A Novel Rapidly Growing *Mycobacterium* Species Causing an Abdominal Cerebrospinal Fluid Pseudocyst Infection

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Nontuberculous mycobacteria (NTM) are a rare cause of ventriculoperitoneal shunt infections. We describe the isolation and identification of a novel, rapidly growing, nonpigmented NTM from an abdominal cerebrospinal fluid pseudocyst. The patient presented with fevers, nausea, and abdominal pain and clinically improved after shunt removal. NTM identification was performed by amplicon and whole-genome sequencing.

**Keywords.** abdominal; pseudocyst; ventriculoperitoneal; cerebrospinal; *Mycobacterium*.

CASE REPORT

A 21-year-old female with a history of multiple ventriculoperitoneal shunt (VPS) revisions presented with a 14-day history of fevers, headache, vomiting, and abdominal pain. This occurred 4 weeks after her last revision. Her original VPS was placed at age 17, and she subsequently had 5 revisions of the VPS due to mechanical issues. She had neither any prior history of VPS infections nor any infectious history necessitating antimicrobial therapy. Upon presentation, she had a temperature of 38°C, respiratory rate of 16 breaths/minutes, pulse rate of 114 beats/minute, and blood pressure of 120/69 mmHg. Her abdomen was tender but no meningeal signs were elicited. Her white blood cell count (WBC) was 19 500/µL with left shift. Both the erythrocyte sedimentation rate and C-reactive protein were elevated to 72 mm/hours (normal, <10), respectively. A computed tomography (CT) scan of the brain showed hydrocephalus indicating VPS failure. Abdominal CT showed the distal tip of the VPS in a large abdominal cerebrospinal fluid pseudocyst (ACP) (Figure 1A) (All images were reproduced with permission from the patient and the family.) A cerebrospinal fluid (CSF) sample was first obtained from a lumbar puncture that revealed a normal glucose of 78 mg/dL, normal protein of 7 mg/dL, and a WBC count of 1/µL. The CSF Gram stain was negative with no evidence of meningitis. She was still empirically started on vancomycin, piperacillin-tazobactam, and metronidazole, and her VPS was externalized. Her ACP was drained by direct aspiration, and thick purulent material was obtained. Five milliliters of this aspirate was sent to our microbiology laboratory for bacterial, acid-fast bacilli (AFB), and fungal cultures. Repeat CT scan of the abdomen after the procedure showed that 90% of the cyst had been aspirated. Her symptoms resolved within 48 hours after the aspiration. Bacterial and fungal cultures of blood, urine, and CSF were negative. The ACP aspirate culture was positive for a rapidly growing nontuberculous mycobacterium (NTM). She received a total of 2 weeks of empiric intravenous antibiotics. At the end of the 2 weeks, a repeat CT of the abdomen showed near-resolution of her ACP. She then received a new ventriculoatrial shunt and was doing so well postsurgery that she was discharged without any outpatient antibiotic therapy pending the susceptibilities of the NTM. Close follow-up at 1, 2, and 4 weeks revealed no infectious symptoms. Repeat abdominal imaging at 2, 4, and 6 months showed no recurrence of the ACP. She did not require any additional antibiotics on her outpatient follow up even after the availability of the susceptibility data for the NTM.

METHODS

Gram stain of the ACP aspirate showed numerous neutrophils and Gram-positive bacilli. The Kinyoun stained smear was semiquantitated using the Kent/Kubica scale [1] with a grade of 3+ and showed numerous AFB (Figure 1B). The specimen was plated on Middlebrook 7H10 Agar (MA), Trypticase soy agar (TSA), Chocolate agar (CA), Colistin Nalidixic Agar (CNA), and MacConkey Agar (MAC), and cultures were incubated at 37°C in the presence of 10% CO₂. Heavy growth of colonies was noted on MA, TSA, CA, and CNA within 3 days of plating (Figure 1C). The rest of the aerobic, fungal, and anaerobic cultures were negative. The AFB isolate was subjected to matrix-assisted laser desorption ionization time-of- flight mass spectrometry (MALDI-TOF MS). Spectra were analyzed using the Bruker database (Mycobacteria database version 1; Bruker Daltonics) and the Biotyper Software (version 3.0; Bruker Daltonics). A logarithmic score ranging from...
0 to 3 determined the best match based on the mass/charge ratio (m/z) and relative intensity of each ionized protein. Scores were interpreted as ≥2.000 for genus and species level identification, 1.700 to 1.999 for genus-level identification, and <1.7 as unreliable for identification. The MALDI-TOF MS gave a score of 1.84 for *Mycobacterium vaccae*. Due to the low identification score, genetic analysis was performed. Sequencing of the full-length 16S ribosomal ribonucleic acid (rRNA) gene, *hsp65*, and region V of *rpoB* did not identify the isolate when ≥99.99% homology was set for 16S rRNA and ≥97.00% for *hsp65* and *rpoB* [2–4]. These sequences have been deposited in GenBank under accession numbers KU948043 (*hsp65*) and KU948044 (*rpoB*).

Genomic deoxyribonucleic acid was then sequenced on a MiSeq (Illumina, San Diego, CA) resulting in 2 × 250 base pair (bp) reads. Raw read sequences have been deposited to National Center for Biotechnology Information (NCBI)’s Sequence Read Archive under accession numbers SRR2878342 and SRR2914050. Reads were trimmed by FastqMcf [5], and viral contaminants were removed using Bowtie2 [6]. De novo assembly was performed by means of SPAdes 2.5 [7], and all contigs were screened for contamination using KRAKEN 0.10.4 [8]. Contigs were annotated with the NCBI Prokaryotic Genome Annotation Pipeline, and rRNA genes were identified using RNAmmer 1.2 [9]. Acquired antimicrobial resistance elements were detected by SSTAR 1.0 [10]. The genome assembly has been deposited to the NCBI under accession LMVQ00000000.

An identified 16S rRNA gene was aligned using MUSCLE 3.8.31 [11] against 21 other mycobacteria, and a neighbor-joining tree was constructed using the Tamura-Nei substitution model (Figure 2). One hundred bootstrap iterations were performed for generating a consensus tree. Tree-building analyses were performed by MEGA6.06 for Mac OS X [12].

RESULTS

Our genome assembly consists of 592 contigs with a cumulative length of 6 095 505 bp. No acquired resistance determinants were identified, indicating that the observed antibiotic resistance is probably due to intrinsic mechanisms (Table 1). The largest proportion of contigs (n = 409; 69%) mapped to
Mycobacterium vanbaalenii. Mycobacterium vanbaalenii was also the most closely related species to the patient’s isolate by hsp65 (95.61%) and rpoB (95.62%) gene sequencing. The 16S rRNA gene sequences of any validated species were less than 99.99%, similar to the patient’s, with the closest being Mycobacterium flavescens (98.6%), suggesting that our isolate is a distinct Mycobacterium species (Figure 2). Therefore, we designated the isolate as Mycobacterium NAZ190054.

DISCUSSION

The development of ACP is a well known complication of both VPS placement as well as VPS revisions [13]. Ventriculoperitoneal shunt bacterial infections are more common in children compared to adults and are mostly due to Staphylococcus aureus, Staphylococcus epidermidis, and Propionibacterium acnes [13, 14]. Mycobacterial central nervous system (CNS) infections are rare with the most prevalent cause being Mycobacterium tuberculosis [15]. Nontuberculous mycobacteria are ubiquitous in nature. Many are capable of producing a biofilm that makes surgical sites and inserted foreign devices common sources of infections. Mycobacterium avium complex seems to be the most commonly reported NTM causing CNS disease [16]. Based on the literature [17, 18], Mycobacterium fortuitum seems to be the most frequently encountered, rapidly growing NTM causing VPS infection. Mycobacterium abscessus VPS

| Antimicrobial      | MIC (µg/mL) |
|--------------------|-------------|
| Amikacin           | ≤1          |
| Cefoxitin          | 32          |
| Ciprofloxacin      | ≤0.12       |
| Clarithromycin     | >16         |
| Doxycycline        | ≤0.12       |
| Imipenem           | ≤2          |
| Linezolid          | ≤1          |
| Moxifloxacin       | ≤0.25       |
| Tigecycline        | 0.06        |
| Tobramycin         | ≤1          |
| Trimethoprim-sulfamethoxazole | ≤0.25/4.75 |

Abbreviation: MIC, minimum inhibitory concentration; sp nov, new species.

* Antibiotic susceptibility performed using the Sensititre Rapid Growing Mycobacteria Susceptibility Plate (RAPMYCO) Trek Diagnostics Systems, Cleveland, OH.

Figure 2. 16S rRNA gene neighbor-joining tree showing the relationship between Mycobacterium NAZ190054 and other mycobacteria.
infections have also been described [19]. Most of these NTM infections required prolonged appropriate antibiotic therapy with removal of the offending infected VPS [19]. However, our isolate is closely related to *M. vanbaalenii* and *M. flavescent*, both of which are non-pathogenic mycobacteria [20, 21]. The close relationship to these NTM organisms suggests that *M. NAZ190054* has low clinical virulence. This relationship has also been independently evaluated using PathogenFinder [22], giving us a probability of 0.294 for being human pathogenic, which further supports that *M. NAZ190054* is probably an opportunistic and not a true human pathogen.

**CONCLUSIONS**

Multiple factors implicate *M. NAZ190054* as a cause of the ACP infection: the aspirated fluid was purulent with heavy neutrophilic predominance; Gram staining and AFB staining showed a monomicrobial infection; and all other aerobic and anaerobic bacterial and fungal cultures were negative. Our patient has no prior history of infections to suggest an impaired immunity, nor does she have any gastrointestinal disorders to explain the pathogenesis of the NTM. She does, however, have the risk factors for development of an iatrogenic ACP infection due to numerous revisions of her VPS. Even after nontargeted antibiotic therapy, the patient’s symptoms resolved after the drainage of her ACP despite no long-term antibiotic treatment and with no recurrence on follow up. The NTM also caused an ACP infection and VPS malfunction without any CNS involvement. This suggests that *Mycobacterium NAZ190054* has low clinical virulence.

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