

Case Report

Catheter-associated bloodstream infection caused by *Leifsonia aquatica* in a haemodialysis patient: a case report

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Leifsonia aquatica is an aquatic coryneform rod that is capable of forming biofilms in environmental water sources. It has rarely been associated with human infections and its pathogenicity and clinical significance are uncertain. We describe a case of catheter-related bloodstream infection in a haemodialysis patient. The isolate grew on conventional media as a yellow-pigmented colony, but identification required molecular methods. Although the strain displayed reduced sensitivity to vancomycin, the clinical outcome was favourable after catheter removal and intravenous treatment with this antibiotic. Our report gives further evidence of the capability of this aquatic bacterium to cause human infection.

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Introduction

Leifsonia aquatica is a non-spore-forming Gram-positive rod that is typically found in environmental water habitats (Funke *et al.*, 1997; Suzuki *et al.*, 1999; Evtushenko *et al.*, 2000). It was first described by Leifson (1962) and has been isolated from samples of distilled water, from municipal water supplies and water from private wells (Sulpher *et al.*, 2008; Beckwith *et al.*, 1986). Although the species was classified under the genus *Corynebacterium* for almost 40 years, it has recently been reclassified because of its chemotaxonomic and genetic differences to corynebacteria (Suzuki *et al.*, 1999; Funke & Bernard, 2007).

Macroscopically, *L. aquatica* grows in opaque and butyrous colonies, which often produce a yellow pigment after extended incubation. The organism is motile by peritrichous flagella and is catalase and oxidase-positive. Microscopically, it can vary from rod-shaped to coccoid forms; the latter are predominantly found in older colonies. *L. aquatica* produces acid from glucose, sucrose, fructose, arabinose, galactose

and mannose by oxidative metabolism. It is urease and aesculin negative, but produces hydrogen sulfide and has DNase activity (Winn *et al.*, 2006). *L. aquatica* does not hydrolyse gelatin or casein, which helps differentiate it from species of the genus *Microbacterium* (Winn *et al.*, 2006). In the API Coryne system, *L. aquatica* ferments carbohydrates, but since some species of *Microbacterium* produce acid oxidatively, phenotypic and biochemical characteristics alone do not provide enough information for definitive identification and other tests such as PCR and sequencing methods are required (Winn *et al.*, 2006; Almuzara *et al.*, 2006; Dempsey *et al.*, 2007).

Information about the pathogenicity and clinical relevance of *L. aquatica* is scarce and only few reports regarding the isolation of this species in human clinical samples have been published (Meyer & Reboli, 2010). Older clinical data involving *Corynebacterium aquaticum* have to be interpreted with caution due to inconsistencies in identification and confusion with other genera, especially species of the genus *Microbacterium* formerly assigned to the genus *Aureobacterium* (Takeuchi & Hatano, 1998; Meyer & Reboli, 2010; Grove *et al.*, 1999). Here we present a case of catheter-associated bloodstream infection in a haemodialysis patient caused by *L. aquatica*.

Abbreviations: AVF, arteriovenous fistula; CVC, central venous catheter; UPGMA, unweighted pair group method with arithmetic mean.

The GenBank/EMBL/DDBJ accession number for the partial 16S rRNA gene sequence of *Leifsonia aquatica* strain LEAQ01 is JQ253798.

Case report

A 79-year-old man with end-stage renal disease secondary to nephrosclerosis and hypertension was referred to the Hospital Militar in Santiago, Chile, because of a history of fever for approximately 2 months, mostly occurring at the end of the dialysis sessions. For the 10 months prior to admission he had been in a thrice-weekly ambulatory dialysis program, which used a tunnelled central venous catheter (CVC) (Ash Split Cath, MedComp) in his right innominate vein. Later on, a right arteriovenous fistula (AVF) was placed and used for dialysis for 3 months, until it became dysfunctional. Therefore, 3 months prior to admission and 1 month before the presentation of fever, haemodialysis was again performed through the tunnelled CVC. On examination, the patient presented in a stable clinical condition, was without respiratory distress or fever, and was haemodynamically stable (heart rate 85 bpm, arterial blood pressure 125/72 mmHg). He was pale and had a discrete haematoma on the AVF area. Signs of endovascular infection and cardiac murmurs were absent. The insertion site of the CVC was unremarkable. Despite the history of prolonged fever, the patient was not hospitalized, but aerobic blood cultures were taken by peripheral venipuncture and from the CVC. Laboratory examinations revealed a total white cell count of 6000 mm^{-3} , a haemoglobin value of 11.7 mg dl^{-1} and a platelet count of $169\,000 \text{ mm}^{-3}$. The sedimentation rate was 75 mm h^{-1} , C-reactive protein was 37.9 mg dl^{-1} (reference value, $1\text{--}10 \text{ mg dl}^{-1}$), alkaline phosphatase (ALP) was 240 U l^{-1} , gamma-glutamyl transpeptidase (GGT) was 82 U l^{-1} , albumin was 4.3 g dl^{-1} and blood urea nitrogen (BUN) was 33 mg dl^{-1} . After 29.9 h of incubation, blood cultures (TREK Diagnostic Systems) taken from the CVC were positive, showing coryneform Gram-positive rods. Contamination was suspected and a new series of blood cultures was drawn during the next dialysis session 48 h later. The new blood cultures, taken from two CVC lumina and one peripheral venepuncture, tested positive after 16.5, 19.2, and 40.9 h of incubation, respectively, fulfilling the criterion for catheter-related bloodstream infection (Blot *et al.*, 1999). The bacterium was motile and Gram staining of blood culture bottles revealed curved Gram-positive rods, which grew in entangled masses (Fig. 1). Subcultures on 5% sheep's blood agar revealed tiny, non-haemolytic, white colonies after overnight incubation at 35°C in 5% CO_2 . There was no growth on MacConkey agar. Colonies were catalase- and oxidase-positive. After 4 days of incubation, colonies were yellow-pigmented and had a mucous-like appearance (Fig. 2). The API Coryne system (bioMérieux) identified all the isolates as *Microbacterium/Leifsonia aquatica* (98.7% probability, profile 2470004). The isolate produced acid from one isolate of the first series of blood cultures, which was further analysed at the Laboratory of Molecular Microbiology of the Pontifical Catholic University in Santiago, Chile. DNA was extracted using the QIAmp DNA Mini kit (Qiagen). A 1380 bp fragment of the 16S rRNA gene was PCR amplified using the universal primers

P10F (5'-AGTTTGATCCTGGCTCAG-3') and P13R (5'-AGGCCCGGAACGTATTAC-3') (Vanechoutte *et al.*, 2000; Wilck *et al.*, 2001). The PCR product was purified with a QIAquick purification kit (Qiagen) and purified DNA was directly sequenced by using a Big Dye Terminator v3.1 Cycle Sequencing kit in an ABI 310 sequencer (Applied Biosystems) using three primers: P13R, P534R (5'-ATTACCGCGGCTGCTGG-3') and PSRV3-1F (5'-CGGTCCAGACTCCTACGGG-3') (Vanechoutte *et al.*, 2000; Wilck *et al.*, 2001; Qin & Urdahl, 2001). The sequence obtained was deposited in GenBank (accession no. JQ253798) and compared with sequences in the GenBank NCBI database (<http://www.ncbi.nlm.nih.gov>) using BLAST software, Bioinformatic Bacterial Identification (BIBI) software (Devulder *et al.*, 2003; Cole *et al.*, 2009) and the Ribosomal Database Project (RDP; <http://rdp.cme.msu.edu/>). Bioinformatic identification of our clinical isolate against public databases, based on ~1300 bp of the 16S rRNA gene, resulted in a species match of $\geq 99\%$ with *Leifsonia aquatica* (accession no. NR_043412.1) using BLAST. Also, BIBI software showed the best species match as *L. aquatica* (accession no. AF299364). However, according to the RDP tool, our isolate was classified as *Leifsonia* spp. The evolutionary relationship of our isolate and seven isolates of various related species was inferred using the unweighted pair group method with arithmetic mean (UPGMA) (Sneath & Sokal, 1973) (Fig. 3). The evolutionary distances were computed using the maximum composite likelihood method (Tamura *et al.*, 2004), which gave the number of base substitutions per site. Phylogenetic analyses were conducted in MEGA4 software (Tamura *et al.*, 2007). The dendrogram revealed that our isolate was more related to other *L. aquatica* strains and *L. naganensis* than to other species such as *L. poae* or *L. xyl*i, and was even less related to *Corynebacterium aurimucosum*.

Our strain was also analysed by MALDI-TOF MS. A colony isolated from blood agar was deposited on a target plate (Bruker Daltonics) and covered with $1 \mu\text{l}$ of matrix. MALDI-TOF-MS was performed, in three replicates, with a Microflex LT tabletop mass spectrometer (Bruker

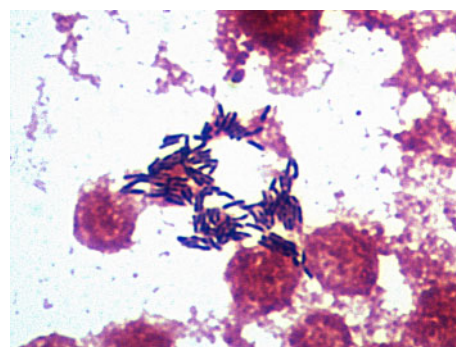


Fig. 1. Growth pattern of *L. aquatica* culture on blood agar after 29.9 h of incubation, showing Gram-positive rods growing as entangled masses; single bacterial cells were not observed.

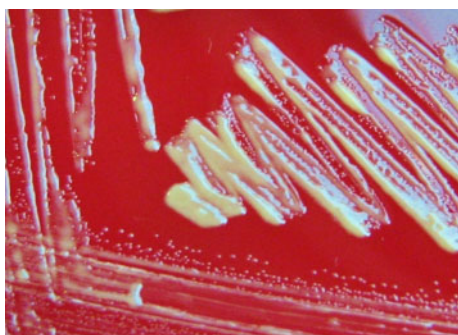


Fig. 2. Yellow-pigmented mucous-like colonies of *L. aquatica* after 4 days of incubation on blood agar at 35 °C in 5% CO₂.

Daltonics) using FlexControl software (version 3.0) (Bruker Daltonics) (Saffert *et al.*, 2011; van Veen *et al.*, 2010). Instrument parameter settings were used according to the manufacturer's instructions (Saffert *et al.*, 2011). Each spectrum was the sum of the ions obtained from 240 laser shots performed in six different regions of the same well (Carbounelle *et al.*, 2007). Captured spectra were analysed using the MALDI Biotyper 3.0 software package (Bruker Daltonics) (Seibold *et al.*, 2010). The identification criteria outlined by the manufacturer were as follows: a score of ≥ 2.000 indicates species level identification, a score of 1.700–1.999 indicates identification to the genus level, and a score of < 1.700 permits no identification. Our isolate was identified as *L. aquatica* with a maximum score of 1.736 in the first run. A second-best match was not provided as this returned a score of 1.341, which was not reliable for identification. The second and third runs gave score values of 1.663 and 1.606, respectively.

Although Clinical Laboratory Standards Institute (CLSI) interpretative guidelines for *in vitro* testing of this organism are not available, we performed susceptibility tests using E-test strips (bioMérieux) for penicillin G, cefotaxime and

vancomycin on Mueller–Hinton agar supplemented with 5% sheep's blood and inoculated with a 0.5 McFarland suspension of the organism. After 24 h of incubation at 35 °C in 5% CO₂, the MICs for penicillin G, cefotaxime, and vancomycin were 0.5, 1 and 4 $\mu\text{g ml}^{-1}$, respectively.

After the second set of blood cultures showed growth, the patient received empirical therapy with intravenous vancomycin (1 g every 5 days) and the CVC was removed. Dialysis was continued through the AVF. Antibiotic therapy was continued for 2 weeks and the patient remained asymptomatic during the next months of follow-up.

Discussion

Leifsonia aquatica is a coryneform bacterium, the clinical relevance of which is uncertain as it is rarely isolated in human infections. As this case report illustrates, its isolation might be misinterpreted as contamination, because other coryneform organisms belong to the typical microbiota of human skin and mucous membranes. It can also be confused with other genera and/or species since its identification, like in other Gram-positive rods, can be misleading using routine phenotypic tests (Funke *et al.*, 1997). In the present case, the commonly used API Coryne system was inconclusive and identification was confirmed by molecular methods (16S rRNA gene sequencing). Limitations of the API Coryne system in the identification of *L. aquatica* and related species have been reported previously (Winn *et al.*, 2006; Lau *et al.*, 2002; Saweljew *et al.*, 1996; Giammanco *et al.*, 2006). Another study including 178 clinical isolates of Gram-positive rods revealed that 25% of the strains required additional tests for their identification and 9% were incorrectly classified (Almuzara *et al.*, 2006). Misidentifications frequently occurred in yellow-pigmented species such as *Leifsonia* spp., *Microbacterium* spp. (including former *Aureobacterium* spp.) and *Cellulomonas* spp. (Almuzara *et al.*, 2006). Analysis by 16S rRNA sequencing is a valuable tool for identification of *L. aquatica* but has a

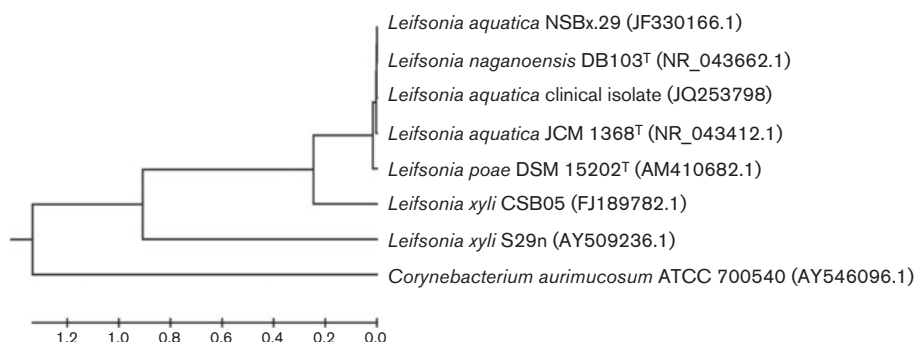


Fig. 3. Dendrogram resulting from UPGMA cluster analysis of 1269 bp of the 16S RNA gene sequence of the *L. aquatica* clinical isolate, as well as that of six closely related species and one unrelated species (*Corynebacterium aurimucosum*). GenBank accession numbers are given in parentheses. Branch lengths represent genetic distance. Bar, substitutions per nucleotide position.

limitation in that it cannot differentiate between this species and *L. naganensis*. Still, phenotypic characteristics such as motility and pigmentation help to separate these two species (Dastager *et al.*, 2009). Proteomic analysis by MALDI-TOF mass spectrometry was able to identify the isolate as *L. aquatica*, although the score provided indicated that this identification was only reliable to a genus level. This reflects on the limitations of the database, which, at the time of analysis, contained 3995 spectra, but only one for *L. aquatica* (DSM 20146^T) and no entry for *L. naganensis*.

Interestingly, we observed a particular growth pattern in blood culture media; the bacteria were only found as convoluted masses and not as single cells (Fig. 1). Furthermore, it was difficult to obtain isolated colonies on solid media. The tendency of cells to coaggregate, which has been described as a factor in the formation of aquatic biofilms (Rickard *et al.*, 2003), might serve as an additional criterion in the identification of this species.

Diagnostic problems might also contribute to the uncertainty regarding the pathogenicity of *L. aquatica*. To our knowledge, only three clinical case reports have been published in which identification was corroborated by 16S rRNA sequencing. In an outbreak of CVC infections with aquatic bacteria in a haemodialysis unit in Italy, *L. aquatica* was isolated in eight symptomatic and two asymptomatic patients, and in some cases was identified together with other bacterial species (D'Amico *et al.*, 2005). Contaminated solutions were the most probable source of this outbreak. Sulpher *et al.* (2008) described another catheter-related case of *L. aquatica* bacteraemia in a haemodialysis patient suffering from fever of unknown origin. In another publication, *L. aquatica* and various other environmental bacteria were detected in samples of 10 removed hip replacement devices. Nevertheless, these bacteria did not grow in culture and were detected solely by 16S rRNA gene amplification techniques. Furthermore, there was no association between detection of bacteria and infection of the respective prosthetic joint. The authors suggested the existence of a biofilm-forming microflora colonizing the hip prostheses (Dempsey *et al.*, 2007). These findings clearly need further confirmation, since neither *L. aquatica* nor the other isolated environmental species have yet been isolated in relation to prosthetic joint infections.

The available data, together with our case, highlight that *L. aquatica* is an aquatic, biofilm-forming bacterium, which is capable of causing sporadic cases or outbreaks of catheter-related infections in dialysis patients. Infections with other aquatic bacteria have also been described in patients undergoing peritoneal dialysis (Levitski-Heikkilä & Ullian, 2005). In general, dialysis patients seem to be especially vulnerable to this group of micro-organisms, probably because of their impaired immunity together with the frequent use of catheters and contact with dialysate water. The problem of waterborne infections in dialysis units and other healthcare settings has recently been reviewed (Exner *et al.*, 2005). As with other aquatic micro-organisms, *L. aquatica*

can grow under a wide range of environmental conditions (e.g. low temperatures and alkaline pH) and might also be able to pass through polycarbonate water filters (Phelps *et al.*, 1980). Long-term catheters are frequently colonized with biofilm-forming micro-organisms, which are most frequently derived from the skin microflora of the patient or healthcare personnel (Donlan, 2001). Catheter infections with waterborne micro-organisms, such as *L. aquatica*, might originate from contaminated infusions or from colonization after contact with environmental or household fresh water. Another potential source of infection with *Leifsonia* might be via the oral cavity, where species of this genus have also been isolated recently (Hung *et al.*, 2011).

The optimum method for management of *L. aquatica* infections is uncertain. In the present case, the isolate had an increased MIC for vancomycin, which is routinely used in catheter-related infections. This finding is consistent with previous reports (Funke *et al.*, 1997), although its clinical importance is unknown. Since other coryneform bacteria usually have lower vancomycin MIC values and in staphylococcal infections, MIC values $>1.5 \mu\text{g ml}^{-1}$ have been associated with reduced clinical outcomes (Aguado *et al.*, 2011), vancomycin might not be the most effective treatment for *L. aquatica* infections. Resistance to penicillin G and daptomycin has also been described in this species (Sulpher *et al.*, 2008; Goldstein *et al.*, 2003). It is of note that *L. aquatica* seems to have low virulence; in our patient, as in other cases, the infection presented as prolonged fever with a benign clinical outcome, and even asymptomatic bacteraemia has been reported (Sulpher *et al.*, 2008; D'Amico *et al.*, 2005). As in other reports, the outcome of this patient was favourable after removal of the infected catheter and empirical treatment with vancomycin. As the species is able to form biofilms, catheter removal might be the crucial factor for treatment.

The present report gives further proof of the ability of *L. aquatica*, an environmental coryneform bacterium, to cause human infection. Dialysis patients using long-term CVCs might be prone to infection with this aquatic species due to its ability to form a biofilm.

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