Legionella jordanis lower respiratory tract infection: Case report and review

Donald C. Vinh¹, Richard Garceau², Gabriela Martinez³, Debbie Wiebe⁴, Tamara Burdz⁴, Aleisha Reimer⁴, Kathryn Bernard⁴*

¹ Department of Medical Microbiology, McGill University Health Centre, Montreal, Quebec, Canada
² L’Hôpital Regional George Dumont, Moncton, New Brunswick, Canada
³ Laboratoire de Santé Publique du Québec, Ste-Anne-de-Bellevue, Québec, Canada
⁴ Special Bacteriology Section, National Microbiology Laboratory, Public Health Agency of Canada (PHAC), Winnipeg, Manitoba, Canada

* Address of correspondence: Kathryn Bernard, National Microbiology Laboratory, PHAC, Winnipeg, Manitoba R3E 3R2, Canada. Kathy_Bernard@phac-aspc.gc.ca

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Abstract:

*Legionella jordanis* was first described in 1982 from environmental sources and is otherwise a very rare human pathogen. Here, *L. jordanis* was recovered from a broncho-alveolar lavage of a patient who presented with an indolent lower respiratory infection associated with constitutional symptoms. It represents the first culture positive case involving this species in Canada.
Case Report:

A 53-year old man was assessed for chronic cough that had become increasingly productive with non-purulent sputum over the last year. In the last 3 months, he also noted constitutional symptoms, with headache, myalgia, malaise, and 1 episode of watery diarrhea but denied any fever, night sweats, or weight loss. He had previously worked in the quality control chemistry laboratory of the regional paper-mill industry but had been re-assigned to administrative duties ~ 1 year prior to onset of cough. To his knowledge, there were no similar symptoms in co-workers, family members or close contacts. He denied smoking, alcohol abuse, or risk factors for HIV. Initial evaluation of his cough consisted of a chest radiograph which was interpreted as normal. One month later, a computed tomodensitometry of the chest revealed bilateral “tree-in-bud” pattern, suggesting a chronic, indolent pulmonary infectious process. A broncho-alveolar lavage (BAL) was then performed, and specimens were sent for Gram stain, routine bacteriologic culture, culture for *Legionella* spp., stain and culture for *Mycobacteria* spp., and stain and culture for fungi. Direct microscopy with Gram stain revealed heavy leukocytes with mixed organisms. Within 3 days, 2 organisms were identified: *Streptococcus pneumoniae* from the blood agar plate, and ~ 15 small clear colonies of a gram-negative bacillus on the buffered charcoal yeast extract (BCYE) agar incubated aerobically at 37 °C, suggestive of *Legionella* spp. This strain was sent to a regional then federal reference center for further characterization.

The patient was prescribed moxifloxacin 400 mg orally once daily for 28 days. After completion of treatment, he reported complete resolution of his constitutional symptoms. Although sputum production significantly decreased, his cough persisted.
Despite routine culture for *Legionella* spp. on all bronchoscopy specimens, no other clinical isolates of *Legionella* spp. were identified in the treating medical centre in the 7 years preceding and the 8 months subsequent to this case.

**Microbiologic Identification:**

The isolate obtained from the BAL was forwarded to the Laboratoire de Sante Publique de Quebec (LSPQ), the regional reference centre, where it was confirmed that it was a member of the *Legionella* genus. The organism was subsequently sent to the National Microbiology Laboratory (NML), the reference centre for Canada, for species identification. Standard and extended microbiological testing procedures (14) were carried out at both institutions and are herein described. The isolate (NML 060502) grew in a candle jar atmosphere on BCYE with L-cysteine at 25 °C, 35 °C, and 42 °C, as well as on BCYE with L-cysteine and polymyxin, anisomycin, and vancomycin (PAV) supplement at 35 °C. No growth occurred on BCYE with L-cysteine at 50 °C, BCYE without L-cysteine at 35 °C, and sheep blood agar in 7 days at 35 °C. The isolate demonstrated growth with browning on tyrosine-supplemented buffered yeast extract agar (BYET), as described previously (5). The organism demonstrated a dull autofluorescence under long-wave UV light. Gram stain revealed short to long, faint-staining gram-negative asporogenous bacilli. The isolate was catalase and β-lactamase positive with weak and slow (4 weeks) gelatinase production. It was oxidase negative and failed to utilize dextrose, reduce nitrate or to hydrolyse urea and sodium hippurate.

The organism was non-reactive by indirect immunofluorescent antibody (IFA) testing (MONOFLUO® *Legionella pneumophila* test kit; Bio-Rad (Montreal, QC)) and direct immunofluorescent antibody (DFA) testing with monovalent FITC-conjugated
anti-*Legionella* (serogroups 1 – 14) rabbit sera (Pro- Lab Diagnostics, (Richmond Hill, On) for *L. pneumophila*. Commercially-available, polyclonal anti-*Legionella* antibodies for DFA testing of individual, non-*pneumophila* species (m-TECH, Alpharetta, Ga) were used as described by the manufacturer. The results of these assays showed strong reaction of this strain with antisera to *L. jordanis*, and weak to strong cross-reaction with *L. longbeachae* serogroup (sg) 2, *L. hackeliae* sg 1 and sg 2, *L. erythra*, *L. rubriluscens*, and *L. parisiensis*. No reactivity was observed using antisera targeting *L. anisa*, *L. bozemanae* sgs 1 and 2, *L. cherrii*, *L. dumoffii*, *L. feeleii* sgs 1 and 2, *L. gormanii*, *L. jamestowniensis*, *L. longbeachae* gr 1, *L. micdadei*, *L. maceachernii*, *L. oakridgensis*, *L. sainthelensi*, *L. santicrucis*, *L. spiritensis*, *L. steigerwaltii* and *L. wadsworthii*.

Cellular fatty acid composition analysis was done after 48h growth on BCYE as described (3) except that ver 4.5 of the software was used for the MIDI Sherlock system (MIDI, Newark De), MIDI’s CLIN library and MIDI Library Generation System (LGS). The profile obtained had a similarity index value of 0.45 to the entry in CLIN ver 4.5 for *L. jordanis* and was highly consistent with the *L. jordanis* type strain BL – 540 (=ATCC 33623T) after in-house LGS analysis (data not shown).

Genetic based sequencing targeting the 16S rRNA (2) and macrophage infectivity potentiator (*mip*) genes (9) was undertaken, to accurately assign this isolate to species. The 16S rRNA gene product for this isolate was 1483 base pairs (bp) in size. The sequence was tested using BLAST software (www.ncbi.nlm.nih.gov/BLAST) and demonstrated 99.7% sequence identity with *L. jordanis* (type strain BL – 540 GenBank accession Z32667). Similarly, the sequence of the *mip* gene product for this isolate was
639 bp in size and demonstrated 100% sequence identity with *L. jordanis* (ATCC 33623T, GenBank accession U92209). The 16S rRNA and *mip* gene sequences of this clinical isolate *L. jordanis* NML 060502, have been deposited into GenBank under accession numbers EF036512 and EF036513, respectively.

**Discussion:**

This is the second reported culture-proven case of a lower respiratory tract infection in which *Legionella jordanis* was isolated and expands on the clinical manifestations of this uncommon pathogen. The previously reported case occurred in a 79-year old man who also presented with a subacute course of progressive respiratory and constitutional symptoms and in which *L. jordanis* was isolated from open-lung biopsy samples; that patient subsequently developed *S. aureus* bacteremia and later died (13). Although other cases with pneumonia or where clinical presentation was not described due to *L. jordanis* have been reported (1, 6, 8, 12), those diagnoses were made using serology only, without isolation of the pathogen. However, diagnostic serology for *Legionella* spp. suffers from low sensitivity and specificity; in the latter instance, cross-reactivity between serogroups, between the various species of the *Legionella* genus, and even with other genera, have been consistently reported (10). As such, the gold standard for diagnosing any form of *Legionella* spp. infection remains isolation in culture (4), as was done in this case with subsequent speciation by polyphasic approach. Assignment to species using specific fluorescein-labelled antiserum for non-*pneumophila* taxa by DFA alone would have been difficult in this instance, due to the high degree of cross reactivity of this isolate with antisera from different species. Definitive characterization of the
strain as *L. jordanis* had to be corroborated using sequence analyses of 16S rRNA and *mip* genes.

In the absence of a well-recognized syndrome or an experimental model to fulfill Koch’s postulates, the clinical significance of an uncommon isolate, such as *L. jordanis*, is controversial. Possibilities other than infection include a non-pathogenic colonization state or exogenous contamination. However, *Legionella* spp., including *L. jordanis*, are not known to be commensals of the human respiratory tract. To date, the only natural reservoirs identified for *L. jordanis* have been river water, tap water, and sewage (5,7). This feature invites the possibility that acquisition by the patient may have been occupational, although this remains entirely speculative. Specimen contamination seems profoundly unlikely given the absence of any synchronous or metachronous isolates in the microbiology laboratory, despite routine culture for *Legionella* spp. on all bronchoscopy specimens. Furthermore, review of infection control practices demonstrated no breach in procedures during the bronchoscopic examination, although water sampling was not performed. In support of its pathogenicity are the following observations: chronic lung disease is an established risk factor for legionellosis (11), and this patient had radiological evidence of structural airway disease; as well, there was significant improvement in respiratory and constitutional symptoms with therapy possessing a spectrum of activity that includes *Legionella* spp. However, in contrast to the acute manifestations of Legionnaires’ disease due to *L. pneumophila*, it appears that *L. jordanis* may tend to produce a more subacute-to-chronic respiratory infection. Although *S. pneumoniae* may have acutely contributed to his symptoms, it likely does
not explain the protracted course of his respiratory illness nor his chronic constitutional
symptomatology.

In conclusion, we describe an indolent respiratory infection with associated
constitutional symptoms due to *L. jordanis* in a patient with underlying lung disease,
further demonstrating the pathogenicity of this organism.
References:


