v., once) and vancomycin (one dose of 15 mg/kg i.v.). The patient defervesced following empirical therapy, and vancomycin (1,000 mg every 8 h for 2 weeks) was then continued following the results of susceptibility testing (vancomycin MIC of 0.5 μg/ml).

Due to difficulty in identification, *Microbacterium* species have been described as diphtheroids, *Corynebacterium* spp., or CDC group A-4 or A-5 bacteria in previous reports (22). Additionally, *Microbacterium* species have been misidentified using the API Coryne system (bioMérieux) (13, 20, 23) and the RapID CB Plus system (Remel) as noted in this study, rendering these systems of limited utility for identification of yellow-pigmented, Gram-positive rods. The utility of using the 16S rRNA gene target along with phenotypic methods for identification was shown in this study. With the increased use of molecular methods and mass spectrometry in clinical laboratories, *Microbacterium* species isolated from clinical specimens are likely to be correctly identified to the genus or species level more often than in the past. Consequently, human infections linked to *Microbacterium* species, including *M. binotii*, will likely be encountered more frequently.

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