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Case report

Non-*Legionella pneumophila* serogroup 1 pneumonia: Diagnosis of a nosocomial legionellosis with the Biofire Pneumonia plus panel

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ABSTRACT

We report a nosocomial case of *Legionella pneumophila* pneumonia caused by a serogroup 10 strain diagnosed with the Biofire® Pneumonia *plus* panel. Molecular investigations of the environment of the patient allowed us to identify the source of contamination.

83-year-old male was hospitalized in the Cardiology unit for pulmonary and cardiac decompensation. The patient's past medical history was significant for hypertensive cardiopathy, obstructive ventilatory disorder, SARS-CoV-2 pneumonia a few months ago and chronic gastritis. One month after his admission in the cardiology unit, physical examination revealed fever, cough and appearance of an oxygen deficiency. Day 0 laboratory tests showed inflammatory syndrome with elevated C-reactive protein (244 mg/L), elevated procalcitonin (2.18 µg/L), elevated white blood cells (11,4 $\times 10^9$/L) and negative result of the Biofire® respiratory 2.1 *plus* panel on naso-pharyngeal sample. TAP-scanner revealed right upper lobe lung disease with pleural effusion. Urine was sent to the microbiology laboratory and pneumococcal and *Legionella* urinary antigen tests were negative. Due to the pneumonia with pleural effusion noted on TAP-scanner, piperacillin/tazobactam was initiated as empirical treatment. In front with the clinical history of the patient and the absence of bacteriological documentation, a bronchoalveolar lavage (BAL) was collected and sent to the microbiology laboratory. Microscopic examination of the BAL showed an absence of erythrocytes, few polymorphonuclear cells, poor oro-pharyngeal flora; the culture was non-contributive on standard media and negative for mycobacteria. In the same BAL, BIOFIRE® pneumonia *plus* panel detected DNA of *Legionella pneumophila*. The antibiotic therapy was adapted and azithromycin was initiated. In our lab, *Legionella* strain was not recovered on BCYE (Buffer Charcoal Yeast Extract, Oxoid, Ireland) medium agar nor on GVPC (BCYE with antibiotics, Biomérieux, France) medium agar after incubation at 35 °C ± 2 on standard atmosphere so the BAL was sent to the National Reference Centre for *Legionella* (NRCL) and was plated on BCYE, BMPA (Buffered céfaMandole Polymyxine Anisomycine α-cétoglutarate, Oxoid, Ireland) and MWY (WADOWSKY and YEE medium, Oxoid, Ireland). The plates were incubated for 10 days at 35 °C in an aerobic atmosphere (BCYE and BMPA media) or in a 2.5% CO_2 atmosphere (BMPA and MWY media) [1]. On day 3, growth was detected on MWY agar plates with only two typical colonies; the isolate was identified as *Legionella pneumophila* by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) [2]. Agglutination of the isolate identified the strain as a *L. pneumophila* serogroup 10 (Prolex™ Lp serogroups 2-14 Latex polyclonal Reagents, Pro-Lab, Canada). After 14 days of treatment, the evolution of the patient was favorable.

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As the patient was hospitalized for one month in the same unit, it was considered as a nosocomial legionellosis. Investigations on the hospital water systems were conducted to identify the source of contamination. We obtained samples of water from 13 different points of the unit where the patient had stayed (shower, offices) and of the hot water storage heater. Because the hospital's water pipes are known to be colonized with *L. pneumophila,* all water outlets are equipped with 0.22 µm filters to protect patients and staff from exposure. During the investigations, it was noticed that the *patient had removed the protective filter from his vanity's tap. *L. pneumophila* serogroup 10 was isolated in GVPC medium agar from the departure (20 cfu/L) and from the return of the hot water system (45 cfu/L). The two environmental and one clinical *L. pneumophila* serogroup 10 isolates were typed at the NRCL using whole genome sequencing (WGS). Sequence Types (ST) and the Core genome multilocus sequence type (cgMLST)-50 genes defined by the ESCMID Study Group for *Legionella* Infections (ESGLI) were extracted from WGS data [3,4]. WGS data showed that the environmental isolates as the patient's strain shared the ST2490 and the same cgMLST confirming the link between clinical and environmental isolates. in conclusion, we report a case of nosocomial legionellosis diagnosed thanks to the multiplex PCR assay Biofire® FilmArray Pneumonia *plus* panel.

Among *Legionella* species, *Legionella pneumophila* is the most common etiologic agent in both community-acquired and nosocomial human infections. While 60 species and 80 serogroups have been reported, *L. pneumophila* serogroup 1 is the predominant serogroup. The analysis of isolates from patients with *Legionella* pneumonia in Europe and the United States, shows that up to 20% were caused by *L. pneumophila* serogroups 2-14 or *Legionella* other than *L. pneumophila* [5]. This rate is higher among nosocomial cases. Among the 433 clinical isolates from France in 2019, serogroup 10 is the second to last cause of *Legionella* pneumonia. According to Helbig et al., serogroup 10 was the fourth most common cause of *Legionella* pneumonia in Germany [6,7].

Cases of non-*L. pneumophila* serogroup 1 varied in severity from mild to severe and the clinical characteristics were often nonspecific [8]. To predict the probability of *Legionella* pneumonia, Fiumefreddo et al. proposed a six-point scoring system using dichotomized routine clinical and laboratory variables, including fever > 39.4 °C, C-reactive protein (CRP) value > 187 mg/L, Lactate dehydrogenase > 225 mmol/L, thrombocytopenia < 171 × 10^9/L, hyponatraemia (serum sodium < 133 mmol/L) and unproductive cough [9]. Our patient manifested with fever, cough and pleural effusion but no myalgia, no digestive symptoms or disturbance of consciousness. For laboratory findings, our patient showed hyponatraemia, moderate thrombocytopenia (160 × 10^9/L) and elevated CRP. From this pattern of clinical symptoms and laboratory findings, that did not respond to the criteria of this six-point scoring system and it seemed difficult to predict *Legionella* pneumonia in our patient. In absence of clinical evidence, the diagnostic methods used are primordial.

Most cases of *Legionella* pneumonia are diagnosed using a urinary antigen test, which presented the advantage of an excellent specificity but the inconvenient of misidentification of non-*L. pneumophila* serogroup 1 strains. Even though *L. pneumophila* serogroup 1 is responsible for most *Legionella* pneumonia episodes, it is advisable to have techniques to detect other serogroups and species of *Legionella* especially in the hospital environment where the presence of non-serogroup 1 *L. pneumophila* is frequent and a high number of susceptible immunosuppressed patients are present. The microbiological culture remains the "gold standard" *Legionella* pneumonia diagnosis but the sensitivity of detection of *Legionella* by culturing of clinical specimens is highly variable and recovery is long, dependent on the sample type and the experience of laboratory staff. Simplex and multiplex assay of real-time PCR have been developed in the early 2000s with variable sensitivity and specificity (17–100%; 95–100%) [10–13]. The Biofire® FilmArray Pneumonia *plus* panel (BioMérieux S.A., Marcy-l'Etoile, France) is a multiplex PCR assay able to simultaneously identify 27 of the most common pathogens involved in lower respiratory tract infections including three atypical bacteria (*L. pneumophila* [all serotypes], *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*). Performances of the Biofire® FilmArray Pneumonia *plus* panel for *L. pneumophila* are good with sensitivity varying between 90% and 100% [14–16]. For our patient, the Biofire® FilmArray Pneumonia *plus* panel has led to the diagnosis of *Legionella* pneumonia with a BAL sample. Thanks to this result, the antibiotic therapy of the patient was adapted very quickly without waiting the result of the culture.

Legionella nucleic acid detection is being increasingly recognized, standardized and implemented in the laboratory for rapid *Legionella* pneumonia diagnostic and detection. The French NRCL reported that an increased number of isolates of non-*L. pneumophila* serogroup 1 isolated from patients was observed since a few years thanks to the increasing use of multiplex PCR which targeted the *pneumophila* species. According to our experience with this case, molecular tests whether simplex or multiplex, must be systematically included in the first diagnosis tests of *Legionella* pneumonia especially in the nosocomial suspected forms. This allows both rapid patient management and the eradication of the environmental source to avoid an outbreak. The molecular tests are probably gradually replacing serology tests and perhaps soon definition of a confirmed *Legionella* pneumonia will also comprise molecular tests. Our case also confirms that the choice of the multiplex respiratory panel is of importance and must contain the detection of *Legionella sp*. or *Legionella pneumophila* for the diagnosis of community acquired or nosocomial pneumonia.

For epidemiologic studies, it is important to obtain the *Legionella* strains from patients. The comparison of these strains with environmental isolates provides evidence of or disproves the transmission of the strains from a suspected source of infection to humans. In our case, the strain was difficult to isolate. The first investigations in our laboratory did not yield the strain, while standard media agar and standard incubation conditions were used, because of excessive contamination of the media despite standard decontamination procedures. The isolation of the strain only with MWY agar plate questioned the use of the different medium for *Legionella* isolation. For pulmonary samples, the BMPA and the MWY medium agar seem to be the most adapted media in addition of the BCYE standard media [1,17]. Difficulties for isolating strains highlight importance of molecular test for environmental samples also.

For the isolation of the source of contamination, different samples were done at 13 different points of the cardiology unit where the patient had stayed and of the hot water storage heater. The isolation of non-serogroup 1 *L. pneumophila* in the hot water storage has led us to conduct an epidemiological investigation and to compare these strains with the patient's isolate. The results of the molecular comparison allowed us to conclude that the strains were identical and that we have identified the source of contamination of our patient. Today, molecular typing as whole genome sequencing, which replaces the sequence base typing, enables to compare and to identify precisely environmental and clinical strains and allows identifying the environmental source of contamination [18–20].

In conclusion, even though *L. pneumophila* serogroup 1 is responsible for most *Legionella* pneumonia, it is advisable to have molecular techniques to detect other serogroups or other species of *Legionella* especially in the hospital environment, where the presence of non-serogroup 1 *L. pneumophila* is frequent and a high number of immunosuppressed patients are present. This case also highlights the growing importance of the multiplex molecular respiratory panel in the management of patients with suspected nosocomial pneumonia.

Ethical approval

None.

Consent

None.

CRediT authorship contribution statement

All authors contributed to the manuscript conception and design. The first draft of the manuscript was written by Camille Courboules, Nathalie Dournon, Faten El Sayed and Anne-Laure Roux and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest

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