Isolation and Characterization of a Novel *Francisella* sp. from Human Cerebrospinal Fluid and Blood\(^\d\)

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Received 11 April 2008/Accepted 5 May 2008

We describe the isolation of a *Francisella* sp. from normally sterile sites in acutely ill patients in two different states within 2 years. Microbiologic and molecular analyses indicate that this organism represents a novel *Francisella* sp. Clinicians and microbiologists should be aware of this new potential pathogen, as infection may be more common than recognized.

The *Francisellaceae* family is composed of *Francisella novicida*, *Francisella philmoragia*, and *Francisella tularensis* (2, 14). Two subspecies of *F. tularensis*, *F. tularensis* subsp. *tularensis* (type A) and *F. tularensis* subsp. *holarctica* (type B), cause classic tularemia, a zoonotic disease associated with multiple animal hosts, arthropod vectors, and a broad range of clinical manifestations, from skin ulceration to pneumonia (5). *F. philmoragia*, *F. novicida*, and “novicida-like” organisms are associated with saltwater sources and cause human illness primarily in immunocompromised hosts. Clinical symptoms of infection vary from afebrile lymphadenopathy to pneumonia (3, 14, 18, 19). Additional *Francisellaceae*, including *Francisella*-like endosymbionts in several tick species and *Francisella* spp. in soil and water samples, have been identified previously (1, 14), although human illness has not been associated with any of these organisms.

We present a microbiologic and molecular characterization of two isolates obtained from cerebrospinal fluid (CSF) and blood from two unrelated patients in two different states within a 2-year time period. Our results indicate that these isolates represent a new species within the *Francisella* genus and a potential human pathogen.

**Patient 1.** In March 2005, a 15-month-old female with a biopsy-proven hemophagocytic syndrome and a diagnosis of juvenile rheumatoid arthritis was admitted to a Philadelphia hospital with a fever, a rash, and vomiting. Routine cultures were negative, and upon the recognition of hydrocephalus, a CSF shunt was inserted. Culture of the CSF obtained at that time yielded a fastidious gram-negative coccobacillus identified as a *Francisella* sp. by 16S rRNA gene sequencing and cellular fatty acid analysis MIDI Labs, Inc. (Newark, DE). The patient died of multiple-organ failure 3 weeks after admission. She had lived in a suburban setting and had traveled to coastal North Carolina; the only family pet was a hamster.

**Patient 2.** An 85-year-old male with end-stage renal disease who was on hemodialysis was admitted to a Boston hospital in August 2006 with a 5-day history of fever and coughing and with a left-upper-lobe infiltrate. The patient was treated empirically with intravenous vancomycin and cefepime and defervesced the next day. Cultures of blood samples drawn at admission yielded a possible *Haemophilus* sp. or atypical *Francisella* sp. Vancomycin was discontinued, and a 6-day course of cefepime was completed during the hospital stay. The patient was discharged with a 10-day course of oral levoflaxacin and recovered fully. He lived in Boston and denied any history of travel, pet ownership, or exposure to ticks or untreated water.

Tests to evaluate the growth and biochemical properties of members of the genus *Francisella* were performed with the two isolates, designated PA051188 and MA067296. Both isolates grew on cysteine heart agar with 9% charcoalized sheep blood and chocolate agar at 35 and 25°C but showed no growth on sheep blood or MacConkey agar at either temperature after 7 days (Table 1). PA051188 was urease negative, whereas MA067296 had a weak positive urease reaction. Both isolates fermented glycerol and were oxidase positive, but all other tests (those for indole, catalase, sucrose, H₂S, and growth in 6.5% NaCl) were negative (Table 1). Thus, the growth requirements and biochemical reactivity profiles of PA051188 and MA067296 were distinct from those described previously for *Francisellaceae* (Table 1). When PA051188 and MA067296 were tested for growth on *Haemophilus* ID QUAD plates (BD Diagnostic Systems, Franklin Lakes, NJ), both isolates grew in the presence of V factor.

Antibody-based testing demonstrated that PA051188 and MA067296 are antigenically similar to one another yet distinct from *F. tularensis*, *F. novicida*, and *F. philmoragia*. Anti-F.
*F. tularensis* antibodies did not recognize either PA051188 or MA067296 when the isolates were tested by slide agglutination or fluorescein isothiocyanate-based direct fluorescent-antibody assays (Table 1) (16). In contrast, antisera against PA051188 raised in rabbits agglutinated both isolates but not *F. tularensis, F. novicida*, or *F. philomiragia* (Table 1). Convalescent-phase serum from patient 1 was not available; however, serum obtained from the second patient 5 months after the illness.

### Table 1. Biochemical reactivity, slide agglutination, and PCR profiles of PA051188 and MA067296 and other Francisella spp.

<table>
<thead>
<tr>
<th>Organism(s)</th>
<th>Result of biochemical reactivity assay for:</th>
<th>Slide agglutination assay result for:</th>
<th>F. tularensis multtarget TaqMan assay result for:</th>
<th>F. tularensis LRN assay result for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indole</td>
<td>Urease</td>
<td>Oxidase</td>
<td>Catalase</td>
</tr>
<tr>
<td><em>F. tularensis</em> subsp. tularensis (type A)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>F. tularensis</em> subsp. holarctica (type B)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>F. novicida</em></td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>–</td>
</tr>
<tr>
<td><em>F. philomiragia</em></td>
<td>+</td>
<td>–</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>PA051188</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>MA067296</td>
<td>–</td>
<td>W/–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>
| a Results for *F. tularensis* subsp. *tularensis* (type A), *F. tularensis* subsp. *holarctica* (type B), *F. novicida* (including novicida-like organisms), and *F. philomiragia* are from the literature or were determined at the CDC (3, 7, 14, 17). Results for PA051188 and MA067296 were compiled from data from the CDC, state health laboratories, and clinical laboratories. +, positive reaction or growth; –, negative reaction or no growth; ND, not determined; W, weak reaction; V, results varied among strains.
| b Studies reported in the literature evaluated growth in 6% NaCl; however, PA051188 and MA067296 were evaluated with 6.5% NaCl.
| c A negative PCR result was determined by the absence of detectable fluorescence at 45 cycles or by the methods described for the LRN protocol.

**FIG. 1.** Neighbor-joining tree showing the relationships of PA051188 and MA067296 to other Francisellaceae members based on partial sequencing of the 16S rRNA genes. Nodes with bootstrap support (1,000 replications) above 70% are indicated. The percent identity of each sequence to that of *F. tularensis* Schu S4 reference strain (AY968226) and included those with accession numbers AY968225, AJ698866, AY968232, AJ698867, AY968237, AY243028, AY968238, AY968235, AY968236, AY968239, AY243027, AY496933, AB001522, AY805304, AM403242, AY968291, AY968296, AY968289, AY968290, AY968283, AY968301, AY968302, AY968284, AY968285, AY968300, AY968303, and AY968304.
weakly agglutinated the MA067296 isolate obtained from this patient but did not agglutinate PA051188, *F. tularensis*, *F. novicida*, or *F. philomiragia*.

Reactivities in three *F. tularensis* TaqMan PCR assays were assessed using reaction conditions as described previously (9, 11, 17). PA051188 and MA067296 were positive by one of three targets in both the *F. tularensis* TaqMan assay (the ISFtu2 target) and the *F. tularensis* Laboratory Response Network (LRN) assay (the FT2 target). Both of these assays require three positive targets for a sample to be identified as *F. tularensis* (Table 1). Neither isolate was positive with either the *F. tularensis* subsp. *tularensis* (type A) or *F. tularensis* subsp. *holarctica* (type B) TaqMan assays. Thus, these isolates displayed PCR profiles distinct from those of *F. tularensis* strains.

To assess the genetic relationships of PA051188 and MA067296 to other *Francisellaceae*, sequencing of the 16S rRNA and *sdhA* genes was performed using the ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA) (1). PCR conditions for the amplification of the 16S rRNA and *sdhA* genes were as described previously (1, 8, 10). Eubacterial primers 63F, 1387R, 519F, and 537R were used for sequencing *sdhA* sequencing was described previously (1). Sequence analysis and tree development were performed using the Lasergene version 7.0 software programs (DNASTAR, Madison, WI).

The 16S rRNA and *sdhA* gene sequences of PA051188 and MA067296 showed 100% identity, indicating that these two isolates represent the same species. When compared to those of other *Francisellaceae*, the 16S rRNA sequences of PA051188 and MA067296 showed 97% identity to the sequences of *F. tularensis* subsp. *tularensis* (type A), *F. tularensis* subsp. *holarctica* (type B), *F. novicida*, and *F. philomiragia* and were most similar (98%) to sequences cloned from soil samples from Houston (027 clones) (Fig. 1) (1). The *sdhA* sequences showed only 83% identity to sequences from *F. philomiragia*, 84% identity to those from *F. tularensis* and *F. novicida*, 84 to 85% identity to those from uncultured clones from soil from Houston, and 86% identity to those from *novicida*-like organisms (Fig. 2).

Susceptibilities to seven antimicrobials were evaluated using Etest strips (AB Biodisk, Piscataway, NJ) (16). MICs of chloramphenicol, ciprofloxacin, erythromycin, gentamicin, streptomycin, and tetracycline fell within the susceptible range. Additionally, both isolates were screened for growth in the presence of cefepime, an expanded-spectrum cephalosporin used in the treatment of patient 2. Whereas the MICs of cefepime for both PA051188 and an *F. tularensis* control strain indicated resistance (MIC > 256), in vitro activity of cefepime against MA067296 was apparent (MIC = 8). This finding may explain the clinical response of patient 2 after treatment with cefepime, despite the fact that β-lactam antibiotics are generally considered to be ineffective for the treatment of *Francisella* infections (4, 6).

Pulsed-field gel electrophoresis (PFGE) patterns were generated using the restriction enzyme PmeI (15) and compared using BioNumerics software version 3.5 (Applied Maths, Sint-Martens-Latem, Belgium). Although the PFGE patterns for PA051188 and MA067296 were similar, a two-band difference was observed. In addition, the PFGE patterns for these two isolates were distinct from those for *F. tularensis*, *F. novicida*, and *F. philomiragia* strains.

Here, we describe two clinical isolates of *Francisella* that are distinct from *F. tularensis*, *F. novicida*, and *F. philomiragia*, as determined by multiple laboratory methods. Although these isolates share the fastidious growth characteristics and some biochemical properties of *F. tularensis* subsp. *tularensis* (type...
A) and *F. tularensis* subsp. *holarctica* (type B), all *F. tularensis*-specific tests (slide agglutination, direct fluorescent-antibody, and PCR analyses) were negative. Sequence analyses placed these isolates in the *Francisellaceae* family, with only 97% and 83 to 86% identity to their nearest neighbors by 16S rRNA and *sdhA* analysis, respectively. Together, these results suggest that PA051188 and MA067296 represent a new *Francisella* species.

PA051188 and MA067296 appear to be very similar to each other, as assessed by phenotypic, genetic, and antigenic analyses. Differences in PFGE patterns and cefepime susceptibilities, however, suggest that PA051188 and MA067296 are not clonal or associated with a point source event, such as the contamination of hospital equipment or supplies during manufacturing. The mechanism by which these organisms were acquired by either patient is not clear.

Several factors suggest that this *Francisella* sp. contributed to each patient’s illness at the time of organism recovery. Both isolates were recovered in pure culture from normally sterile sites in acutely ill, immunocompromised patients. The potential of this organism to be a human pathogen is further supported by the fact that at least one patient (patient 2) mounted a detectable immune response to the organism. Though the symptoms of the two patients were different, they are consistent with those of infections caused by other *Francisella* spp.

Further analysis is required to understand the pathogenicity and prevalence of this organism. Given that these two isolates were recovered from patients within a 2-year time period, infection with this organism may be more prevalent than recognized. The testing methods described here can be used by clinical laboratories in the future to help identify and classify this organism. Public health laboratories should be on the alert for organisms that display only a positive FT2 PCR in the *F. tularensis* LRN assay. Clinical laboratories may want to consider this *Francisella* sp. if a V-factor-dependent *Haemophilus* sp. is identified on *Haemophilus* ID QUAD plates.

**Nucleotide sequence accession numbers.** The 16S rRNA and *sdhA* gene sequences from MA067296 and PA051188 were deposited in GenBank with the following accession numbers: for 16S rRNA, EU031810 (MA067296) and EU031811 (PA051188), and for *sdhA*, EU031812 (MA067296) and EU031813 (PA051188).

We thank the Pennsylvania Department of Health, the Massachusetts Department of Public Health, Cheryl Gauthier, Nordin Zeidner, Michael Bowen, and Sherif Zaki for their assistance with these investigations.

**REFERENCES**


