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Francisella novicida Bacteremia after a Near-Drowning Accident

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ABSTRACT

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 *F. tularensis* infections.

28 **CASE REPORT**

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

39 On hospital day 10, the patient was transferred to a tertiary care hospital in Pennsylvania for
40 additional neurosurgical evaluation. Two sets of peripheral blood cultures were obtained the
41 day after transfer. The anaerobic bottle of one set yielded a coagulase-negative *Staphylococcus*
42 after 24 hours and the aerobic bottle from the same set yielded a pleomorphic Gram-negative
43 bacillus after3 days of incubation. Chest radiographs demonstrated left lower lobe airspace
44 opacity on hospital days 10–12; bronchoscopy cultures grew *E. aerogenes* and anaerobes.
45 Cefepime, linezolid, and metronidazole were prescribed and indwelling lines were changed but
46 not cultured; his fevers gradually improved. Unfortunately, the patient remained quadriplegic,
47 ventilator-dependent and unresponsive. Following neurosurgical evaluation and review of
48 advanced directives, the family withdrew life support. The patient died of respiratory failure 13
49 days after the initial injury; a post-mortem 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. *Haemophilus* was suspected based on Gram-stain findings and slow and fastidious growth; however, X and V factors were not necessary for growth. Using MIDI® fatty acid methyl ester analysis by gas chromatography (GC-FAME), the organism was identified as *F. tularensis*. The Pennsylvania State Department of Health was notified and the isolate sent to the 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, one chose ciprofloxacin and one 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 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 *F. 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, subsp. *tularensis* (type A) and 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'AAYTTCCAWCCTGTWGGA GT 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 constructed using the Jukes-Cantor algorithm in MEGA (v 5.0) with 1000 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 *F. novicida* and showed 100% identity to the *F. novicida* strain, Fx2 (Figure 1). Sequencing of the *pgm* gene indicated the clinical isolate clustered with *F. novicida* strains as opposed to *F. tularensis* strains (Figure 2). Similarly, sequencing of a 224bp region of the *pdpD* gene (GenBank JX070223) indicated 100% identity to other *F. novicida* strains for which *pdpD* sequence was available (U112, GA99-3549, and Fx1). *F. novicida* strains have a 144 bp insertion in the *pdpD* gene when 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 Laboratory 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 which is transmitted to humans via arthropod bites, contact with infected animals, inhalation of contaminated aerosols or consumption of contaminated freshwater (18).

Tularemia usually presents with fever, cutaneous ulcer and regional lymphadenopathy. Less common syndromes include pneumonia, oculoglandular, or typhoidal tularemia.

Human infection with *F. novicida* is exceedingly rare with only six cases published in the English literature (2, 5, 8, 14). Clinical manifestations of reported cases include two otherwise healthy individuals with regional lymphadenopathy without fever and four immunocompromised patients with fever and non-localizing 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 isolated from 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, one 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* sp. (20), intermittent or low levels of *F. novicida* bacteremia, or progressive immunosuppression of the patient due to decadron 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 megabase pairs 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 lipopolysaccaharide of the two organisms (21). Consequently, *F. novicida* should be suspected when PCR assays, fatty acid analysis, or 16S rRNA gene sequencing are positive for *F. tularensis* but DFA and slide agglutination are equivocal or negative. *F. tularensis* type A and type B specific assays can also be used to distinguish *F. novicida* from *F. tularensis*. Further resolution between *F. novicida* and *F. tularensis* can be completed with sequencing of 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 is recommended (3). If *Francisella* species other than *F. tularensis* are suspected with 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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Figure 1. Neighbor-joining tree showing the relationship of the clinical isolate, PA107858, to other *Francisellaceae* members 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 ID.

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

Figure 1

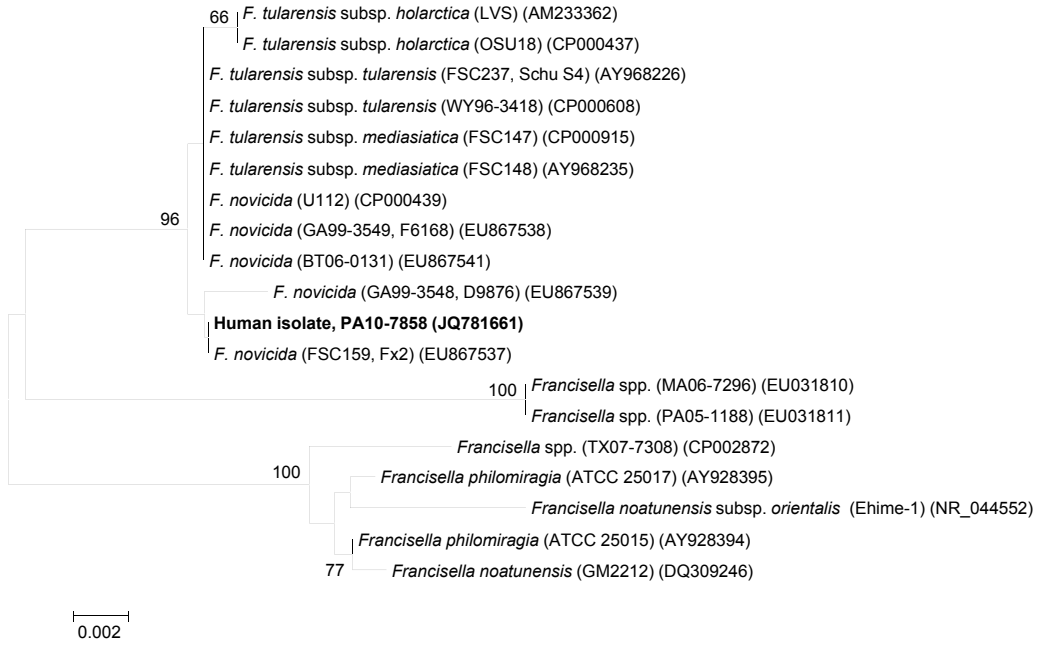

Figure 2