



## Case Report

*Legionella indianapolisensis* sp. nov., isolated from a patient with pulmonary abscessR.F. Relich<sup>a,\*</sup>, B.H. Schmitt<sup>a</sup>, H. Raposo<sup>b</sup>, L. Barker<sup>b</sup>, S.J. Blosser<sup>c</sup>, M. May<sup>b</sup><sup>a</sup> Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana, USA<sup>b</sup> University of New England, Biddeford, Maine, USA<sup>c</sup> Indiana State Department of Health Laboratory, Indianapolis, Indiana, USA

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## ABSTRACT

**Background:** To date, at least 50 species of *Legionella* have been described. These organisms are ubiquitous in nature and have been isolated from diverse ecological environments, including man-made structures such as cooling towers and spas. Legionellae have also been isolated from human and veterinary clinical specimens, and their roles in disease are well-established. This report describes the isolation of a novel *Legionella* species from a respiratory specimen from a patient with influenza and suspected pulmonary embolus.

**Case:** A 68-year-old male presented to an Indianapolis-area hospital with pulmonary disease; upon workup, he was found to have influenza A. Bronchoalveolar lavage fluid was also submitted for conventional bacterial culture and *Legionella* culture. The patient was prescribed a broad-spectrum antibiotic and recovered.

**Results:** A *Legionella*-like bacterium was isolated on buffered charcoal yeast extract agar, and mass spectrometry and comparative 16S rRNA gene sequencing inconclusively identified the isolate as a *Legionella* sp. Further analysis of the 16S rRNA gene confirmed the strain to be a new species, related to *Legionella hackeliae*. Physiochemical and morphological testing were used to confirm the discovery of a novel species, *Legionella indianapolisensis* sp. nov., type strain SMNF-IS.

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## 1. Introduction

Legionellae are rod-shaped, asaccharolytic, fastidious, facultatively intracellular, and obligately aerobic Gram-negative bacteria that are most often found growing in warm water environments, including hot tubs, cooling towers, and water heaters, among other places (Edelstein and Lück, 2015). Infections with these organisms are referred to as legionellosis, an uncommon form of atypical pneumonia. Legionellosis caused by *Legionella pneumophila*, the primary causative agent of Legionnaire's disease, is perhaps best known; however, other species, including *Legionella bozemanii*, *Legionella dumoffii*, *Legionella longbeachae*, and *Tatlockia micdadei* (formerly *Legionella micdadei*), also cause human infections (Cunha and Cunha, 2017). Transmission to humans occurs through the inhalation of organism-laden aerosols (Edelstein and Roy, 2014).

Subsequently, and generally following a 2- to 14-day incubation period, disease symptoms develop and include cough, shortness of breath, fever, and body aches (Edelstein and Lück, 2015). Individuals with compromised respiratory systems and those with underlying immunosuppression are at the highest risk of infection and development of severe disease. Every year, between 8000 and 18 000 people are affected by Legionnaires' disease in the USA, of which approximately 10% die. Those who most often suffer severe and fatal infections include smokers, the immunocompromised, and individuals older than 50 years of age (Burillo et al., 2017). Early detection and rapid initiation of antimicrobial therapy is essential to prevent severe morbidity and mortality.

## 2. Case

The organism was isolated from a 68-year-old male patient who presented to an Indianapolis-area hospital with an influenza-like illness. The patient had a past medical history significant for thrombocytopenia and multiple myeloma. A chest X-ray was performed and revealed left lower lobe infiltrates, and a computed tomography scan showed a right-sided filling defect of the heart.

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Bronchoalveolar lavage (BAL) was performed to determine the cause of the patient's illness. During hospitalization, he was diagnosed with a pulmonary embolus, left lung abscess, and a left lower lobe pneumonia. A nucleic acid amplification test for influenza A was positive, prompting oseltamivir treatment. Gram staining of the BAL fluid did not reveal microorganisms; modified acid-fast (MAF) and other stains were not performed. Four days after the patient was discharged, an unidentified organism grew in culture. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), performed using a Bruker MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA), tentatively identified the isolate as '*Legionella* species'. The patient was treated with a 14-day course of levofloxacin (750 mg/24 h) and recovered fully.

### 3. Results and discussion

The organism isolated from the patient's BAL fluid was cultured on buffered charcoal yeast extract (BCYE) agar according to standard methods. Gram staining of a representative colony revealed faint-staining Gram-negative rods, and MAF staining indicated that the organism was non-acid-fast. The isolate grew on BCYE agar, but not on standard sheep blood agar, chocolate agar, or other routine bacteriological culture media, suggesting that the organism belonged to the genus *Legionella*. The isolate was non-motile, oxidase-positive (weakly), and spot indole-negative. Physiochemical testing of the isolate was attempted using the VITEK 2 system (bioMérieux Inc., Durham, NC, USA); however, the isolate failed to grow in the test card. The isolate did not hydrolyze hippurate or produce catalase and demonstrated an absolute requirement for L-cysteine; the latter phenotype was demonstrated by growth of the isolate on BCYE agar containing 2.5 mM L-cysteine and lack of growth of the isolate on BCYE agar devoid of L-cysteine. The isolate was also positive for  $\beta$ -lactamase activity. A genus-level polyvalent direct fluorescent antibody (DFA) test was positive, but an *L. pneumophila*-specific DFA test was negative. Taken together, these results supported the MALDI-TOF MS-generated identification of *Legionella* species.

Genomic DNA was isolated using a NucliSENS easyMAG instrument (bioMérieux Inc.). The 16S ribosomal RNA gene was amplified using the forward primer 27f (5'-AGAGTTTGATCCT-3')

and the reverse primer 1492r (5'-ACGGCTACCTTGT-3'), as described previously (Weisburg et al., 1991). Three distinct 1.5-kb amplicons were sequenced using standard dideoxy methods (i.e., Sanger sequencing chemistry) by GenScript (Piscataway, NJ, USA). The sequence similarities of the 16S rDNA between the isolate and other *Legionella* species available in GenBank ranged from 94.9% (1362/1435) to 96.7% (1389/1486), with *Legionella hackeliae* being the most closely related species (multiple sequence alignment viewable at doi 10.13140/RG.2.2.19151.79526; input sequence files available at doi 10.13140/RG.2.2.34251.28966). Type species were used whenever possible. Phylogenetic analysis was performed via neighbor-joining method using MEGA 7.0 (Figure 1A).

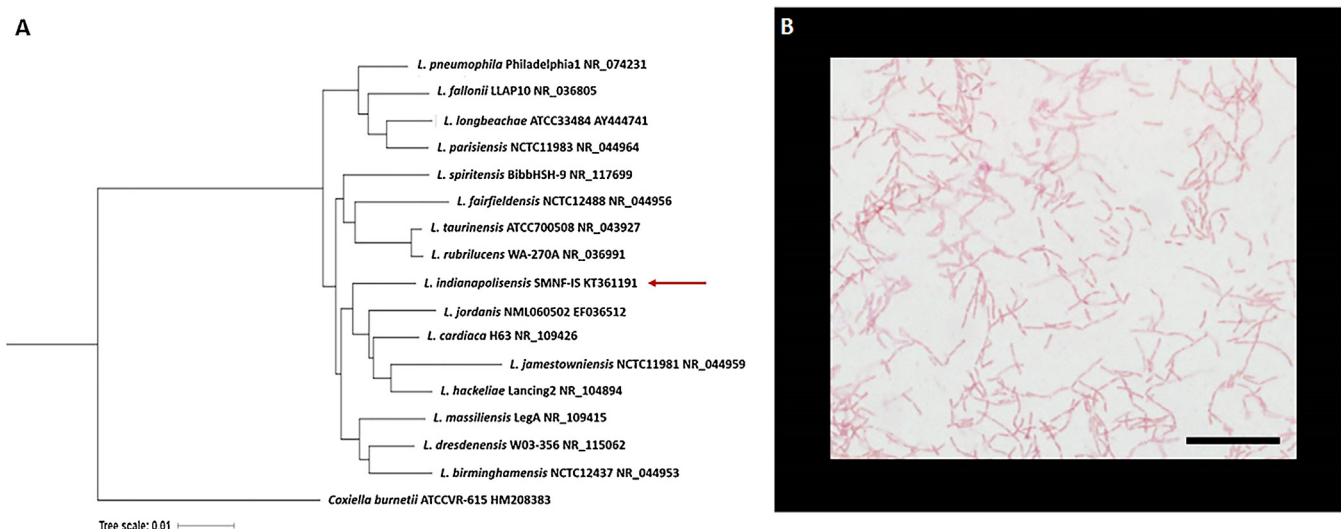
Ribosomal RNA and phylogenetic analyses featuring 15 species in the genus *Legionella* indicated that this isolate represented a novel species, and was appropriately assigned to the genus *Legionella*. Growth on BCYE agar but not sheep blood agar, exogenous L-cysteine requirement, lack of catalase, sodium hippurate hydrolysis, and genus-level DFA testing results further support this assignment. The ribosomal RNA gene sequence and mass spectral profile are unique to this organism, as is the lack of motility. Based on these data, we propose the formal name *Legionella indianapolisensis*, sp. nov., strain SMNF-IS, after the geographic location of its isolation.

#### 3.1. Description of *L. indianapolisensis* sp. nov., strain SMNF-IS

*L. indianapolisensis* sp. nov. (in di an ap' o li sen' sis) N.L. neut. Of Indianapolis, the geographic site of the index case. Cells are Gram-negative, rod-shaped, and non-acid-fast (Figure 1B). Colonies are white with a ground-glass appearance. Non-motile. Aerobic. Non-sporulating. Chemoorganotroph. Sodium hippurate is hydrolyzed. Oxidase is weakly produced. Unique 16S rRNA gene sequence (GenBank accession number **KT361191**) distinct from the most closely related species in the genus *Legionella*. First isolated from the bronchoalveolar lavage fluid from a man with pulmonary abscess and concurrent influenza A in Indianapolis, Indiana, USA. The type strain is SMNF-IS.

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**Ethical approval:** The work presented herein abides by all ethical standards set forth by Elsevier and each of the author's institutions.



**Figure 1.** Phylogeny and morphology of *Legionella indianapolisensis*. (A) Phylogenetic analysis using the neighbor-joining method showing the position of *L. indianapolisensis* (arrow) within the genus *Legionella*. *Coxiella burnetii* served as the outgroup. (B) Gram stain showing pleomorphic Gram-negative bacilli (scale bar = 10  $\mu$ m).

*Conflict of interest:* All authors declare no conflicts of interest pertaining to the work presented herein.

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