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Legionella pneumophila serogroup 5 infection in the presence of multiple environmental contamination. The importance of a bacteriological diagnosis

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Abstract

Legionella pneumophila is a pathogen that causes severe pneumonia in humans; *L. pneumophila* serogroup 1 accounts for at least 90% of infections. This is not linked to an environmental predominance of *Legionella pneumophila* 1, but may be due to a greater virulence of the strain. *L. pneumophila* sg 5 has also been reported, albeit less frequently, to be a cause of the disease. We report a case of *L. pneumophila* sg 5 occurring in a large hospital in southern Italy (Apulia region), where both *L. pneumophila* sg 1 and sg 5 were detected in the water supply; the nosocomial origin was demonstrated by molecular subtyping (PFGE). An environmental investigation, performed immediately after diagnosis of the case of legionellosis, identified a low *L. pneumophila sg 5* contamination level. Our experience highlights that in hospital, risk assessment, in order to institute control measures for *Legionella,* should be carried out not only in response to a case of the disease and/or in risk wards only, as described in the Italian Guidelines, but periodically in every ward. The present study confirms that, although in the community *L. pneumophila* sg 1 is the most frequent strain isolated in both outbreaks and isolated cases, in hospital other serogroups and species may often cause infection because of the high susceptibility of the hosts.

Key words: Legionella spp, legionellosis, bacteriological diagnosis, epidemiology

Introduction

Legionella pneumophila is a pathogen that causes severe pneumonia in humans. It is frequently found in water distribution systems and has been repeatedly associated with both hospital and community-acquired infections, especially in immunocompromised patients. *L. pneumophila* serogroup (sg) 1 accounts for at least 90% of infections [1], followed by serogroup 6 [2]. *L. pneumophila* sg 5 has also been reported, albeit less frequently, as a cause of disease [3-12]. The high frequency of *L. pneumophila* 1 isolation from clinical samples is not linked to an environmental predominance but may be due to a higher virulence of the strain or a more efficient intracellular growth [13, 14]. It must, however, be noted that a precise etiological diagnosis is biased by the difficulty of isolating the germ when the patient has already received antibiotics that mask the identity of the pathogen [15, 16]. For *Legionella* pneumoniae this problem is compounded by the patient's difficulty in bringing up sputum and by, in general, limited laboratory experience in actually isolating *Legionella* from clinical specimens [17, 18]

We report a case of *L. pneumophila* sg 5 occurring in a large hospital in southern Italy (Apulia region), where both *L. pneumophila* sg 1 and sg 5 were detected in the water supply; the nosocomial origin of the strains was demonstrated by molecular subtyping.

Case report

The patient. In June 2001, a 64-year-old woman was admitted to a General Medical ward in a hospital located in Southern Italy, for investigation. She had a history of *IgM myeloma* and *Waldestrom's Disease* since December 1993, for which she had received immunosuppressive therapy followed by interferon. In 1999 she was treated with chemotherapy.Antibiotics + cortisone were given in December 2000 for a persistent non productive cough, but with no clinical response. In May 2001, one month prior to this admission, she

had been admitted to another hospital for fever (39 °C), dyspnoea, chest pain and osteomyalgia. CT scans showed a pulmonary infiltrate in the right middle and lower lobe, with pleural exudates at the right base, as well as left focal areas. Bacteriological and cytological investigations of bronchial fluid samples were negative. Neither during the first admission (May 2001) nor during the second (June 2001) did the patient undergo any *Legionella* tests. In July 2001, one month after the second admission, due to persistence of the productive cough, the patient was transferred from the General Medical to the Pneumology ward of the same hospital. Her respiratory conditions worsened, showing extensive bilateral pulmonary consolidation. Her white blood cell count was 13,490/mm^3, with a neutrophil count of 97%. Pleural exudates and expectorate were negative for common bacterial cultures. Serological tests for chlamydia, mycoplasma, cytomegalovirus, varicella-zoster virus, Epstein-Barr virus (EBNA and VCA), adenovirus, enterovirus and influenza viruses were also negative. Legionnaires' disease was suspected, and sputum, urine and serum specimens were examined for *Legionella* infection, with positive results. Despite Ciprofloxacin therapy (500 mg every 12 h), the patient died at the beginning of August.

Laboratory investigations. A sputum specimen was plated on selective BCYE-GVPC agar (Biolife, Italy), and cultured at 30°C in an atmosphere supplemented with 2.5% CO_2. The urinary antigen was detected by an enzyme immunoassay (EIA), using a commercially available kit for the *in vitro* detection of soluble *Legionella* antigen in urine (Biotest, Italia). Antibody titers were determined by an indirect immunofluorescence assay firstly using a polyvalent kit for *L. pneumophila* sg 1 to 6 (MarDx, Carlsbad, CA, USA purchased from Arnika, Italy) and subsequently using antigens prepared at the ISS, Rome, with *L. pneumophila* sg 1, Philadelphia 1 strain, and with the patient's isolate.

Environmental culturing for *Legionella spp* was performed in the patient's home and in the hospital wards where the patient had been exposed to water supplies (sinks, basins and shower taps).

Legionella reagents by latex agglutination (Oxoid, Italy) were used to screen suspected clinical and environmental *Legionella* colonies. Polyclonal reagents for direct immunofluorescence (MarDx, Carlsbad, CA, purchased from Arnika, Italy) were used for the identification of *Legionella* strains, and monoclonal antibodies (Institut für Medizinische Mikrobiologie und Hygiene, Dresden, Germany) were used for definitive serogrouping and subtyping of *L. pneumophila* sg 1 strains [19].

Genotyping. The genomic profiles from the clinical and environmental strains were compared by PFGE. The assay was performed as previously described [20], using the *Not* I low-cutting restriction enzyme (Roche, Italy).

Results

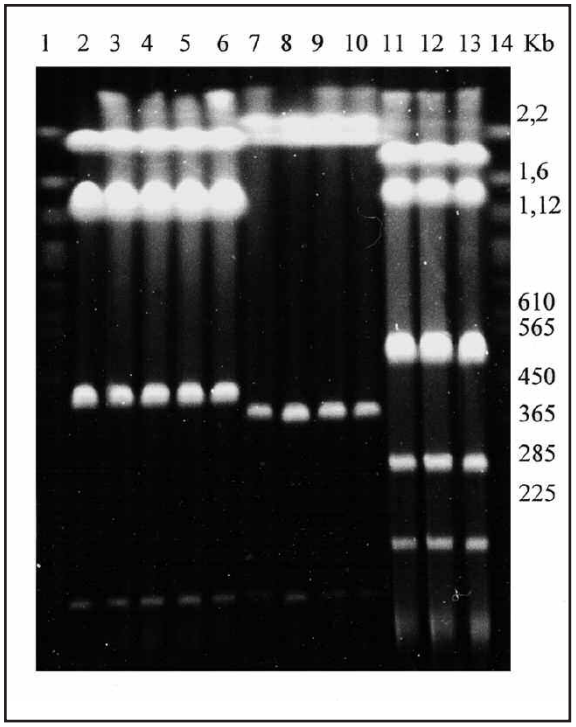
Culture of the patient's specimen yielded *L. pneumophila* sg 5 and her urinary antigen test was positive (ratio to negative, 2.6). Two serum samples were collected in the Pneumology Ward: the first one 5 days after admission, the second 21 days later. A seroconversion between collection of these two samples (1:256 to 1:2048) was demonstrated both with the polyvalent *L. pneumophila* antigen and with the *L. pneumophila* sg 5 clinical isolate. Both serum samples were negative (<1:16) when tested against *L. pneumophila* sg 1. Cultures of the patient's home water supply did not yield *Legionella* isolates. *L. pneumophila* sg 1 subgroup Bellingham (100 - 400 cfu/L) and sg 5 (400 cfu/L) were isolated from the water in the hospital's General Medical Ward and *L. pneumophila* sg 5 (200-30,000 cfu/L as well as *Legionella bozemanii* (100-400 cfu/L) were isolated from water in the Pneumology ward.

The patient's isolate and *L. pneumophila* sg 5 isolated from the medical ward showed a very similar genomic pattern. These strains showed a distinct genomic profile from that of the *L. pneumophila* sg 1 isolated from the same ward and from the *L. pneumophila* sg 5 isolated from the Pneumology ward (Figure 1).

Discussion

Legionella is a widely distributed microorganism in man-made environments and multiple species and serogroups can co-exist in the same water supply. *L. pneumophila* sg 1 is a frequent cause of pneumonia, whereas confirmed cases of *L. pneumophila* sg 5 are rare. Definitive documentation of the infectious agent can only be obtained by culture of the microorganism from clinical specimens. In the present case *L. pneumophila* sg 5 was isolated from the sputum of a patient at a hospital where nosocomial *Legionella* infections had not been previously detected. The urinary antigen test was positive, but this test detects specific *Legionella* antigen and recognizes all *L. pneumophila* serogroups with a relatively wide spectrum of cross-reactivity, as well as other *Legionella* species [6, 21, 22]. For this reason, taking into account only the positive urinary antigen, it was not possible to identify the specific species and serogroup of *Legionella* that had caused the disease. Antibody titres against a

Figure 1. Pulsed-field gel electrophoresis of Not I-cleaved genomic DNA of clinical and environmental *L. pneumophila* strains.



Lanes: 2, patient's *L. pneumophila* serogroup 5 isolate; 3 to 6, environmental *L. pneumophila* serogroup 5 isolates from the hospital general Medicine ward; 7 to 10, *L. pneumophila* serogroup 1 isolated from the hospital general Medicine ward; 11 to 13, *L. pneumophila* serogroup 5 isolated from the hospital Pneumology ward; 1 and 14, molecular size markers (*Saccharomyces cerevisiae* YPH 755 chromosomes; Bio-Rad, Italy).

commercially available polyvalent antigen showed seroconversion of *L. pneumophila* sg 1 to 6. No seroconversion was seen using antigens prepared with *L.pneumophila* sg 1 Philadelphia, whereas there was strong evidence of antibodies to *L. pneumophila* sg 5 in the patient's serum samples when tested against the clinical isolate. Moreover, the similarity between the PFGE clinical pattern and some of the environmental strains of *L. pneumophila* sg 5 demonstrated that the infection was indeed due to *L. pneumophila* sg 5. The PFGE also showed that the *L. pneumophila* sg 5 strains isolated from the two hospital wards had different genomic patterns, and that the infection was acquired in the General Medical Ward (Figure 1).

The data reported herein underlines the necessity to confirm the diagnosis of a suspected *Legionella* pneumonia by bacteriological culture. This will avoid an erroneous etiological diagnosis, facilitate the detection of the source of the infection, and contribute to a better understanding of the incidence of *L. pneumophila* sg *non-1* infections.

In the present study, the environmental investigation carried out immediately following the case of Legionnaires' disease identified a low *L. pneumophila* sg 5 contamination level. In fact, as recently demonstrated by other authors [23], even if the environmental contamination level is low it is possible to observe cases of disease. This highlights the fact that, in our opinion, risk assessment in order to institute control measures for *Legionella* should be carried out in hospitals not only in response to a case of disease and/or only in risk wards, as reported in the Italian Guidelines, but periodically in every ward, in order to prevent the disease [18].

Our experience underlines that although *L. pneumophila* sg 1 is considered the most pathogenic serogroup, *L. pneumophila* sg 5 can be responsible for the disease if both *L. pneumophila* sg 1 and sg 5 are present in the environment. In accordance with other studies [24, 10], although in the community *L. pneumophila* sg 1 is the most frequent strain isolated in both outbreaks and isolated cases, in hospitals other serogroups and species may often cause isolated cases or outbreaks of infection because of the high susceptibility of the hosts.

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