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Emergence of a Microbe in a New Geographic Area

Genetically divergent *Francisella philomiragia* associated with septic arthritis, Montevideo, Uruguay

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ABSTRACT

Here we report a case of septic arthritis associated with a genetically divergent *Francisella philomiragia* strain in a patient with chronic rheumatoid arthritis and Adult-onset Still's disease (AOSD) in Maldonado, Uruguay. In this study mass spectrometry together with whole-genome sequencing using Oxford Nanopore technology allowed for the correct identification of the etiologic agent.

1. Case presentation

A fifty-two-year-old man with Adult-onset Still's disease (AOSD), chronic rheumatoid arthritis and bilateral hip and knee arthroplasty (under treatment with 15 mg/mL prednisone daily and anakinra) suffered a short contusive wound in the middle third of the left leg while doing sports in oceanic waters, which was self-treated with topical antibiotics. Ten days after, on January 31st, 2022, the patient made an emergency consultation due to left knee pain of 48 hours evolution with increased usual pain, accompanied by inflammatory elements such as heat and redness. The patient denied having a fever. Initial physical examination revealed joint effusion and pain on passive mobilization. Knee X-ray showed no bone lesions. Evaluated by a traumatologist, diagnostic and therapeutic arthrocentesis was performed, draining 50 cc of haemopurulent fluid. Of the tests requested, the following protrudes: C-reactive protein of 277 mg/L, erythrocyte sedimentation rate of 43 mm/h and leukocytosis of 8000/mm^3, predominance of neutrophils and ferritin of 919 ng/ml. Surgical cleaning was performed on February 1st, 2022. Immediately after, the patient started antibiotic treatment with vancomycin and ceftazidime. Given that the patient was coming from another country, after the emergency consultation, he returned to his country to continue the assistance.

The haemopurulent surgical sample was processed for further microbiological investigation. Direct gram staining showed abundant polymorphonuclear leukocytes (PMNs), with small and thin gram-negative bacilli that were very difficult to observe. The sample was plated in Tryptic Soy sheep blood agar, MacConkey agar and chocolate agar in aerobiosis and CO_2 at 35 °C; and in Thioglycolate broth. In addition, an aliquot of the sample was cultured in a BactAlert® FAN aerobic blood culture bottle, which was positive within the first 24 hours. Bacterial growth was documented between 48 and 72 hours in aerobic blood agar and in CO_2 at 35 °C. Colonies were bright and convex, of around 2 mm. Gram staining of bacterial colonies showed small and pleomorphic short gram-negative coccobacilli. The catalase and oxidase tests were positive, the indole test negative and the nitrocefin disc beta-lactamase test was positive.

Antibiotic susceptibility (Table 1) and bacterial identification were first assessed using an automated VITEK 2 equipment, which initially failed to provide identification. In a second instance it identified the isolate as *Sphingomonas paucimobilis/Francisella* and in a third instance *S. paucimobilis* with an identification accuracy of 94 %. As a preliminary result, the isolate was reported as *S. paucimobilis* but given the inconsistency of the results, it was decided to submit it for mass spectrometry and whole-genome sequencing analyses.

The strain was sent to the Analytical Biochemistry and Proteomics Unit and the Microbial Genomics Laboratory of the Institut Pasteur

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Table 1
Sensititre® microdilution and Disc Diffusion antibiotic sensitivity results on MH agar (BD).

Antimicrobial compound	MIC µg/ml	Disc Diffusion (mm)	Interpretation
Ampicillin	>32	6	Resistant
Cephalothin	≥64	6	Resistant
Ceftazidime	≤1	29	Susceptible
Cefotaxime	≤1	27	Susceptible
Imipenem	≤1	30	Susceptible
Meropenem	≤1	32	Susceptible
Gentamicin	≤4	31	Susceptible
Amikacin	≤4	36	Susceptible
Colistin	≥8		Resistant
Ciprofloxacin	≤0.06	40	Susceptible
Trimethoprim/ sulfamethoxazole	>2/38	6	Resistant

Montevideo for downstream identification. The Analytical Biochemistry and Proteomics Unit (Institut Pasteur de Montevideo and IIBCE) analyzed the strain using electrospray ionization (ESI) mass spectrometry. Proteomics analysis led to the identification of 2114 peptides assigned to 416 proteins of *Francisella philomiragia* (Supplementary Table 1). In parallel, the Microbial Genomics Laboratory using Oxford Nanopore Technologies, obtained the whole genome sequence as previously described [1]. Based on these analyses, the strain was reclassified as *F. philomiragia.* Further genomic characterization revealed that this strain, named *F. philomiragia* 265916 represents a phylogenetically divergent strain from other *F. philomiragia* sequenced so far (Fig. 1A). Also, Average Nucleotide Identity (ANI) further confirmed that *F. philomiragia* 265916 is a genetically distinct strain which departs from the average genomic distance shared between previously sequenced genomes of this species (Fig. 1B).

The genetic divergence of this *F. philomiragia* strain was further explored relative to other complete genomes available using the BV - BRC database (bv-brc.org). The genetic profile for antibiotic resistance genes, LPS modifying enzymes and virulence factors was explored against seven genomes of representative *Francisella* sp. and against sixty seven *F. philomiragia* strains (Supplementary Tables 1 and 2). The representative strains included three different species: *Francisella* sp. TX007308, *F. guangzhouensis* and *F. noatunensis* subsp. *orientalis* isolated from Italy, USA, Costa Rica and China. The sixty seven *F. philomiragia* genomes selected were predominantly isolated from the USA (50 genomes), Thailand (5), Japan (2), China (1) and Others (3). CARD database found a class A beta lactamase (EC. 3.5.2.6) in the isolated strain *F. philomiragia* from Montevideo, similar to that found in other *F. philomiragia* strains. Using BV-BRC annotation, 27 additional features involved in antibiotic resistance were identified in this strain. These features included: OxyR, glycerophosphoryl diester phosphodiesterase (EC 3.1.4.46) and alanine racemase (EC 5.1.1.1). These features were found in a similar profile in other *F. philomiragia* strains but were less represented in other representative *Francisella* sp. genomes. The chemical structure of *Francisella* sp. LPS is unique as compared to other Gram negative bacteria. In fact, the absence of phosphate moieties in its structure has been associated with low endotoxicity 3,6. The unique structural modification in *Francisella* sp. LPS has been linked to colistin resistance 7. In the isolated strain the observed colistin resistant phenotype is accompanied by a genome with higher copy number of LPS modifying enzymes. Among these enzymes, the strain harbors four copies of Lipid A biosynthesis lauroyl acyltransferase gene (EC 2.3.1.241), two copies of undecaprenyl phosphate galactosephosphotransferase (EC 2.7.8.6) and Lipid A disaccharide synthase (EC 2.4.1.182).

The genetic potential of virulence factors was also considered for the *F. philomiragia* strain isolated in this report showing a unique profile of three virulent genes. BV-BRC database annotation found three copies of phosphoribosylaminoaimidazole-succinocarboxamide synthase (EC 6.3.2.6), three copies of an uncharacterized MFS-type transporter and a hypothetical protein with no annotation. Additionally, two copies of an alanine transaminase (EC 2.6.1.2), chromosome (plasmid) partitioning protein ParB and biotin synthase (EC 2.8.1.6). These genes were found in other *Francisella* sp. genomes but not in this high copy number. Taken together, the genomic content of this divergent lineage contains a different genetic profile in virulence factors as well as LPS modifying enzymes as compared to other representative *Francisella* species and *F.*

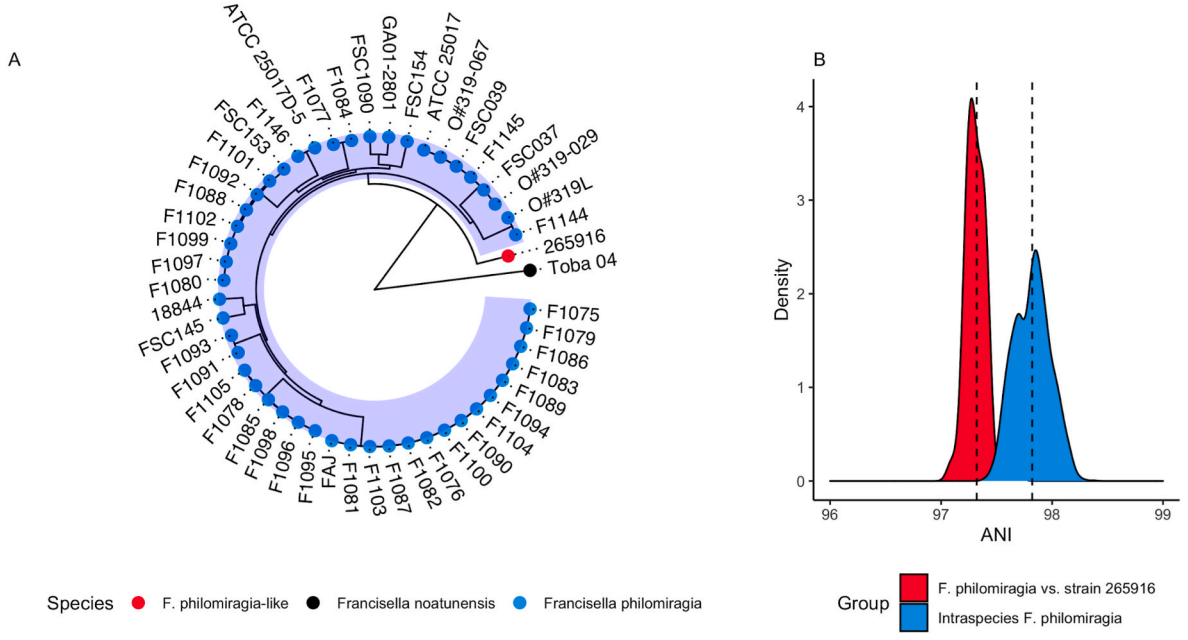

Fig. 1. Comparative genomic analysis of *P. philomiragia* 265916. A) Phylogenetic analysis based on a concatenated alignment of 128,154 SNPs identified in 48 publicly available *F. philomiragia* genomes and *F. philomiragia* 265916 strain. The genome of *F. noatunensis* Toba 04 was used as an outgroup. B) Average Nucleotide Index (ANI) comparing *F. philomiragia* 265916 with other publicly available *F. philomiragia* genomes. Intraspecies ANI values for previously available genomes are clearly higher than when comparing these genomes with *F. philomiragia* 265916.

philomiragia genomes.

2. Discussion

F. philomiragia is categorized as a rare opportunistic human pathogen. These bacteria are found in soil and aerosol samples but are most commonly known in the aquatic environment for the infection of various species of fish causing francisellosis disease. In humans, *F. philomiragia* has been associated largely with infections in near-drowning victims and systemic infections in immunocompromised patients [2]. There are many aspects of this bacteria that makes it fastidious and hard to treat in the clinic. In fact, most *F. philomiragia* strains sequenced thus far present at least one copy of the class A beta lactamase (EC 3.5.2.6) and show phenotypic resistance to Trimethoprim/sulfamethoxazole [3–6]. Firstly, the lipopolysaccharide (LPS) layer of *Francisella* cell wall is unusual as compared to other Gram-negative bacteria and makes this bacterium naturally resistant to many last-line antibiotics like colistin [7] (Table 1). Secondly, treatment can be further complicated due to the facultative intracellular lifestyle of the bacterium, which can infect and replicate within macrophages and other cells in the host [4]. Lastly, *F. philomiragia* has been known to survive for several weeks in the environment due in part to the ability to form biofilms.

Adding to the treatment complications, regular bacterial identification procedures can be misleading for this bacterium. Previous misidentification with other species such as *F. tularensis, S. paucimobilis*, *Aggregatibacter* and *Pseudomonas* had been reported. Of note, inaccurate taxonomic classification with the close relative *F. tularensis*, currently classified by the US Centers for Disease Control and Prevention (CDC) as category A bioterrorism agent, can lead to a false biosafety alarm [8]. Increased awareness and a timely identification of *F. philomiragia* allows for treatment with improved prognosis and lower mortality among infected patients.

In the reported case, a patient with Still's disease (AOSD) and chronic rheumatoid arthritis with hip and bilateral knee arthroplasty presented septic arthritis associated with *F. philomiragia*. Proper identification was only possible using nanoLC-MS/MS and subsequent WGS analysis of the isolate. WGS of the strain allowed the genomic comparison with other *F. philomiragia* strains and related *Francisella* species. The genomic analysis revealed that the strain can be classified within the *F. philomiragia* species, but revealed it belongs to a diverging lineage from those reported thus far. Further genomic comparison of the strain revealed a unique profile of virulent genes and LPS modifying enzymes which can provide important information for new cases. Importantly, this genome-driven classification could be performed in a three days lapse, which allows for an improved management of a putative high risk bacterial pathogen. In this case report, bacteria was treated with extreme caution and immediately discarded in rigid and soft red containers depending on the type and subsequently sent to incineration. Together, this report highlights the importance of incorporating WGS in clinical microbiology to improve pathogen identification and subsequent patient treatment and management in a timely manner.

Data availability

Genomic data generated in this study is available under GenBank accession number PRJNA934943. Proteomic data generated in this study is available via ProteomeXchange with identifier PXD039709.

CRediT authorship contribution statement

Nadia Riera: Conceptualization, Formal analysis, Writing – original draft. **Cecilia Salazar:** Conceptualization, Formal analysis, Writing – review & editing. **Bernardina Rivera:** Conceptualization, Formal analysis, Methodology, Writing – review & editing. **Antonio Galiana:** Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Rosario Durán:** Conceptualization, Methodology, Resources, Writing – review & editing. **María Magdalena Portela:** Methodology, Writing – review & editing. **Virginia Antelo:** Methodology, Writing – review & editing. **Beatriz Pi:** Methodology, Writing – review & editing. **Óscar González:** Methodology, Writing – review & editing. **Gregorio Iraola:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2023.101210.

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