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Herbaspirillum Species Bacteremia in a Pediatric Oncology Patient▽

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***Herbaspirillum* species, an organism commonly found in soil, has only recently been linked to disease in humans. We report *Herbaspirillum* bacteremia in a 2-year-old female patient following a hematopoietic stem cell transplant for relapsed acute lymphoblastic leukemia.**

CASE REPORT

We report the case of a 2-year-old female diagnosed with acute lymphoblastic leukemia (ALL) classified as high-risk due to *MLL* gene rearrangement. A double-lumen Broviac catheter was placed for intravascular access. The child went into clinical remission after standard five-drug induction chemotherapy, but cytogenetic analysis of her bone marrow following induction revealed the presence of persistent leukemic cells, conferring a risk for relapse. As a result, she underwent a matched, unrelated donor hematopoietic stem cell transplant (MUD HSCT). The child's course of treatment was uncomplicated until posttransplant day 162, when she was admitted with a 2-day history of fever (maximum temperature, 103.5°F) and diarrhea. She presented to her local hospital emergency room, where she was found to be tachycardic and febrile. She received two intravenous normal saline fluid boluses and was empirically started on ceftriaxone (50 mg/kg of body weight) prior to transfer to our hospital.

On admission, the patient was hemodynamically stable, with an unremarkable physical examination. Admission laboratory results included a white blood cell (WBC) count of 3,400/mm^3, with 83% neutrophils, 1% bands, 11% lymphocytes, 5% monocytes, an absolute neutrophil count of 2,822, and a hemoglobin level of 9.5 g/dl. She was empirically started on cefepime (50 mg/kg) administered every 8 h. Given the history of diarrhea, stool was sent for enteric culture and *Clostridium difficile* toxin testing. These studies were negative, and the child's diarrhea resolved spontaneously on admission.

Isolator blood cultures were drawn on admission from both the red and the white central venous line lumens, and within 36 h, the quantity (CFU/ml) of a long, thin, oxidase-positive, weakly catalase-positive, Gram-negative bacillus was too numerous to count. At this point, gentamicin (2.5 mg/kg/dose every 8 h) was added to the antibiotic therapy.

Two days later, an Isolator blood culture from the white lumen was still growing the same organism in a quantity too numerous to count, while 39 CFU/ml was recovered from the red lumen. Blood cultures drawn on the third day from both central venous line lumens and a peripheral site were sterile. Antibiotic therapy was changed to meropenem at a dose of 20 mg/kg every 8 h for a total of 7 days following the negative blood culture.

The Vitek 2 Gram-negative identification system identified the organism as *Burkholderia cepacia* complex. The corresponding Kirby-Bauer disk diffusion susceptibility testing showed that the isolate was colistin resistant (consistent with *B. cepacia*) but susceptible to piperacillin-tazobactam, ticarcillin-clavulanic acid, trimethoprim-sulfamethoxazole, meropenem, ceftazidime, gentamicin, amikacin, tobramycin, ciprofloxacin, and cefepime—a susceptibility pattern not consistent with that of *B. cepacia*. The isolate was then inoculated onto (i) the Remel Uni-N/F Tek plate for identification of oxidase-positive, nonfermenting Gram-negative bacilli, (ii) oxidation-fermentation polymyxin B-bacitracin-lactose (OFPBL) agar, and (iii) the BD Phoenix Gram-negative identification panel. The Phoenix panel identified the isolate as *Cupriavidus pauculus* and then on repeat testing as *Ochrobactrum anthropi*. The Uni-N/F Tek plate was positive for xylose, mannitol, urease, and *o*-nitrophenyl-β-D-galactopyranoside (ONPG). It was negative for hydrogen sulfide production, lactose, maltose, acetamide, esculin, DNase, and indole. The isolate was nonpigmented. The combination of the susceptibility pattern and the biochemical reactions was not compatible with any of the automated identifications.

DNA was extracted from a pure culture of the isolate using the BiOstic bacteremia DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA). Amplification of the 16S rRNA gene was performed using the primers 27F (AGA GTT TGA TCC TGG CTC AG) and 1391R (GAC GGG CGG TGW GTR CA), and the product was sequenced (5). The sequence was compared with the GenBank "nr/nt" database and the Ribosomal Database Project and had >99.9% identity (742/743 nucleotides) with *Herbaspirillum* spp. The next-closest match was *Ralstonia* spp., with 97.0% sequence homology (720/743 nucleotides) to our isolate. The biochemical features of our isolate were consistent with what has been previously described for *Herbaspirillum* spp., namely, being oxidase positive and weakly catalase positive, in addition to being ONPG and urease positive (6, 7).

The patient's central venous line was removed prior to discharge and was not replaced, as this was her third line infection in 6 months, and it was almost 180 days after her stem cell infusion. The organisms associated with the previous infections were *Pseudomonas putida*, coagulase-negative *Staphylococcus*

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spp., and *Candida parapsilosis*, all of which were isolated 5 months prior to this admission.

Given the myeloablative effects of the conditioning regimen prior to stem cell infusion, followed by the chronic administration of immunosuppressive therapy to minimize the chance of rejection, recipients of MUD HSCT are immunosuppressed for at least 1 year posttransplantation. In addition to being immunosuppressed, patients may have indwelling catheters in place during this time. While a wide spectrum of organisms, including those causing opportunistic infections, are known to cause disease in this population, *Herbaspirillum* species has not been previously reported.

Herbaspirillum species was first described about 25 years ago (1, 6) as a Gram-negative, rod-shaped member of the *Betaproteobacteria* class. It is a nitrogen-fixing soil and plant bacterium previously not considered a human pathogen (1, 3). Its close phylogenetic and phenotypic resemblance to *Burkholderia cepacia* complex has often resulted in misidentification (2, 4). Although one of the earliest documented human infections with this organism was reported in 2005 from a wound isolate in a 49-year-old homeless man with a history of chronic liver disease (8), the advent of new technology has led to the reclassification as *Herbaspirillum* of previously identified and other, unclassified human isolates obtained as early as 1978 from infections of the ear, eye, knee, urine, oropharnyx, gastrointestinal tract, blood, and respiratory tract (2). Between 2000 and 2007, the *Burkholderia cepacia* Research Laboratory and Repository at the University of Michigan, Ann Arbor, isolated *Herbaspirillum* from 28 sputum cultures and one blood isolate referred from 23 cystic fibrosis (CF) treatment centers in the United States; 19 (68%) of the isolates had been initially identified as *Burkholderia* (4). Recent theories about infections contributing to aortic aneurysms led to the isolation of multiple organisms, including *Herbaspirillum* species, from aneurismal walls. Though this infectious etiology remains unproven, it is a cause for great concern (5). In light of the fact that automated systems will provide an identification for this organism (albeit an incorrect one), it is possible that this organism may be an underrecognized cause of opportunistic infection.

Although stem cell transplant recipients are prone to a wide spectrum of infections, *Herbaspirillum* species has not been previously described as a cause of infection in this patient population. It is uncertain how our patient was exposed to this soil-based organism, but she lives with her parents and grandparents on a large farm, and we speculate that she likely encountered the organism in that environment.

This case demonstrates the utility of sequence-based identification of organisms, especially those that are not in the databases of commercial identification systems or are not readily identifiable using conventional phenotypic methodology.

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