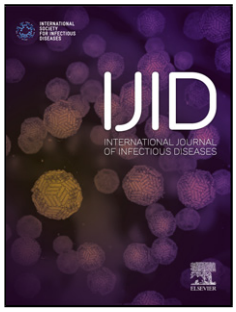

The relevance of molecular genotyping to allocate cases in a suspected outbreak of *Legionella* pneumonia in patients with prolonged immunosuppressive therapy

Borella Paola, Vecchi Elena, Incerti Federica, Marchesi Isabella, Meacci Marisa, Frezza Giuseppina, Fregni Serpini Giulia, Mansi Antonella, Paduano Stefania, Bargellini Annalisa

PII: S1201-9712(19)30441-2

DOI: https://doi.org/10.1016/j.ijid.2019.11.002

Reference: IJID 3823

To appear in: *International Journal of Infectious Diseases*

Received Date: 11 September 2019

Revised Date: 31 October 2019

Accepted Date: 1 November 2019

Please cite this article as: Borella P, Vecchi E, Incerti F, Marchesi I, Meacci M, Frezza G, Fregni Serpini G, Mansi A, Paduano S, Bargellini A, The relevance of molecular genotyping to allocate cases in a suspected outbreak of *Legionella* pneumonia in patients with prolonged immunosuppressive therapy, *International Journal of Infectious Diseases* (2019), doi: https://doi.org/10.1016/j.ijid.2019.11.002

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

Short title: Genotyping and origin of *Legionella* pneumonia

Borella Paola[1], Vecchi Elena[2], Incerti Federica[1], Marchesi Isabella[1], Meacci Marisa[3], Frezza Giuseppina[1], Fregni Serpini Giulia[4], Mansi Antonella[5], Paduano Stefania[1], Bargellini Annalisa[1]

[1]Department of Biomedical, Metabolic and Neural Sciences, Section of Public Health, University of Modena and Reggio Emilia, Modena, Italy;

[2]Hospital Hygiene, University Hospital, Modena, Italy;

[3]Unit of Clinical Microbiology, University Hospital, Modena, Italy;

[4]Unit of Virology and Molecular Microbiology, University Hospital, Modena, Italy;

[5]INAIL Research Area, Department of Occupational and Environmental Medicine, Epidemiology and Hygiene, Monte Porzio Catone (Rome), Italy.

Corresponding author

Paola Borella, MD, Full Professor of Hygiene , Dept. of Biomedical, Metabolic and Neural Sciences University of Modena and Reggio Emilia, Via G. Campi,287 - 41125 Modena, Italy, phone n.+39 059 2055474, E-mail: paola.borella@unimore.it

Highlights

- Difficulty in classifying cases based on incubation time in immunosuppressed patients
- Genotyping as a useful tool to properly allocate *Legionella* cases
- Importance of storing environmental *Legionella* strains isolated over time

Abstract

Three cases of pneumonia by *L. 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's rooms. Water samples collected from the home 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 the routine water sampling along the decade 2009-2019 and recovered after long term storage at -20°C. These "historical" strains exhibited high grade of similarity and stability over time, regardless of disinfection systems.
The different molecular profile showed among 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 which the symptomatology could be insidious and the incubation period could be prolonged. Moreover, the genotyping allowed us to exclude that the cases were linked each other's.

Keywords: Legionnaires' disease; Immunosuppressed patients; Sequence-Based Typing; Cluster; Environmental strains; Clinical strains

Introduction

Cases and clusters of Legionnaires' diseases (LD) are globally increasing.[1] Greater diagnostic capability, variety of possible sources of contagion, higher number of at-risk persons, travelling habits and influence of climate change can explain this increase.[2] The connection with the environmental source is carried out on the basis of the incubation period (2-10 days), although longer periods have been described.[3,4]

Here, we describe three cases of LD diagnosed at the University Hospital Policlinico of Modena between April and May 2018, whose origin was unclear. The suspect 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 for liver transplantation (2017) due to HBV cirrhosis. From 4 to 12 April 2018, he was hospitalized in the transplant surgery ward for biliary stenosis, attributed to liver rejection. He was again hospitalized on 17 April to perform the screening for a second transplant. At 9th day of hospitalization (26 April), the patient developed fever with negative chest x-ray. On 4 May, since the fever persisted antibiotic therapy was modified (levofloxacin), and he was discharged for familiar reasons, but the next day readmitted with fever and respiratory symptoms. On 8 May, radiography showed pulmonary consolidation and *Legionella* urinary antigen test (EIA Binax LUA, Alere, Scrborough, Inc. Maine, USA) was positive. The diagnosis of LD was confirmed by isolation of *Legionella pneumophila* sg 1 (Lp1) in bronchoalveolar lavage with cultural method (ISO 11731:2017) and serological typing (ProLab Diagnostics, Merseyside, UK). The course of pneumonia was favourable, and on 19 May the patient underwent a second liver transplant. Considering the typical incubation period, the infection could have had a nosocomial origin.

Other two cases were diagnosed in patients hospitalized the same day, both with a history of previous admission in our hospital. One was a 56-year-old man on immunosuppressive therapy for kidney transplant since 1998 for polycystic kidney disease. From 23 April to 5 May, the patient was hospitalized in the nephrology unit, and returned on 21 May with dyspnoea reporting fever since 3 days and diarrhoea since a week. Chest computed tomography revealed pulmonary consolidations. On 24 May, 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, if we consider gastrointestinal symptoms as related to LD, the incubation period may be compatible with a nosocomial origin.

The third case was an 84 years-old woman with recent diagnosis of Henoch Schonlein purpura. From 19 April to 8 May, she was hospitalized in the nephrology unit for acute renal failure due to proliferative necrotizing glomerulonephritis, and steroid therapy was initiated. On 21 May, fever

and respiratory symptoms occurred, and on 23 May, for worsening of respiratory symptoms and onset of gastrointestinal disorders, she was admitted to hospital with diagnosis of pneumonia. *Legionella* urinary test was positive and Lp1 was isolated in the sputum. Antibiotic therapy obtained healing of LD. Considering the incubation period, this case would be classified as community-acquired, but given the previous hospitalisation 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.[5] The sequence type (ST) has been defined with the Sequence-Based Typing (SBT) method (http://www.ewgli.org/). The clinical isolates exhibited a low degree of similarity with different SBT profiles: ST 18 (2,10,9,13,2,5,6), new ST 2632 (21,14,28,10,15,26,6) and new ST 2631 (21,14,28,15,15,26,6).

Environmental investigation

The hospital water distribution system was monitored regularly according to the hospital's *Legionella* sampling and management plan. Nephrology and transplants surgery wards are located in the hospital central building where hot water is treated by monochloramine; after the installation of monochloramine devices, all water samples resulted negative for *Legionella* spp (<25 cfu/L) in both wards. Additional nineteen water samples collected during the epidemiological investigation in the patients' rooms were all *Legionella* spp negative, a result confirmed by qPCR and EMA-qPCR.[6]

Water samples collected from two cooling towers operating in that period showed a contamination by *L. pneumophila* sg 6 (Lp6) at concentration < 1.000 cfu/L.

Given the lacking of environmental isolates, clinical strains were compared with environmental Lp1 and Lp6 isolated in the hospital during the routine water sampling along the decade 2009-2019 and recovered after long term storage at -20°C. These "historical" strains were selected on spatial and

temporal criteria as represented in Figure 1. The genotyping of the isolates was made by using RAPD-PCR and rep-PCR followed by SBT method as for clinical strains.[5] The molecular analysis of ten Lp1 strains isolated in hot water revealed one sequence type (ST1), with high similarity grade between the strains (A profile), but different from 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 environmental survey at home, whereas for case two and three, domestic water analyses were negative for *Legionella* spp.

Discussion

The reported cases highlight the difficulty of identifying the source of infection in patients with multiple co-morbidities, chronic therapy with immunosuppressant, and repeated hospital admissions.[7] In these patients, the symptomology of LD is insidious, frequently severe, often characterized by extra-pulmonary (gastrointestinal) symptoms. Considering the timing of hospitalization before the symptoms onset (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.[8] A possible role of oropharyngeal colonization was also described, especially in immunocompromised patients.[9] Moreover, these two patients have been hospitalized in nephrology ward in the same period, therefore a suspected nosocomial outbreak was plausible.

The water samples collected in the involved wards just after the cases appearance were all negative for legionellae, also due to the disinfection treatments adopted in the hospital and previously

described.[10] The molecular typing of Lp1 isolated in the hospital water from 2009 to 2019 showed high grade of similarity and stability over time, regardless of disinfection, in line with other authors.[11] 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 one with the other, and no increase in LD was notified in that period in Emilia Romagna Region, we excluded also a community–acquired cluster. Nevertheless, even if the environmental, epidemiological and molecular analyses were done, the source of infection remains unknown.

The study suggests that the application of molecular typing may be a useful tool to discriminate hospital *vs* community-acquired cases, and, in our experience, also to exclude that the cases were linked each other's.

Ethical approval: approval was not required

Funding source: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest: none

Acknowledgments. We are grateful to Dr. Roberto Cagarelli for the information on LD notified in 2018 in the Emilia Romagna Region and to Monica Barbieri for her assistance in the re-examination of the medical records.

References

1. Herwaldt LA, Marra AR. *Legionella*: a reemerging pathogen. *Curr Opin Infect Dis* 2018;31:325-333.
2. Walker JT. The influence of climate change on waterborne disease and *Legionella*: a review. *Perspect Public Health* 2018;138:282-286.
3. Bargellini A, Marchesi I, Marchegiano P, et al. A Culture-Proven Case of Community-Acquired *Legionella* Pneumonia Apparently Classified as Nosocomial: Diagnostic and Public Health Implications. *Case Rep Med* 2013:303712.
4. Cassier P, Benet T, Nicolle MC, et al. Community-acquired Legionnaires' disease in a renal transplant recipient with unclear incubation period: the importance of molecular typing. *Transpl Infect Dis* 2015;17:756–760.
5. Patton TG, Katz S, Sobieski RJ, Crupper SS. Genotyping of clinical *Serratia marcescens* isolates: a comparison of PCR based methods. *FEMS Microbiol Lett* 2001;194:19-25.
6. Mansi A, Amori I, Marchesi I et al., *Legionella* spp. survival after different disinfection procedures: comparison between conventional culture, qPCR and EMA-qPCR. *Microchem J* 2014;112:65-69.
7. Turner DP, Boswell TC, Lee JV, Slack RC, Burden RP. Community-acquired Legionnaires' disease in an immunocompromised patient masquerading as a hospital-acquired infection. *J Hosp Infect* 2001;47:76-77.
8. Marrie TJ, Bezanson G, Haldane DJ, Burbridge S. Colonisation of the respiratory tract with *Legionella pneumophila* for 63 days before the onset of pneumonia. *J Infect* 1992;24:81-86.
9. Pedro-Botet ML, Sabria M, Sopena N, García Núñez M, Morera J, Reynaga E. Environmental legionellosis and oropharyngeal colonization by *Legionella* in immunosuppressed patients. *Infect Control Hosp Epidemiol* 2002;23:279-281.

10. Marchesi I, Ferranti G, Bargellini A, et al. Monochloramine and chlorine dioxide for controlling *Legionella pneumophila* contamination: biocide levels and disinfection by-products (DBPs) formation in hospital water networks. *J Water Health* 2013;11:738-747
11. Casini B, Valentini P, Baggiani A, et al. Molecular epidemiology of *Legionella pneumophila* serogroup 1 isolates following long-term chlorine dioxide treatment in a university hospital water system. *Hosp Infect* 2008;69:141-147.

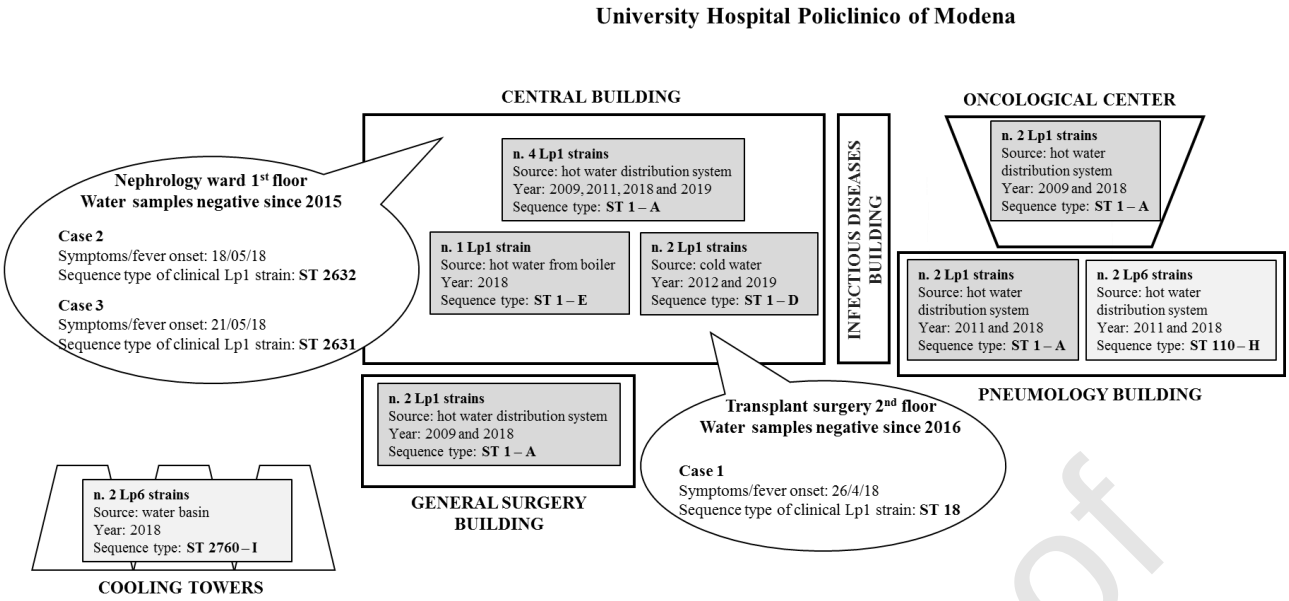

Figure 1. Scheme of the hospital buildings (figure not to scale) showing the temporal and spatial distribution of environmental *L. pneumophila* serogroup 1 and 6 strains isolated in the years 2009-2019, with their molecular profile.