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Case report

Francisella novicida infection in a patient with pulmonary infection and pancreatitis in Italy

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ABSTRACT

Tularemia is a rare but potentially life threatening zoonotic disease caused by *Francisella tularensis*. *F. novicida*, previously considered a subspecies of *F. tularensis*, is currently considered a separate species. Human infections related to *F. novicida* are exceedingly rare but can cause morbidity and mortality in debilitated or immunocompromised individuals.A 42-year-old male presented at the hospital with vomiting, dehydration, constipation and pain in the right iliac fossa. He was first diagnosed with pancreatitis and admitted for further analysis. Chest computerized tomography scan showed the presence of parenchymal consolidation in the left upper and lower lobes of the lung with pleural effusion. Blood cultures isolated a Gram-negative coccobacillus, that was at first identified by MALDI-TOF as *Francisella tularensis.* Serological analysis for the detection of total antibodies against *F. tularensis* and Real-Time PCR targeting the gene coding for 23 kDa, resulting negative. Subsequently, PCR targeting *helicases* and *tul4* genes, and the Regions of Difference *RD1* and *RD6* were performed allowing the identification of *F. novicida*. The isolate was further genetically characterized by whole genome sequencing (WGS).This is the first reported case of human infection caused by *F. novicida* in Italy.Given the rarity of human cases and the lack of specific symptoms, this pathogen is difficult to identify and the diagnosis can be extremely challenging. In this case report, despite the lack of amplification of the gene encoding for 23 kDa protein, the identification of *Francisella* species was achieved with the amplification of different genes and characterized by WGS.

Introduction

Francisella tularensis is a highly infectious Gram- negative coccobacillus, obligately aerobic and non-spore forming [1]. It is the causative agent of the zoonotic disease known as tularemia.

Other *Francisella* species, such as *F. novicida*, *F. philomiragia* and *F. hispaniensis*, were also reported as etiological agents of disease in immunocompromised individuals. *Francisella novicida* was considered the fourth subspecies of *F. tularensis* but its classification at the species level was extensively debated. Currently it is regarded as a separate species [2].

F. novicida is weakly virulent in humans and the infection is usually associated with debilitated or immunocompromised patients. Moreover, *F. novicida* has not been associated with animals or arthropod vectors but it is limited to salt water, brackish water and soil [3]. Although only few human cases were reported in literature [4–11] the investigations revealed that water-borne infection was the primary mode of transmission.

Here we described the first reported case of human infection by *F. novicida* in Italy.

Case report

A 42-year-old previously healthy Senegalese male, accessed to the

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Emergency Department of Rimini Hospital on 24th September, 2019 with a 3 days history of vomiting, dehydration, constipation and pain in the right iliac fossa. His past medical history did not report previous pathologies, he was employed as milker in a farm in a rural area and he referred a two-months trip to Senegal until the 27th August. Initial vital signs included normal temperature, blood pressure between 75 and 120 mmHg, respiratory rate of 20 breaths per minute and oxygen saturation of 99 %.

Laboratory investigation at admission revealed a clinical picture of metabolic ketoacidosis with acute kidney failure (creatinine level of 1.77 mg/dL), diabetic decompensation (glucose level of 612 mg/dL) and elevation of lipases (to 1895 U/L), so he was admitted for further evaluation with a presumed diagnosis of pancreatitis. Initial chest x-ray and abdomen echo were normal and blood samples were collected for cultures.

On the first day of hospitalization supportive therapy was administrated including an empirical antibiotic with ceftriaxone, intravenous fluids and insulin.

On hospital day 3 the patient developed higher fever to 39.5 °C with an increase of C-reactive protein values of 395.4 mg/L. The patient got negative test results to pneumococcal and *Legionella* urinary antigens, and the peripheral blood smear resulted negative for malaria parasites. A computed tomography scan (CT) of the chest revealed the presence of extensive parenchymal consolidation in the left upper and lower lobes of the lung with pleural effusion in the latter (Fig. 1). Serological examinations for *Salmonella typhi, S. paratyphi* and *Brucella* spp. were performed, resulting negative.

In the next days the patient clinically improved very quickly with the adjustment of the therapy with piperacillin/tazobactam and the acute renal failure was normalized. The blood cultures turned positive for a single type of colony corresponding to a Gram-negative coccobacillus, which was identified from a representative number of colonies (n = 5) by MALDI-TOF mass spectrometry as *Francisella tularensis*. No other bacterial colonies were detected. Antimicrobial susceptibility was assessed on the *Francisella* isolate according to the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution method for *F. tularensis*, using Vitek® system (bioMérieux) (Table 1). Based on the results, the antibiotic treatment of the patient was switched to levofloxacin.

Serological analysis to detect total antibodies against *F. tularensis* was performed using a single-assay chemiluminescence test (CHT) (Tularaemia VIRCLIA® Vircell Microbiologists), but no positive reaction was observed. Generally, the serological assay is not able to detect antibodies of other species, such as *F. novicida* [12].

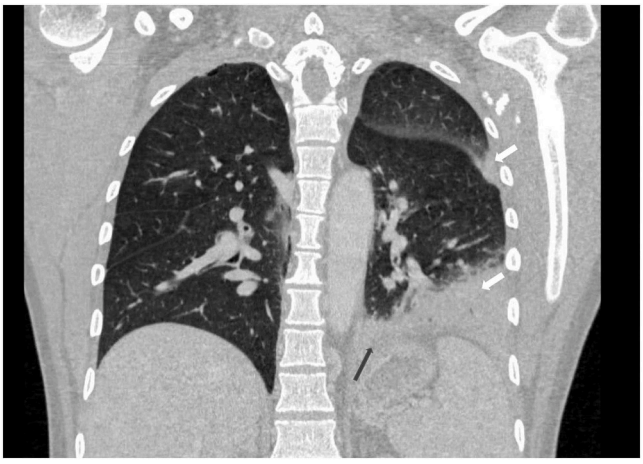

Fig. 1. Chest computed tomography (CT) imaging showing parenchymal consolidation in the left upper and lower lobes of the lung (white arrows) with associated pleural effusion (black arrow).

Table 1
Selected antibiotics tested against *F. novicida* strain isolated from blood samples.

Antimicrobial	MIC[a] value (μg/ml)	Interpretation
Tetracycline	1	S[b]
Chloramphenicol	1.5	S
Levofloxacin	0.047	S
Doxycycline	1	S
Streptomycin	1	S
Gentamicin	0.064	S

[a] MIC, minimum inhibitory concentration, [b] S= susceptible

On day 10 of hospitalization, the patient was discharged with a diagnosis of pneumonia and the recommendation to rest.

The isolate was sent to the National Reference Center for Tularemia in Pavia to confirm and characterize the *Francisella* isolate.

Real-Time PCR targeting the gene coding for 23 kDa of the *Francisella tularensis* group [13], resulting negative. Subsequently, end-point PCR targeting *helicases* and *tul4* genes, and the Regions of Difference *RD1* and *RD6* were performed [14], allowing the identification of *F. novicida*.

The genome of *F. novicida* was sequenced, libraries were prepared by Illumina® DNA Prep kit, (M) Tagmentation (Illumina) and sequenced (2 ×250 bp) on MiSeq platform (Illumina). The genome was assembled with Spades (Galaxy Version 3.15.4 +galaxy1) and Unicycler (Galaxy Version 0.4.8.0), and annotated both with Artemis and Prokka v 1.14.5 [15].

The presence of *F. novicida* was confirmed both with taxonomic classification of the reads using Kraken2 and with phylogenetic analysis inferred on *pgm* genes (Fig. 2) (PhyML with 100 bootstrap pseudo-replicates, MEGA v.7). Further bioinformatic analysis, performed to understand the mis-assignation of the subspecies using PCR-based approaches, indicated a high variability in the gene region encoding for 23 kDa showing numerous point mutations (nr.13) in the primers and probe pairing region. A deeper genome analysis based on the comparison with databases of antibiotic resistance (ResFinder 4.1) [16] and virulence (Virulence Factor Database, VFDB) [17] genes, revealed: i) the absence of known genetic determinants responsible for antibiotic resistance, indeed *Francisella* is intrinsically resistant to many classes of antibiotics due to the nature of its LPS and the many enzymes and membrane channels it produces [18]; ii) the presence of virulence factors typically of *Francisella* like genes involved in secretion systems (T6SS genes), capsule formation (e.g. *cap* genes), nutritional virulence (e.g. biotin metabolism genes), iron uptake (e.g. *Francisella* siderophore locus), intracellular survival (e.g. acp genes) and adherence and invasion (type IV pile genes); iii) a virulence pattern similar to those of reference *F. novicida* Fx1 strain (NC_017450) with the presence of some *wbt* genes (B,C,L,M) and the absence of others (namely *wbt* D, E,F,G,H,N, O,P,Q) and with the lack of the *FmvB* gene, involved in magnesium uptake, like the reference *F. novicida* U112 strain (NC_008601). Interestingly, *F. novicida* isolate of this study possess a *cas9* gene, that is part of the CRISPR-Cas systems, a prokaryotic adaptive immune system that facilitate protection of bacteria and archaea against infection by external mobile genetic elements [19], and that is used for mammalian genome editing [20]. Raw reads of the newly sequenced *Francisella novicida* was deposited at EBI under Project number PRJEB61682.

Discussion

Francisella tularensis is the *Francisella* species most commonly associated with human infection. Human may be contaminated through arthropod bites, after direct contact with infected animals, inhalation of contaminated aerosols or ingestion of contaminated water or food [1]. Clinical manifestations vary according to the mode of infection, from an asymptomatic infection to severe diseases. Early symptoms usually correspond to influenza-like illness, the disease may then evolve to one of the six classical clinical presentations: glandular, ulceroglandular, oropharyngeal, oculoglandular, pneumonic and typhoidal [1]. Human

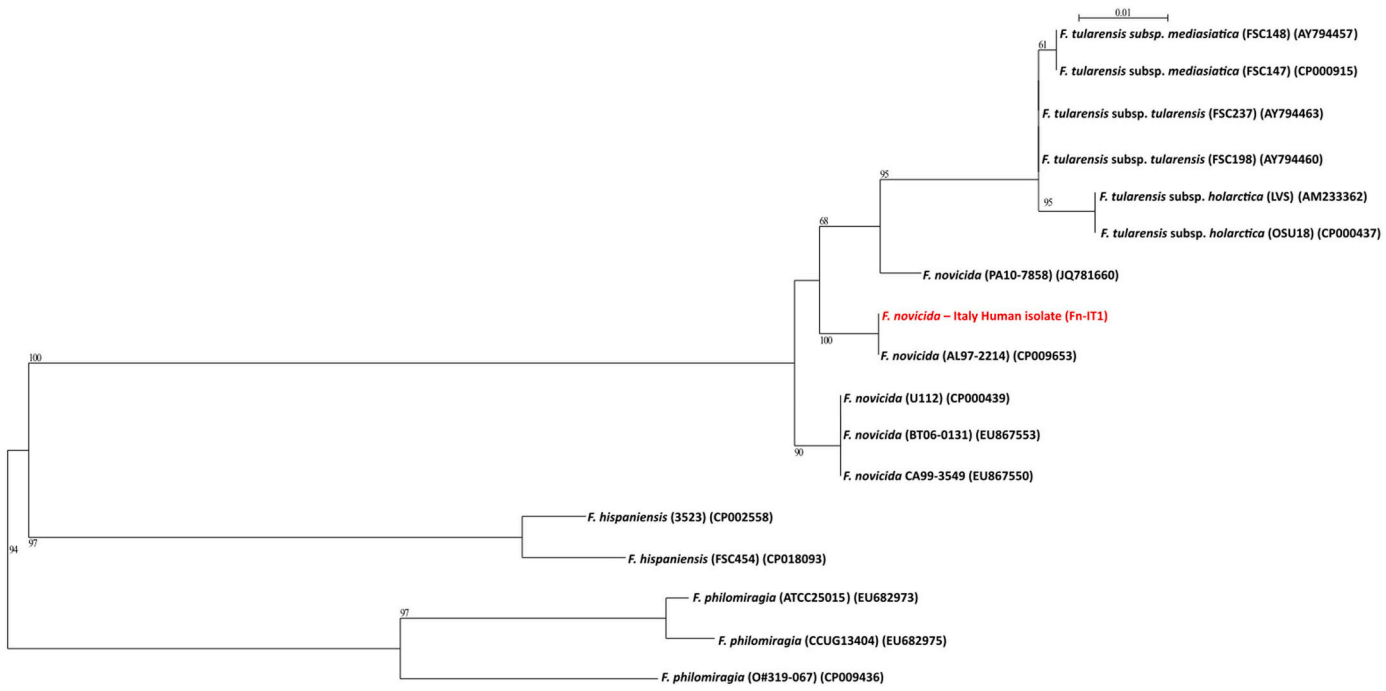

Fig. 2. Phylogenetic analysis based on the *pgm* genes of *F. novicida* isolated in this study (shown in red) compared with selected sequences available in NCBI GenBank database. Nodal support bootstrap values > 60 % are indicated only.

infections from the other pathogenic species, *F. hispaniensis, F. novicida,* and *F. philomiragia* were rarely reported and frequently linked to waterborne transmission.

Only sporadic human infection caused by *F. novicida* were described in Australia, Taiwan, Thailand and USA [4–11]. Clinical presentation that was generally non-specific, strictly dependent upon immune system status of the host and the presence of underlying diseases, included fever, regional lymphadenopathy, bacteraemia, pneumonia, skin and soft-tissue infection.

Therefore, given the rarity of human cases and the lack of specific symptoms, the diagnosis can be extremely challenging and should be based on the combination of laboratory tests based mainly on bacterial culture, PCR and sequencing.

In this case report, since the lack of amplification of the gene encoding for 23 kDa protein specific of the *F. tularensis* group, the identification of *Francisella* species was achieved with the amplification of different genes suitable for the discrimination of *Francisella* species and characterized by WGS to typify the isolate in terms of genetic content.

As regards the source of infection, remained unknown. However, the incubation period of the pathogen, ranging from 1 to 14 days, oriented towards the hypothesis of an autochthonous infection. *F. novicida* is known to be associated with aquatic habitats and closely linked to waterborne transmission. An outbreak of *F. novicida* bacteraemia occurred in a correctional facility was associated with the consumption of ice from contaminated ice machine and scoop [5]. Several studies reported the isolation of *F. novicida* from environmental water samples and the infection through contact with contaminated salt water was implicated as a cause of *F. novicida* bacteremia. Brett et al. [4] reported a case of a patient that likely became infected after a near-drowning accident in the seawater.

The probable source of the pathogen is unknown in this case. So far, the pathogen was never identified in natural infected animals, excluding the animals of the farm as vehicles of infections. However, livestock are a prevalent source of water illness, especially when animal waste is not safely managed and personal hygiene measures are not observed. Additionally, although transmission via arthropods was never associated with this specific *Francisella* species, it could be considered given the nature of farm work, even though the patient did not report a tick bite.

Therefore, this study demonstrates the need of accurate epidemiological investigations following suspected *Francisella* infections, in order to acquire proper data on the factors involved in the transmission of the pathogen in Italy.

Ethics approval

Not applicable.

Funding

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Consent

Consent Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

CRediT authorship contribution statement

Vittorio Sambri: Investigation. **Carlo Biagetti:** Investigation. **Paola Prati:** Conceptualization. **Olivieri Emanuela:** Writing – original draft, Conceptualization. **Sara Rigamonti:** Writing – original draft, Conceptualization. **Erika Scaltriti:** Methodology. **Nadia Vicari:** Methodology, Conceptualization. **Moira Bazzucchi:** Methodology. **Claudio Marco Lodola:** Methodology. **Arianna Torri:** Investigation.

Declaration of Competing Interest

None.

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