



Case Report

The relevance of molecular genotyping to allocate cases in a suspected outbreak of *Legionella* pneumonia in patients with prolonged immunosuppressive therapy



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ABSTRACT

Three cases of pneumonia caused by *Legionella pneumophila* serogroup 1 (Lp1) in immunosuppressed patients with repeated hospitalization were suspected as a healthcare-associated cluster. The environmental investigation did not reveal the presence of legionellae in the hospital patient rooms. Water samples collected from the homes of two patients were also negative for *Legionella* spp. In the absence of environmental strains potentially involved in the infections, we proceeded to genotype environmental Lp1 strains isolated in the hospital during routine water sampling during the decade 2009–2019 and recovered after long-term storage at -20°C . These 'historical' strains exhibited a high grade of similarity and stability over time, regardless of the disinfection systems. The different molecular profiles shown among the clinical and environmental strains excluded a nosocomial outbreak. The study suggests that the application of molecular typing may be a useful tool to discriminate hospital vs community-acquired cases, mostly for severely immunosuppressed patients in whom the symptomatology could be insidious and the incubation period could be prolonged. Moreover, the genotyping allowed us to exclude any link between the cases.

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Introduction

Cases and clusters of Legionnaires' diseases (LD) are increasing globally (Herwaldt and Marra, 2018). The greater diagnostic capability, variety of possible sources of contagion, higher numbers of at-risk persons, travelling habits, and influence of climate change are possible explanations for this increase (Walker, 2018). The connection with the environmental source is conducted on the basis of the incubation period (2–10 days), although longer periods have been described (Bargellini et al., 2013; Cassier et al., 2015).

This report describes three cases of LD diagnosed at the University Hospital Policlinico of Modena between April and May 2018, whose origin was unclear. The suspicion of a nosocomial cluster was refuted by comparing the molecular characteristics of clinical and environmental strains.

Case series report

The first case was a 67-year-old man, treated with immunosuppressive therapy following a liver transplantation (2017) due to hepatitis B virus (HBV) cirrhosis. From April 4 to April 12, 2018, he was hospitalized in the transplant surgery ward for biliary stenosis, attributed to liver rejection. He was again hospitalized on April 17 to perform the screening for a second transplant. On day 9 of hospitalization (April 26), the patient developed a fever with negative chest X-ray. On May 4, since the fever persisted, antibiotic therapy was modified (levofloxacin); he was then

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discharged for family reasons. However, he was readmitted the next day with fever and respiratory symptoms. On May 8, radiography showed pulmonary consolidation and a *Legionella* urinary antigen test (EIA Binax LUA; Alere Inc., Scarborough, Maine, USA) was positive. The diagnosis of LD was confirmed by isolation of *Legionella pneumophila* serogroup 1 (Lp1) in bronchoalveolar lavage with culture methods (ISO 11731:2017) and serological typing (ProLab Diagnostics, Merseyside, UK). The course of his pneumonia was favourable, and on May 19 the patient underwent a second liver transplantation. Considering the typical incubation period, the infection could have had a nosocomial origin.

A further two cases of LD were diagnosed in patients hospitalized on the same day, both with a history of previous admission to the hospital. One was a 56-year-old man on immunosuppressive therapy since 1998, following a kidney transplant he had received due to polycystic kidney disease. From April 23 to May 5, the patient was hospitalized in the nephrology unit; he returned on May 21 with dyspnoea, reporting fever for 3 days and diarrhoea for a week. Chest computed tomography revealed pulmonary consolidations. On May 24, after a positive *Legionella* urinary antigen test confirmed by isolation of Lp1 from sputum, levofloxacin was started with subsequent clinical improvement. Given his previous hospitalization and the possibility that the gastrointestinal symptoms were related to LD, the incubation period was considered potentially compatible with a nosocomial origin.

The third case was an 84-year-old woman with a recent diagnosis of Henoch–Schönlein purpura. From April 19 to May 8, she was hospitalized in the nephrology unit for acute renal failure due to proliferative necrotizing glomerulonephritis, and steroid therapy was initiated. On May 21, fever and respiratory symptoms occurred, and on May 23, due to worsening of respiratory symptoms and the onset of gastrointestinal disorders, she was admitted to the hospital with a diagnosis of pneumonia. A *Legionella* urinary test was positive and Lp1 was isolated in the sputum. Antibiotic therapy led to the resolution of the LD. Considering the incubation period, this case would be classified as community-acquired; however, given the previous hospitalization and the immunosuppressive therapy, we could not exclude a healthcare-associated infection.

The clinical isolates were genotyped using the RAPD-PCR method with two different primers and rep-PCR (Patton et al., 2001). The sequence type (ST) was defined using the sequence-based typing (SBT) method (<http://www.ewgli.org/>). The clinical isolates exhibited a low degree of similarity with different SBT profiles: ST18 (2,10,9,13,2,5,6), new ST2632 (21,14,28,10,15,26,6), and new ST2631 (21,14,28,15,15,26,6).

Environmental investigation

The hospital water distribution system is monitored regularly according to the hospital's *Legionella* sampling and management plan. The nephrology and transplant surgery wards are located in the central hospital building where the hot water is treated with monochloramine. Following the installation of the monochloramine devices, all water samples tested negative for *Legionella* spp in both wards (<25 CFU/l). An additional 19 water samples collected during the epidemiological investigation in the patients' rooms were all *Legionella* spp-negative, a result confirmed by qPCR and EMA-qPCR (Mansi et al., 2014).

Water samples collected from two cooling towers operating during that period showed contamination by *L. pneumophila* serogroup 6 (Lp6) at a concentration of <1000 CFU/l.

Given the lack of environmental isolates, clinical strains were compared with environmental Lp1 and Lp6 isolated in the hospital during routine water sampling in the decade 2009–2019 and recovered after long-term storage at –20 °C. These 'historical' strains were selected on spatial and temporal criteria, as shown in Figure 1. The genotyping of the isolates was performed by RAPD-PCR and rep-PCR followed by SBT method, as for the clinical strains (Patton et al., 2001). The molecular analysis of 10 Lp1 strains isolated in hot water revealed one sequence type (ST1), with high similarity grade between the strains (A profile), but different from the clinical strains; two Lp1 strains from cold water and one isolated from a boiler supplied with cold water showed the same sequence type (ST1), but a low similarity grade (D and E profile, respectively). The Lp6 strains from cooling towers and from the pneumology building had a different ST (new ST 2760-I and 110-H profile, respectively).

The first patient denied consent to carry out an environmental survey at his home. For case patients two and three, domestic water analyses were negative for *Legionella* spp.

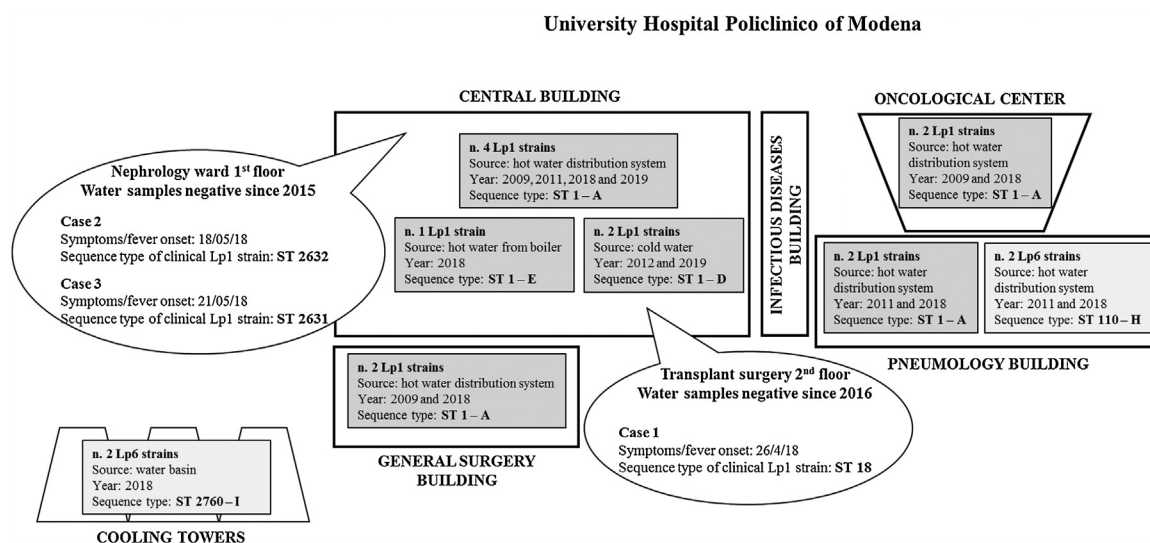


Figure 1. Schematic diagram of the hospital buildings (figure not to scale) showing the temporal and spatial distribution of environmental *Legionella pneumophila* serogroup 1 and 6 strains isolated in the years 2009–2019, with their molecular profile.

Discussion

The reported cases highlight the difficulty of identifying the source of infection in patients with multiple co-morbidities, chronic therapy with immunosuppressants, and repeated hospital admissions (Turner et al., 2001). In these patients, the symptomology of LD is insidious, frequently severe, and often characterized by extrapulmonary (gastrointestinal) symptoms. Considering the timing of hospitalization before the onset of symptoms (9 days), we initially classified the first case as possible hospital-acquired. For the other two cases, fever occurred 13 days after hospital discharge, suggesting a community origin. However, the literature suggests that the incubation time may not be strictly limited to 10 days in these patients, but lasts up to 63 days (Marrie et al., 1992). A possible role of oropharyngeal colonization was also described, especially in immunocompromised patients (Pedro-Botet et al., 2002). Moreover, these two patients had been hospitalized in the nephrology ward during the same period; therefore a suspected nosocomial outbreak was plausible.

The water samples collected from the involved wards just after the cases occurred were all negative for legionellae, also due to the disinfection treatments adopted in the hospital and described previously (Marchesi et al., 2013). The molecular typing of Lp1 isolated in the hospital water during the period 2009–2019 showed a high grade of similarity and stability over time, regardless of disinfection, in line with other authors (Casini et al., 2008). The molecular stability of these environmental isolates and the lack of correspondence with the clinical strains did not support the hypothesis of a healthcare-associated cluster. Considering that the three patients lived in different geographical areas of Modena province, had no connection with one another, and no increase in LD was notified during that period in Emilia Romagna Region, we also excluded a community-acquired cluster. Nevertheless, even though environmental, epidemiological, and molecular analyses were done, the source of infection remains unknown.

This study suggests that the application of molecular typing may be a useful tool to discriminate hospital vs community-acquired cases, and furthermore, as in our experience, to exclude any link between the cases.

Ethical approval

Approval was not required.

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Conflict of interest

None.

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