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Catheter-Related *Microbacterium* Bacteremia Identified by 16S rRNA Gene Sequencing

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We describe the application of 16S rRNA gene sequencing in defining two cases of catheter-related *Microbacterium* bacteremia. In the first case, a gram-positive bacillus was isolated from both the blood culture and central catheter tip of a 39-year-old woman with chronic myeloid leukemia. The API Coryne system identified the isolate as 98.9% *Aureobacterium* or *Corynebacterium aquaticum*. In the second case, a gram-positive bacillus was recovered from five sets of blood cultures from both central catheter and percutaneous venipuncture of a 5-year-old girl with acute myeloid leukemia. The isolate was identified by the API Coryne system as 99.7% *Cellulomonas* or *Microbacterium* species. Further phenotypic tests failed to identify the two isolates. 16S rRNA gene sequencing showed 99.4% similarity between the first isolate and *Microbacterium oxydans* and 98.7% similarity between the second isolate and *Microbacterium trichotecenolyticum*, indicating that both isolates were *Microbacterium* species. *Microbacterium* infections are rarely reported in the literature. Although the central venous catheter was previously proposed to be a source of bacteremia, the first case in this report represents the first culture-documented case of catheter-related *Microbacterium* bacteremia.

Identification of rarely encountered bacteria often poses problems in clinical microbiology laboratories. As a result, epidemiology, clinical spectrum, treatment, and outcome have been poorly defined for infections caused by rare bacteria. A comparison of the gene sequences of bacterial species has shown that the 16S rRNA gene is highly conserved within a species and among species of the same genus. Thus, it can be used as the new "gold standard" for the classification and identification of rare bacteria and bacteria with ambiguous biochemical profiles (19, 20). Recently, this technique was used for defining infections caused by some rare bacteria, including a strain of *Mycobacterium neoaurum* isolated from a neutropenic patient with catheter-related bacteremia (31), a strain of *Arcobacter cryaerophilus* from a traffic accident victim with bacteremia (33), and a strain of *Arcobacter butzleri* from a patient with acute bacteremic appendicitis (15). We have also detected the 16S rRNA gene sequence of *Pseudomonas veronii*, which was not known to cause infections in humans, in the involved lymph node tissue of a patient with intestinal inflammatory pseudotumor (5). In this report, we describe the application of 16S rRNA gene sequencing in defining two cases of catheter-related *Microbacterium* bacteremia associated with hematological malignancies.

All clinical data were collected prospectively. Clinical specimens were collected and processed according to standard protocols. All suspect colonies were identified by standard conventional biochemical methods (17) and the API Coryne system (bioMerieux, Lyon, France). The MICs of penicillin and vancomycin against the two isolates were tested by the E test method (AB Biodisk, Solna, Sweden) with Mueller-Hinton blood agar plates.

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Bacterial DNA extraction and PCR amplification and DNA sequencing of the 16S rRNA genes were performed as described previously (25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35). LPW398 (5′-GGCGTGCTTACCACATG-3′) (nucleotides 14 to 30 of the *Microbacterium liquefaciens* 16S rRNA gene; GenBank accession no. X77444) and LPW58 (5′-AGGCCCGGG AACGTATTCAC-3′) (nucleotides 1332 to 1351) (34) were used as the PCR and sequencing primers. The sequences of the PCR products were compared with known 16S rRNA gene sequences in the GenBank database by multiple sequence alignments with the CLUSTAL W program (24), and phylogenetic tree construction was performed by using PileUp and the neighbor-joining method with GrowTree (Genetics Computer Group, Inc.).

Case 1. A 39-year old female with chronic myeloid leukemia was admitted to the hospital for allogeneic bone marrow transplantation in 1998. While receiving conditioning chemotherapy, she developed a fever of 38°C. Physical examination did not reveal any obvious focus of infection. Cultures were performed with blood from both percutaneous venipuncture and a central catheter. The total white cell count was 4.9×10^9/liter, with neutrophils at 2.7×10^9/liter, lymphocytes at 1.4×10^9/liter, and monocytes at 0.6×10^9/liter. The hemoglobin level was 11.6 g/dl, and the platelet count was 177×10^9/liter. The alanine aminotransferase level was 60 U/liter, and the aspartate aminotransferase level was 44 U/liter. The serum urea, creatinine, bilirubin, and alkaline phosphatase levels were within normal ranges.

On day 1 postincubation, blood cultures of samples from both the central catheter and percutaneous venipuncture were positive for a motile gram-positive bacillus. The bacterium grew on blood agar and chocolate agar to yellow-pigmented colonies of 1 mm in diameter after 24 h of incubation at 37°C in ambient air or in 5% CO_2. The MICs of penicillin and vancomycin were 1 and 4 mg/liter, respectively. The isolate was identified by the API Coryne system as 98.9% *Aureobacterium*

TABLE 1. Phenotypic characteristics of the two isolates from this study and available information for *Microbacterium* spp., *L. aquaticum*, and *Cellulomonas* spp.

Phenotypic characteristic	Result[a] for:				
	Isolate 1	Isolate 2	*Microbacterium* spp.[b]	*L. aquaticum*[b]	*Cellulomonas* spp.[b]
Motility	+	−	V	+	V
Production of:					
Catalase	+	+	+	+	+
Oxidase	−	−	−	−	−
Nitrate reduction	−	−	V	V	+
Hydrolysis of:					
Urea	−	−	V	−	−
Esculin	+	+	V	V	+
Gelatin	−	−	+	−	+
Casein	−	−	V	−	−
Acid production from:					
Glucose	−	+	+	+	+
Maltose	−	+	+	V	+
Sucrose	−	+	V	V	+
Lactose	−	−	V	+	NA
Mannitol	−	−	V	+	V

[a] +, positive; −, negative; V, variable; NA, not available.
[b] See reference 17.

(now reclassified as *Microbacterium*) or *Corynebacterium aquaticum* (now reclassified as *Leifsonia aquaticum*). Further phenotypic tests used to distinguish among the blood culture isolate, *Microbacterium* species, and *L. aquaticum* are summarized in Table 1. In view of the contradicting profiles and rarity of the two genera, 16S rRNA gene sequencing was performed.

Intravenous vancomycin was administered, and the central catheter was removed 2 days later. The central catheter tip yielded a gram-positive bacillus with the same biochemical profile and antibiogram as the blood culture isolate. The fever subsided, and the patient underwent marrow infusion 1 week later with engraftment on day 14 posttransplant.

Case 2. A 5-year-old girl with acute myeloid leukemia was admitted to the hospital because of fever for 2 days. Physical examination revealed an oral temperature of 38.7°C and hepatomegaly. Cultures were performed with blood from both percutaneous venipuncture and a central catheter. The total white cell count was 5.9×10^9/liter, with neutrophils at 3.6×10^9/liter, lymphocytes at 1.7×10^9/liter, and monocytes at 0.3×10^9/liter. The hemoglobin level was 13.2 g/dl, and the platelet count was 183×10^9/liter. The results of liver and kidney function tests were within normal limits.

On day 1 postincubation, blood cultures of samples from both the central catheter and percutaneous venipuncture were positive for a nonmotile gram-positive bacillus. The bacterium grew on blood agar and chocolate agar to pale yellow-pigmented colonies of 1 mm in diameter after 24 h of incubation at 37°C in ambient air or in 5% CO_2. The MICs of penicillin and vancomycin were 0.125 and 0.75 mg/liter, respectively. The isolate was identified by the API Coryne system as 99.7% *Cellulomonas* or *Microbacterium* species. Further phenotypic tests used to distinguish among the blood culture isolate, *Microbacterium* species, and *Cellulomonas* species are summarized in Table 1. 16S rRNA gene sequencing was performed because of the ambiguous profiles.

Treatment with intravenous penicillin G was begun. The fever subsided after 5 days of antibiotic therapy, but the bacteremia persisted for 5 more days. Five sets of blood cultures of samples taken at different times over a period of 10 days all yielded the same gram-positive bacillus. Therefore, the central catheter was removed. However, culture of the central catheter tip failed to recover any organism. A transthoracic echocardiogram did not show any vegetation. Treatment with oral penicillin was continued, and the patient remained afebrile. Subsequent blood cultures were negative.

PCR and sequencing of the 16S rRNA genes (1,227 bp) of the blood culture and catheter tip isolates from the first patient revealed that they possessed the same sequence. The analysis revealed 99.4% similarity between the isolate and *Microbacterium oxydans* (GenBank accession no. Y17227), 99.1% similarity between the isolate and *Microbacterium* (*Aureobacterium*) *liquefaciens* (GenBank accession no. X77444), 98.9% similarity between the isolate and *Microbacterium* (*Aureobacterium*) *testaceum* (GenBank accession no. X77445), but only 94.1% similarity between the isolate and *Leifsonia* (*Corynebacterium*) *aquaticum* (GenBank accession no. D45057). PCR and sequencing of the 16S rRNA gene (1,233 bp) of the blood culture isolate from the second patient revealed 98.7% similarity between the isolate and *Microbacterium trichotecenolyticum* (GenBank accession no. Y17240), 97.4% similarity between the isolate and *Microbacterium arabinogalactanolyticum* (GenBank accession no. Y17228), 97.2% similarity between the isolate and *Microbacterium esteraromaticum* (GenBank accession no. Y17231), but only 92.3% similarity between the isolate and *Cellulomonas gelida* (GenBank accession no. AF282627). Based on phylogenetic affiliations, the two isolates both belong to the genus *Microbacterium*, and their 16S rRNA gene sequences share 97.3% similarity (Fig. 1).

The classification of the genus *Microbacterium*, previously designated CDC (Centers for Disease Control and Prevention) coryneform group A-4 and A-5 bacteria, has been confusing. The genus *Microbacterium* was first proposed in 1919, whereas the genus *Aureobacterium* was introduced only relatively recently based on biochemical features and G+C content (7). In 1994, Takeuchi and Yokota demonstrated that species of the two genera are phylogenetically intermixed and closely related in physiological and chemotaxonomic features other than the diamino acid in the cell wall (22). Based on comparative 16S rRNA gene sequence analyses, Takeuchi and Hatano proposed the union of the two genera in a redefined genus *Microbacterium* in 1998 (23).

Although *Microbacterium* infections have been reported with increasing frequency in the last few years, the epidemiology and pathogenic potential of this rarely encountered genus are yet to be described. This shortcoming is related to the difficulty in the identification of closely related coryneform bacterial strains to the genus level in most clinical laboratories. Even when isolated from clinical specimens, they are often disregarded as contaminants because of their relatively low infection rates. Moreover, because of the confusing taxonomic classification, a combined analysis of *Microbacterium* and *Aureobacterium* infections in the literature has not been reported.

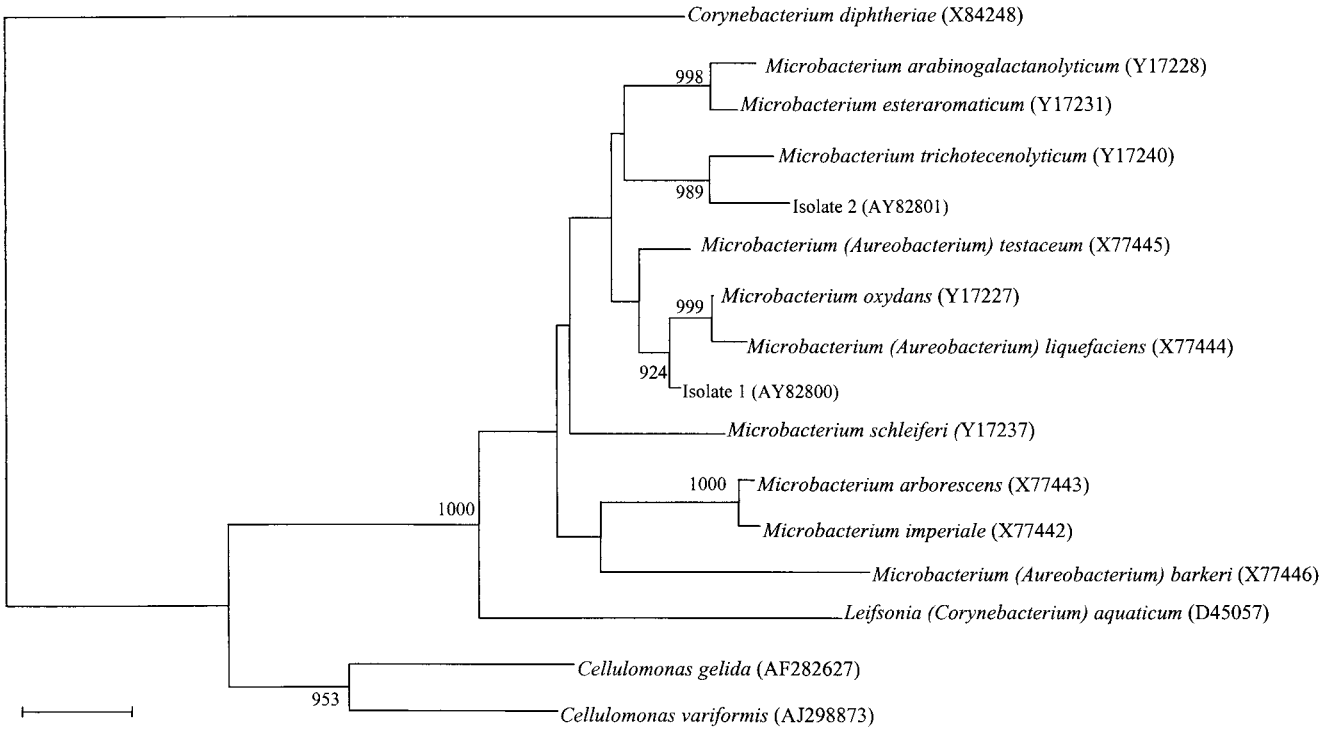

FIG. 1. Phylogenetic tree showing the relationship of the two isolates from our patients to related species. The tree was inferred from 16S rRNA sequence data by the neighbor-joining method and bootstrap values calculated from 1,000 trees. The scale bar indicates the estimated number of substitutions per 100 bases with the Jukes-Cantor correction. Names and accession numbers are given as cited in the GenBank database.

Despite its rarity, *Microbacterium* often causes serious infections. Including the present cases, only 18 cases of *Microbacterium*, *Aureobacterium*, or CDC coryneform group A-4 and A-5 infections with clinical details have been described in the English literature (Table 2) (MEDLINE search, 1966 to 2001) (1, 2, 3, 4, 6, 12, 13, 14, 16, 18, 21); isolates have also been obtained from blood, soft tissues, maxillary sinus, surgical drain, cerebrospinal fluid, peritoneal fluid, and epidural abscess, but the reports had limited clinical details (10). Among the 18 cases, the male/female ratio was 10:8; 5 of the 8 females were victims of a nosocomial outbreak of bacteremia, and the males accounted for more of the sporadic cases (9 of 12). The median age was 49.5 years (range, 5 to 77). Excluding the 3 cases of endophthalmitis, all of the other 15 cases were either primary or secondary bacteremia, among which 5 were catheter-related bacteremia. The presence of a central venous catheter was also associated with the nosocomial outbreak, although the authors believed that the source was probably contaminated multidose vials used to admix infusion components (1). However, in none of the previous reports was the organism isolated from any of the catheters. The first case in our present report represents the first culture-documented case of catheter-related *Microbacterium* bacteremia. Eighty-seven percent of patients (13 of 15) with bacteremia had underlying malignancies, of which hematological malignancy was the most common diagnosis. *Microbacterium* infections are associated with significant mortality and morbidity. Three of the 18 patients died despite antibiotic therapy, and the infections in all 3 patients with endophthalmitis were complicated by impaired vision. While most of the isolates were susceptible in vitro to β-lactams, two were resistant to vancomycin, one recovered from a patient with catheter-related bacteremia and one recovered from a patient who died of bacteremic cellulitis (4, 18). *Microbacterium* species have been isolated from a wide variety of environmental sources, including plants, dairy products, soil, sewage, humidifiers, and air samples in hospitals (11). To better characterize their clinical significance and treatment recommendations, *Microbacterium* species should be considered whenever yellow-pigmented, coryneform bacteria are isolated, especially from hospitalized patients, immunocompromised patients, and patients with central venous catheters.

Most of the isolates in previous reports were identified either by phenotypic tests in reference laboratories or by 16S rRNA gene analysis, suggesting that identification of *Microbacterium* is difficult in most clinical microbiology laboratories. First, the descriptions of many *Microbacterium* species are based on a single strain or very few strains. Therefore, phenotypic species identification is almost impossible, and it is unlikely that there will be a commercial identification system for these bacteria. Second, cell wall peptidoglycan analysis is not available in many clinical microbiology laboratories and does not necessarily lead to reliable species identification (21). As in the present report, *Microbacterium* was misidentified as *C. aquaticum* and *Cellulomonas* species previously, and the identities had to be finally confirmed by 16S rRNA gene analysis (13, 21). While *Cellulomonas* is a genus of coryneform bacteria with poorly defined pathogenicity, *C. aquaticum* was previously a member of another genus phenetically closely related to *Microbacterium* but was recently reclassified under the new

TABLE 2. Clinical reports of patients with *Microbacterium* infections

Reference or source	Patient		Underlying condition	Presentation	Bacteria	Diagnostic system(s)	Antimicrobial therapy	Outcome
	Sex[a]	Age (yr)						
14	M	38	Penetrating metallic foreign body	Endophthalmitis	*Corynebacterium* (CDC group A-4)	CDC	Gentamicin + methicillin	Impaired vision
2	M	77	Abdominal surgery	Hematogenous endophthalmitis	*Corynebacterium* (CDC group A-4)	CDC	Cefotaxime + gentamicin; 5-flucytosine + ketoconazole	Impaired vision
16	M	67	None	Native valve endocarditis	*Corynebacterium* (CDC group A-4)	CDC	Ampicillin	Responded
4	M	11	Acute myeloid leukemia	Catheter-related bacteremia	*Corynebacterium* (CDC group A-5)	CDC	Ticarcillin-clavulanic acid + tobramycin + vancomycin; cefotaxime	Responded
3	M	64	Myelodysplastic syndrome	Catheter-related bacteremia	*Corynebacterium* (CDC group A-4)	Louisiana State Reference Laboratory	Ticarcillin-clavulanic acid + gentamicin + ciprofloxacin; ticarcillin-clavulanic acid + vancomycin; ceftriaxone	Responded
12	M	34	Penetrating metallic foreign body	Endophthalmitis	*Microbacterium* sp.	16S rRNA gene analysis	Cefazolin; tobramycin + gentamicin; gentamicin + cefotiam + clindamycin	Impaired vision
21	M	39	Acute myeloid leukemia	Cellulitis, bacteremia	*Aureobacterium* sp.	API ZYM, cell wall analysis	Vancomycin; amikacin + erythromycin; imipenem + rifampin	Died
1	M	75	NM[a]	Bacteremia	*Aureobacterium* sp.	16S rRNA gene analysis	Imipenem + penicillin + ciprofloxacin	Died
1	F	50	Non-Hodgkin's lymphoma	Bacteremia	*Microbacterium* sp.	CDC	Vancomycin + tobramycin + ceftazidime	Responded
1	F	31	Pheochromocytoma	Bacteremia	*Microbacterium* sp.	CDC	NM	Responded
1	M	64	Pancreatic adenocarcinoma	Bacteremia	*Microbacterium* sp.	CDC	NM	Responded
1	F	49	Non-small-cell lung carcinoma	Central nervous system infection, bacteremia	*Microbacterium* sp.	CDC	Tobramycin + nafcillin	Died
1	F	48	Acute myeloid leukemia	Bacteremia	*Microbacterium* sp.	CDC	NM	Responded
1	F	77	Non-Hodgkin's lymphoma	Bacteremia	*Microbacterium* sp.	CDC	NM	Responded
6	F	73	Chronic renal failure on hemodialysis	Bacteremia	*Aureobacterium* sp.	Cell wall analysis	Teicoplanin + netilmicin; amoxicillin	Responded
13	M	68	Multiple myeloma	Catheter-related bacteremia	*Aureobacterium* sp.	16S rRNA gene analysis	Ceftazidime + gentamicin; imipenem + gentamicin; vancomycin + imipenem + gentamicin	Responded
This study (case 1)	F	38	Chronic myeloid leukemia	Catheter-related bacteremia	*Microbacterium* sp.	16S rRNA gene analysis	Vancomycin	Responded
This study (case 2)	F	5	Acute myeloid leukemia	Catheter-related bacteremia	*Microbacterium* sp.	16S rRNA gene analysis	Penicillin G	Responded

[a] M, male; F, female.
[b] NM, not mentioned.

genus *Leifsonia* as *L. aquaticum* (Fig. 1) (8). Although 16S rRNA gene analysis confirmed that our two isolates were *Microbacterium* species, the 16S rRNA genes in different *Microbacterium* species are highly conserved. As a result, nucleotide sequence analysis and phylogenetic analysis of multiple genes would be required for final species description. In fact, under some rare circumstances, 16S rRNA sequence identity may not be sufficient to guarantee species identity, as in the case of very recently diverged species (9). However, as PCR and sequencing techniques are becoming more readily available in clinical laboratories, 16S rRNA gene analysis is probably the most practical approach to identifying these pathogens at least to the genus level.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences determined here have been deposited in the GenBank database under accession numbers AY82800 and AY82801.

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