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Characterization of a novel *Francisella* sp. from blood and urine of a patient with an unusual clinical presentation

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Aims: Characterization of a novel *Francisella* sp. (FnSp1) isolated from human blood and urine.

Methodos: Two isolates of a gram negative coccobacile with the same biochemical characteristics were obtained from blood and urine of a 43 year old male who was admitted to the hospital with the initial diagnosis of acute obstructive pyelonephritis. Empiric treatment was started with Aztreonam and Clavulanic acid/Amoxicillin. Five days later, the patient's condition worsened with severe sepsis. After a preliminary identification of the isolate (FNSp-1) as *Francisella* spp., the treatment was changed to Tobramycin and the patient recovered in five days. Subsequent analyses were performed by agglutination with specific anti-*F. tularensis tularensis* (Ftt) immune serum (BBL), serologic response of the patient against its own isolate (western blotting and microagglutination), protein and biochemical profiles, antimicrobial susceptibilities, PFGE, reactivity to different PCR protocols, including SSTR9 and *pdpD*, sequencing of 16S rRNA, *lpnA* and VNTR M19, and finally Multilocus Sequence Analysis (MLSA) using *tpiA*, *dnaA*, *mutS*, *prfB* and *putA*.

Results: Microagglutination was negative for Ftt (strain B38), *Ft holarctica* (strain LVS) and *Ft novicida* (strain UTAH-112). Western blotting with soluble fractions of the 3 subspecies and the patient's isolate disclosed a protein band specific of FnSp1. Biochemical profile was closer to Ftn than to Ftt or Fth and protein profile was unique for FnSp1. The PFGE pattern with *PmeI* was distinct from those for Ftt, Fth, Ftn and *F. phylomiragia*. The size of the SSTR9 was similar to that of *Ftn* Fx1, and no amplification was obtained for *pdpD* as happens with *Fth* LVS. The sequence of 16S rRNA showed a high homology to Ftt (99.9%), the sequence of *lpnA* was close to that of *Ftn*-like strain 3523 and that of M19 showed similarities to both Ftt and Ftn. Interestingly, the MLSA pattern showed a high divergence of FnSp1 to any of the described subspecies.

Conclusions: Microbiological and molecular analyses of this strain indicate that it can represent a novel *Francisella* sp. Since the recognition of this agent can be misled by routine identification methods, attention should be paid to identify additional isolates to assess the actual role of this microorganism in human disease. Due to the scarce number of strains and of clinical cases caused by this agent, this report provides additional data that could be of clinical and microbiological relevance.