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Catheter-Associated *Nocardia higoensis* Bacteremia in a Child with Acute Lymphocytic Leukemia[▽]

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A 23-month-old child with leukemia who was receiving chemotherapy developed fevers. Serial blood cultures from a central venous catheter and a peripheral venous site grew an organism identified by 16S rRNA gene sequencing and phenotypic analysis as *Nocardia higoensis*, an opportunistic organism isolated once previously from a pulmonary infection in Japan.

CASE REPORT

A 23-month-old male child with B-cell acute lymphocytic leukemia (ALL) was being treated with a maintenance chemotherapy regimen that included methotrexate, 6-mercaptopurine, and pegaspargase. He had a Broviac central venous catheter (CVC) that was used for intravenous (i.v.) access with his chemotherapy. Despite his illness, he was an active child without a history of previous or recurrent infections. Two days prior to hospital admission he developed fevers of up to 39.4°C, and blood cultures were obtained (BD Bactec 9240 blood culture instrument and BD Bactec Peds Plus media bottles; Becton Dickinson, Franklin Lakes, NJ) from the central venous line at a referring hospital. He was treated empirically with i.v. ceftriaxone and monitored as an outpatient. The blood cultures became positive at about 48 h of incubation for growth of a Gram-positive bacillus that could not be identified by the referring hospital. He was subsequently admitted to St. Louis Children's Hospital (St. Louis, MO) and was administered broad-spectrum antibiotic therapy with i.v. vancomycin and i.v. cefepime.

The complete blood count (CBC) at the time of admission included a white blood cell count of 4.7×10^9 cells/liter (reference range, $[6.0 \text{ to } 17.0] \times 10^9$ cells/liter), hemoglobin of 10.3 g/dl (reference range, 10.5 to 13.5 g/dl), and a platelet count of 244×10^9 cells/liter (reference range, $[144 \text{ to } 440] \times 10^9$ cells/liter). He was not neutropenic (absolute neutrophil count, 3.9×10^9 cells/liter; reference range for a healthy child, $[1.0 \text{ to } 10.0] \times 10^9$ cells/liter), but he was lymphopenic (absolute lymphocyte count, 0.47×10^9 cells/liter; reference range, $[1.2 \text{ to } 11.5] \times 10^9$ cells/liter). A complete metabolic panel was normal, with no evidence of liver or renal dysfunction. He was febrile with an ill appearance but maintained normal blood pressure and normal mental status, and there was no concern for sepsis. Isolator blood cultures (Wampole) were obtained from his Broviac catheter, plated on sheep blood agar and chocolate agar, and were positive for growth at 24 to 48 h of

incubation. A modified Kinyoun stain (12) identified the presence of a beaded, partially acid-fast organism that initially grew as short rods but upon extended incubation on sheep blood agar (>72 h) exhibited colonies with salmon-red pigment and aerial hyphae and microscopically exhibited longer, filamentous morphology with branching. Based on these characteristics, the organism was presumed to be a rapidly growing *Mycobacterium* or *Nocardia* species. Empirical antibiotic therapy was changed to oral clarithromycin and i.v. imipenem, awaiting further identification and susceptibility testing. A total of seven positive blood cultures were obtained over a period of 6 days, including five Isolator blood cultures from the Broviac catheter and two cultures obtained from a peripheral venous site. The BD Bactec FX blood culture instrument and Bactec Peds Plus and anaerobic lytic/F medium (Becton Dickinson) were used for peripheral blood cultures. The infected Broviac catheter was removed, and peripheral blood cultures remained positive for growth of this organism for 24 h after catheter removal before becoming sterile. The patient became afebrile following removal of the infected catheter and remained afebrile for the remainder of his hospitalization. Magnetic resonance imaging (MRI) of his brain and computed tomography (CT) imaging of his chest, abdomen, and pelvis identified no definitive evidence of disseminated infection.

In order to expedite identification of the organism to optimize antimicrobial therapy, 16S rRNA gene sequencing was performed on the isolate based a laboratory-developed method. The full-length 16S rRNA gene was amplified using the primers 27F (AGAGTTTGATCCTGGCTCAG) and 1391R (GACGGGCGGTGWGTRCA) (3). The product was sequenced bidirectionally, and the resulting 1,250 bp of high-quality sequence were aligned with nucleotide sequences in the Ribosomal Database Project (RDP) and GenBank by using BLAST (http://blast.ncbi.nlm.nih.gov/blast.cgi). The result was 100.0% sequence identity to *Nocardia higoensis* sp. nov., a *Nocardia* isolate reported only once previously, in association with human pulmonary disease in Japan (6). A second closely related *Nocardia* species reported by Kageyama et al. (6), *N. shimofusensis* sp. nov., was found to have 11 bp mismatches with our isolate near the 3′ end of the gene sequence, with overall 99.1% sequence identity. The next nearest match was *Nocardia farcinica*, with 98.7% sequence homology to our iso-

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TABLE 1. *Nocardia higoensis* susceptibility testing[a]

Antibiotic(s)	Kirby-Bauer test		Microdilution test	
	Zone size[b] (mm)	Interpretation	MIC(s) (μg/ml)	Interpretation
Amikacin	40	Susceptible	≤1	Susceptible
Amoxicillin-clavulanate	25	Susceptible	16/8	Intermediate
Ceftriaxone	ND		16	Intermediate
Ciprofloxacin	30	Susceptible	0.25	Susceptible
Clarithromycin	40	Susceptible	2	Susceptible
Doxycycline	ND		1	Susceptible
Ertapenem[c]	30		ND	
Gentamicin	30	Susceptible	ND	
Imipenem	40	Susceptible	4	Susceptible
Kanamycin	34	Susceptible	ND	
Linezolid	ND		≤1	Susceptible
Meropenem[c]	30		ND	
Minocycline	ND		≤1	Susceptible
Moxifloxacin	ND		≤0.25	Susceptible
Tigecycline[c]	40		0.5	
Tobramycin	30	Susceptible	≤1	Susceptible
Trimethoprim-sulfamethoxazole	ND		≤0.25/4.75	Susceptible

[a] Susceptibility testing was performed by the Mycobacterial/Nocardia Research Lab (Director, Richard Wallace), University of Texas Health Center (Tyler, TX). ND, not determined. The methods for Kirby-Bauer disk diffusion susceptibility and broth microdilution MIC determinations have been previously published (16, 17).

[b] The broth MIC is the only susceptibility method approved for *Nocardia* isolates by the Clinical and Laboratory Standards Institute (previously known as the NCCLS) (14).

[c] There are currently no CLSI standard MIC breakpoints for ertapenem, meropenem, or tigecycline for *Nocardia* and, therefore, there are no interpretation criteria available for disk diffusion or microdilution results.

late. Both RDP and GenBank provided identical sequence matches. Biochemical characteristics supported the genetic identification of the isolate as *N. higoensis* and matched previously reported features for that species, including urease activity (which is relatively rare in *Nocardia* spp.), a negative xanthine decomposition reaction, a negative casein reaction, and growth in lysozyme broth (6).

The initial study describing *N. higoensis* and *N. shimofusensis* reported that these two species can be differentiated by the ability to grow at elevated temperatures: *N. shimofusensis* will grow at 37°C but not 45°C, whereas *N. higoensis* will grow at both temperatures (6). Our isolate grew at 37°C but not at 42°C, suggesting this property may be variable between isolates of *N. higoensis*.

Upon extended incubation (>5 days), two colony types became evident on blood and chocolate agar media, with enhanced morphology on chocolate agar. One was nonpigmented with a flat, rhizoid morphology, and the other was a salmon-red, chalky colony with aerial hyphae present. A reddish pigmentation is consistent with the colonial morphology previously reported to occur for *N. higoensis* sp. nov. (6). 16S rRNA gene sequencing was then performed on each colony type; they were found to have identical 16S rRNA gene sequences and were deemed to be colony variants of the same species. A laboratory-developed *Nocardia* genus-specific SYBR green PCR assay targeting the *noc* gene was also positive when performed on this organism. The finding that both colony types exhibited identical melting curves provided additional evidence that these were colony variants of an identical species.

Antibiotic susceptibilities among isolates within the genus *Nocardia* are quite unpredictable, and therefore, identification to the species level may be helpful in determining appropriate empirical antibiotic therapy. As very little is known regarding the typical antimicrobial susceptibility profile for *N. higoensis*, the isolate was sent to the Mycobacterial/Nocardia Research

Laboratory at the University of Texas Health Center for identification and susceptibility testing. The isolate was identified as "*Nocardia* spp." by this laboratory; DNA sequence analysis was not repeated at the Texas lab, and further identification to the species level was not reported. *Nocardia* susceptibility testing was performed using Kirby-Bauer disk diffusion and broth microdilution utilizing previously published methods (16, 17). The susceptibility profiles of the two colony types were identical; the results are summarized in Table 1.

While we awaited susceptibility testing results, empirical antibiotic therapy for the patient was modified to i.v. imipenem plus amikacin. After 7 days, oral sulfadiazine was substituted for the amikacin, and the patient received three additional weeks of imipenem and oral sulfadiazine. At that time, susceptibility test results were available, and based on those results, clarithromycin was substituted for imipenem. The oral regimen of sulfadiazine and clarithromycin was continued for the patient, to complete a 6-month course of treatment. This duration was chosen based on his need for ongoing chemotherapy and theoretical risk of relapse. He has not since had any further episodes of bacteremia or evidence of relapse of his infection.

Nocardia spp. are classified within the family *Nocardiaceae*, suborder *Corynebacteriaceae*, order *Actinomycetales*. *Nocardia* is an aerobic, branching, partially acid-fast bacillus with a high G+C DNA content. *Nocardia* is ubiquitous in the environment as a saprophytic component in soil, sand, fresh and salt water, and decaying organic matter (1). Taxonomic classification of *Nocardia* has historically been confusing and remains controversial today due to a paucity of biochemical markers to distinguish closely related species. The introduction of molecular characterization methods (e.g., 16S rRNA gene sequencing)

has improved the resolution of species-level identification. The genus *Nocardia* currently encompasses more than 80 published species in the NCBI Taxonomy Database as of August 2010. Not all of these species have been rigorously characterized by genetic and/or biochemical profiling, and thus the taxonomy of this genus remains in a state of flux.

Nocardia species can colonize skin and the upper respiratory tract without causing disease. Given the ubiquitous presence of *Nocardia* in the environment, it can be difficult at times to attribute infection to *Nocardia* spp. when these organisms are isolated from some specimen types. However, several species of *Nocardia* are known pathogens of humans and animals. Human infections in which *Nocardia* is a recognized pathogen most commonly occur in immunocompromised hosts and typically manifest as severe pulmonary disease or central nervous system infections (e.g., brain abscess or meningitis) (1, 2, 4, 6, 11, 13). Local infections caused by traumatic inoculation can occur in otherwise-healthy individuals. Deficiencies in cell-mediated immunity, such as in cancer patients receiving steroids and/or chemotherapy, transplant recipients on immunosuppressive regimens, and those with AIDS through infection with human immunodeficiency virus (HIV), place individuals at particular risk of invasive and disseminated nocardiosis (2, 4–6, 9, 11, 13, 15). The one patient previously reported to have disease from which *N. higoensis* was first isolated was a 70-year-old female from Japan on steroid therapy for autoimmune hepatitis, and she developed *Nocardia* pulmonary infection (6).

Bacteremia with *Nocardia* species is relatively uncommon, but when associated with disseminated infection it has a relatively high mortality rate. In a previous review of 36 cases of *Nocardia* bacteremia, Kontoyiannis et al. reported a mortality rate of about 50%; the presence of endovascular foreign bodies (i.e., indwelling catheters) was a major risk factor associated with developing *Nocardia* bacteremia (8). Fortunately, catheter-related infection with *Nocardia* bacteremia occurs at a low frequency; about 7% of all invasive *Nocardia* infections in a study at the University of Texas M.D. Anderson Cancer Center involved central venous catheters (15). CVC-associated bacteremia has been associated with a more benign clinical course. Good outcomes have occurred with early removal of the infected catheter and prompt initiation of antibiotic therapy (5, 15). A case series by Kontoyiannis et al. reported resolution of bacteremia in three patients whose CVCs were discontinued in addition to antibiotic treatment of 2 weeks to 3 months duration. In contrast, a single patient who received 4 weeks of antibiotic therapy but did not have the CVC removed experienced relapse of infection 4 months later with repeat bacteremia in addition to pulmonary disease (7). The absolute requirement to remove a *Nocardia*-infected catheter and the optimal duration of antibiotic therapy have been debated by others and, currently, no clear guidelines have been established (5, 10).

The present case involves *Nocardia higoensis*, a species isolated once previously from pulmonary disease in an immunocompromised female in 2001 in Japan (6). To our knowledge, this is the first documented case of bacteremia with *N. higoensis*. Our patient did have clinical risk factors, including leukemia with chemotherapy and concurrent lymphocytopenia and an indwelling CVC, which may have predisposed him to infection. It is possible he acquired this *Nocardia* infection from environmental contamination of his Broviac catheter with dust or dirt while playing. Removal of the infected catheter resulted in clearance of the bacteremia within 24 h, after several days of serially positive blood cultures. Empirical antibiotic therapy was modified quickly based on information provided by molecular identification with 16S rRNA gene sequencing. This case illustrates the utility of this technique in identification of bacteria, particularly those isolated from immunosuppressed patients, in which the isolation of "unusual" microorganisms (which might not be identifiable by conventional phenotypic methods) is not uncommon.

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