Francisella novicida Bacteremia after a Near-Drowning Accident

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We describe a rare case of *Francisella novicida* bacteremia following a near-drowning event in seawater. We highlight the challenges associated with laboratory identification of *F. novicida* and differences in the epidemiology of *F. novicida* and *Francisella tularensis* infections.

CASE REPORT

A healthy 69-year-old male from Pennsylvania suffered severe neck trauma and a near drowning while body surfing along the coast of South Carolina. On admission to the local hospital, he was intubated and was quadriplegic with C1 and C3 vertebral fractures and spinal cord contusion at C3 to C4. Chest computed tomography demonstrated moderate bilateral pulmonary opacities suggesting aspiration. He was treated with ampicillin-sulbactam and dexamethasone. After respiratory cultures yielded *Enterobacter aerogenes* and methicillin-sensitive *Staphylococcus aureus*, antibiotics were changed to ceftriaxone. On hospital day 7, the patient developed a fever of 38.7°C. Blood cultures grew *Staphylococcus epidermidis* on 3 consecutive days, and respiratory culture grew *Serratia marcescens*. Antibiotics were switched to vancomycin and imipenem-cilastatin.

On hospital day 10, the patient was transferred to a tertiary care hospital in Pennsylvania for additional neurosurgical evaluation. Two sets of peripheral blood cultures were obtained the day after transfer. The anaerobic bottle of one set yielded a coagulase-negative *Staphylococcus* after 24 h, and the aerobic bottle from the same set yielded a pleomorphic Gram-negative bacillus after 3 days of incubation. Chest radiographs demonstrated left lower lobe air space opacity on hospital days 10 to 12; bronchoscopy cultures grew *E. aerogenes* and anaerobes. Cefepime, linezolid, and metronidazole were prescribed, and indwelling lines were changed but not cultured; his fevers gradually improved. Unfortunately, the patient remained quadriplegic, ventilator dependent, and unresponsive. Following neurosurgical evaluation and review of advanced directives, the family withdrew life support. The patient died of respiratory failure 13 days after the initial injury; a postmortem exam was not conducted.

The pleomorphic Gram-negative organism recovered from the patient's blood grew on blood and chocolate agar within 1 day of incubation but not on MacConkey agar. The isolate was oxidase negative. A *Haemophilus* sp. was suspected based on Gram stain findings and slow and fastidious growth; however, X and V factors were not necessary for growth. Fatty acid methyl ester analysis by gas chromatography (GC-FAME) (MIDI, Inc., Newark, DE) identified the organism as *Francisella tularensis*. The Pennsylvania State Department of Health was notified, and the isolate was sent to the Pennsylvania State

Public Health Laboratory (PASPHL) on the same day. Due to concern for laboratory transmission of *F. tularensis*, antimicrobial prophylaxis was offered to 16 potentially exposed laboratory staff; 14 opted to take doxycycline, 1 chose ciprofloxacin, and 1 declined prophylaxis.

At PASPHL, the Laboratory Response Network real-time PCR assay was positive for three of three targets suggesting identification as *F. tularensis*. However, direct fluorescent antibody (DFA) testing using fluorescein isothiocyanate (FITC)-labeled anti-whole cell *F. tularensis* was indeterminate, and a commercial slide agglutination test for *F. tularensis* (Becton, Dickinson, Franklin Lakes, NJ) was negative. The isolate was forwarded to the Centers for Disease Control and Prevention (CDC), Division of Vector-Borne Diseases, where a separate real-time PCR assay (24) for *F. tularensis* (previously shown to also detect *Francisella novicida*) was positive for three of three targets, whereas real-time PCR assays for the two subspecies of *F. tularensis* responsible for causing human tularemia, *F. tularensis* subsp. *tularensis* (type A) and *F. tularensis* subsp. *holarctica* (type B) (11), were both negative. A repeated DFA for *F. tularensis* was also negative. DNA sequencing of the 16S rRNA, *pgm*, and *pdpD* genes was performed. Primers and PCR conditions for the amplification and sequencing of the 16S rRNA were as described previously (10). Primers for *pdpD* amplification and sequencing were the same as used for real-time PCR (11). Primers for *pgm* amplification and sequencing were 5′ GADGCTTTWGGTGGBATYRTATTWTC 3′ (forward) and 5′ AAYTTCCAWCCTGTWGGAGT 3′ (reverse). PCR annealing temperatures for *pdpD* and *pgm* were 55°C and 50°C, respectively. All nucleotide positions included in analyses were sequenced at least twice. Neighbor-joining trees were con-
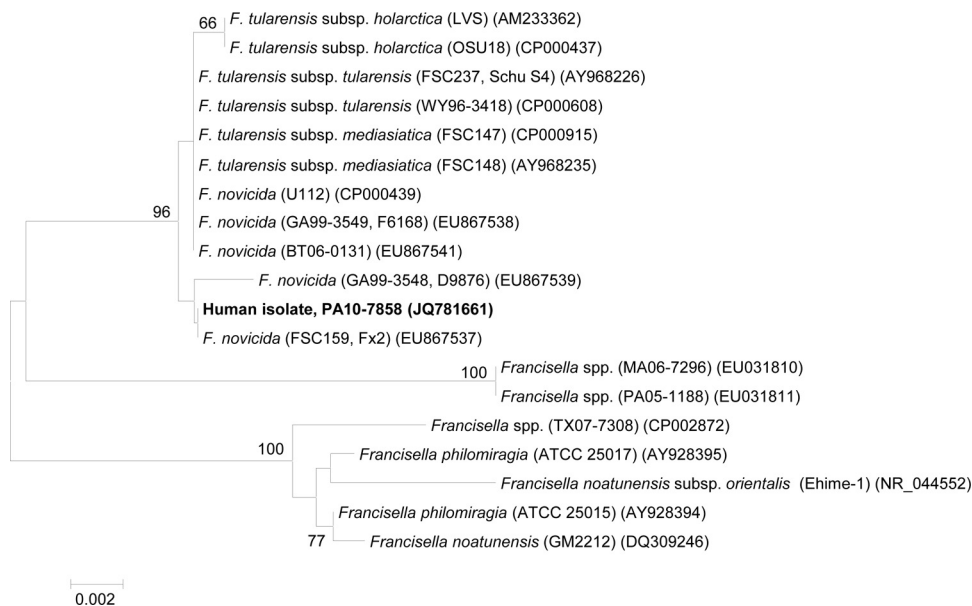
FIG 1 Neighbor-joining tree showing the relationship of the clinical isolate, PA107858, to other members of the *Francisellaceae* based on an 835-bp region of the 16S rRNA gene. Bootstrap support values >60% are indicated. GenBank accession numbers are indicated following the strain designations.

structed using the Jukes-Cantor algorithm in MEGA (version 5.0) with 1,000 bootstrap replicates.

Analysis of the 16S rRNA gene sequences grouped the patient isolate with *F. tularensis* subspp. *tularensis* (type A), *holarctica* (type B), and *mediasiatica* and with *F. novicida* and showed 100% identity to the *F. novicida* strain Fx2 ([Fig. 1]). Sequencing of the *pgm* gene indicated that the clinical isolate clustered with *F. novicida* strains as opposed to *F. tularensis* strains ([Fig. 2]). Similarly, sequencing of a 224-bp region of the *pdpD* gene (GenBank accession no. [JX070223]) indicated 100% identity to other *F. novicida* strains for which *pdpD* sequences were available (U112, GA99-3549, and Fx1). *F. novicida* strains have a 144-bp insertion in the *pdpD* gene compared with *F. tularensis* type A strains, and the *pdpD* gene is entirely absent in *F. tularensis* type B strains ([11], [16]). Taken together, these data identified the isolate as *F. novicida*. Antibiotic susceptibility testing was performed using Clinical and Labora-

tory Standards Institute (CLSI) broth microdilution for *F. tularensis* ([6]); the isolate was susceptible to ciprofloxacin, doxycycline, gentamicin, levofloxacin, streptomycin, and tetracycline.

The *Francisella* species most commonly associated with human infection is *F. tularensis*, the cause of tularemia. This is transmitted to humans via arthropod bites, contact with infected animals, inhalation of contaminated aerosols, or consumption of contaminated freshwater ([18]). Tularemia patients usually present with fever, cutaneous ulcer, and regional lymphadenopathy. Less common syndromes include pneumonia, oculoglandular tularemia, and typhoidal tularemia.

Human infection with *F. novicida* is exceedingly rare, with only six cases published in the English literature ([2], [5], [8], [14]).
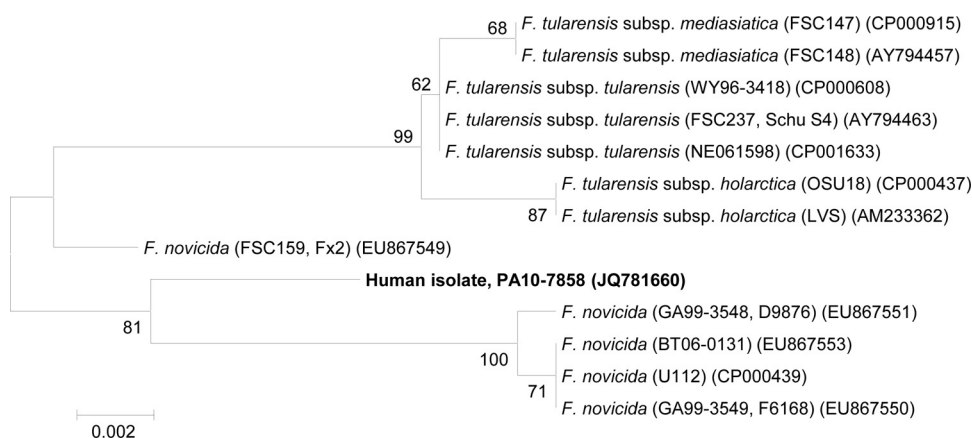

FIG 2 Neighbor-joining tree showing the relationship of the clinical isolate, PA107858, to other members of the *Francisellaceae* members based on a 507-bp region of the *pgm* gene. Bootstrap support values >60% are indicated. GenBank accession numbers are indicated following the strain designations.

Clinical manifestations of reported cases include two otherwise healthy individuals with regional lymphadenopathy without fever and four immunocompromised patients with fever and nonlocalizing symptoms. Sources of human infection with F. novicida remain largely unknown; unlike *F. tularensis*, *F. novicida* has not been shown to be associated with arthropod vectors or animals in nature. *F. novicida* has been detected in brackish and saltwater sources (1, 12, 19); however, this appears to be the first reported case associated with a near-drowning event. In this instance, *F. novicida* was not detected until hospital day 14, 1 day after the patient's death. Potential explanations include faster growth in culture by other organisms, inhibition of growth by other organisms such as *Staphylococcus* spp. (20), intermittent or low levels of *F. novicida* bacteremia, and progressive immunosuppression of the patient due to dexamethasone administration. Although the patient received multiple antibiotics, the patient was treated predominantly with beta-lactams, which have limited to no activity against *Francisella* species (7, 15).

Given the rarity of human illness caused by *F. novicida*, clinical and laboratory identification of cases can be challenging. Despite marked differences in virulence (17), *F. tularensis* and *F. novicida* share an average nucleotide identity of 99.2% over 1.1 Mbp of genome sequence (13). Consequently, *F. novicida* has been considered a subspecies of *F. tularensis*, and controversy exists regarding the nomenclature of *F. novicida* (9). This high level of genetic relatedness limits the ability of many DNA-based assays to accurately differentiate *F. novicida* from *F. tularensis*. Additionally, many bacterial identification systems that use biochemical or fatty acid profiles (including MIDI) do not include *F. novicida* in their databases. These systems may misidentify *F. novicida* and other rare *Francisella* spp. as *F. tularensis*.

In reference laboratories, tests generally available for identifying *F. tularensis* include slide agglutination, DFA staining, PCR, and 16S rRNA sequencing. Polyclonal antibodies to whole killed *F. tularensis*, used in both the direct fluorescence antibody and slide agglutination tests (25), generally react poorly or not at all with *F. novicida* due to differences in the O antigens of the lipopolysaccharides of the two organisms (21). Consequently, *F. novicida* should be suspected when PCR assays, fatty acid analysis, or 16S rRNA gene sequencing is positive for *F. tularensis* but DFA or slide agglutination is equivocal or negative. *F. tularensis* type A- and type B-specific PCR assays can also be used to distinguish *F. novicida* from *F. tularensis*. Further resolution between *F. novicida* and *F. tularensis* can be completed by sequencing genes such as *pdpD*, *sdhA*, *pdpD*, *uup*, *aroA*, *atpA*, *pgm*, *tpiA*, *trpE*, and *parC* (1, 2). Development of PCR assays for *F. tularensis* that do not cross-react with *F. novicida* will be important in the future for eliminating misidentification of *F. novicida* as *F. tularensis*.

Early in the identification process, laboratory manipulation of cultures of suspect *Francisella* species should be minimized and biosafety level 3 precautions should be used due to the risk of laboratory airborne transmission of *F. tularensis* (4, 22). Following potential laboratory exposure to *F. tularensis*, CDC's Select Agent Program should be notified (23) and exposed workers offered antimicrobial prophylaxis or a "fever watch" with immediate treatment if a fever develops (3). If *Francisella* species other than *F. tularensis* are suspected from preliminary testing of isolates, risks and benefits of antibiotic prophylaxis should be considered in the context of the patient history and laboratory data.

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