ORIGINAL ARTICLE

Sequence based typing of *Legionella pneumophila* sg 1 isolated in nosocomial acquired infections in Apulia, Southern Italy

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Key words

Legionella pneumophila sg 1 • Sequence based typing • Nosocomial acquired infections

Summary

Objective. *The present report aims to molecularly characterize seven clinical L. pneumophila (L. pn.) sg 1 isolated from nosocomial acquired infections in Apulia region, using the European Working Group on Legionella Infections (EWGLI), sequence-based typing (SBT) and amplified fragment length polymorphism (AFLP) protocols and to compare the identified sequence types (STs) with those available in the EWGLI database.*
Methods. *In the period, January 2000 - December 2012, 151 cases (136 of community and 15 of nosocomial origin) of Legionnaires' disease were notified to the Regional Center for Epidemiology. With regard to nosocomial cases, 8 were confirmed by the isolation of Legionella spp. from respiratory secretions. These clinical isolates were characterized by amplified fragment length polymorphism (AFLP) and sequence-based typing (SBT), using the EWGLI standardized protocol.*
Results. *The clinical isolates belong to ST42, ST23 and ST1. The AFLP confirms the SBT results. Comparing the STs herein detected with those already in the EWGLI SBT database, the 3 STs are frequent in other European countries.*
Conclusions. *The molecular analysis demonstrates that the 3 STs are the most frequent in Italy and in Europe, supporting the hypothesis that some specific L. pn. sg 1 clones have gained widespread dissemination probably due to a common ecological niche. Further researches are required to investigate the potential changing incidence of STs and the fitness of emerging strains or clonal groups in environmental strains.*

Legionnaires' disease (LD) is a form of pneumonia with no clinical features that clearly distinguish it from other types of pneumonia. Since the transmission occurs via inhalation of aerosols contaminated by *Legionella* spp., the frequent sources of infection are hot water systems, cooling towers and other water disseminators [1, 2]. At the same time, the problem is particularly important in hospital, where medical equipment can also be a potential source of infection (endoscopes, devices for artificial respiration and oxygen therapy, dental devices, etc) [3-5].
Legionella pneumophila (*L. pn.*) is the aetiological agent of approximately 90% of Legionellosis cases, and *L. pn.* serogroup 1 (sg 1) is the predominant one [6, 7]. The characterization of clinical isolates by molecular typing methods is essential for epidemiological investigations. The European Working Group on *Legionella* Infections (EWGLI) recently renamed European Study Group for *Legionella* Infections (ESGLI) has provided a description of a standardized protocol for the molecular identification of *Legionella* spp. firstly by amplified fragment length polymorphism (AFLP) [8] and more recently by sequence-based typing (SBT) [9]. In particular, SBT is a rapid and discriminatory method based on the sequence of seven loci (*flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA* and *neuA*) of *L. pn.* Using the SBT protocol, the EWGLI has built up a database allowing the assignment of the seven ordered alleles represented as a Sequence Type (ST), or allelic profile. Successively, each study participant can submit his own data in order to increase information in the database and verify the circulation of *L. pn.* genotypes in different geographic areas throughout the available *Legionella* isolates SBT map.
Apulia Region, with a land surface of 19,347 sq. km, is located in Southeastern Italy and its Regional Center for Epidemiology (OER) includes in its mission the surveillance of LD and the control of the environmental spread of *Legionella* spp. Information obtained from the environmental and clinical surveillance are maintained in a local computer database with real-time availability of all the information, allowing better programming of the necessary measures for prevention and control. At the same time, all environmental and clinical isolates of *Legionella* spp. are collected.
In the period, January 2000 - December 2012, 151 cases of LD were notified to the OER, according to the national compulsory reporting system: 136 of community and 15 of nosocomial origin. With regard to nosocomial LD cases, patient median age was 68.5 years (range 30-98), and 60% were females. Eight patients (53.3%) were confirmed by two or more diagnostic methods; overall, 80% tested positive for urinary antigen, 53.3% were

Tab. I. Clinical and molecular data of the 7 *L. pn.* sg 1 isolates recovered from nosocomial acquired infections.

Isolate identification	Type of specimen, date of collection	SBT allele number (ST) (flaA, pilE, asd, mip, mompS, proA, neuA)	AFLP pattern type
Leg1u	Sputum, 11 February 2005	2,3,9,10,2,1,6 (ST23)	A
Leg2u	BA, 10 March 2004	4,7,11,3,11,12,9 (ST42)	B
Leg3u	BA, 6 October 2003	4,7,11,3,11,12,9 (ST42)	B
Leg4u	Sputum, 6 August 2002	4,7,11,3,11,12,9 (ST42)	B
Leg5u	BA, 11 September 2003	4,7,11,3,11,12,9 (ST42)	B
Leg6u	BA, 22 March 2003	4,7,11,3,11,12,9 (ST42)	B
Leg7u	Sputum, 15 March 2005	1,4,3,1,1,1,1 (ST1)	C

BA: bronchoaspirate.

confirmed by the isolation of *Legionella* spp. from respiratory secretions, 33.3% showed seroconversion and 13.3% had one single positive antibody titer. All cases diagnosed by cultural examination acquired the infection in the largest Hospital of Apulia region which is composed up of 32 separate buildings with 60 bed-operating units, for a total bed capacity of 1,400. *L. pn*. sg 1 and *L. pn.* sg 5 [10] were respectively isolated from seven and one patients. Since 2000, in order to contain the spread of the disease, the water system of the hospital underwent to a periodical environmental monitoring for the detection of *Legionella* spp. in water samples; moreover, additional environmental investigations are carried on when nosocomial cases are detected. According to the procedures described in the Italian Guidelines [11] treatment of disinfection (i.e., hyper-chlorination) is performed.

The present report aims to molecularly characterize the seven clinical strain of *L. pn*. sg 1 isolated from nosocomial acquired infections in Apulia region, using the EWGLI SBT and AFLP protocols (version 4.2 and 1.2, respectively, http://www.ewgli.org), and to compare the identified sequence types with those available in the EWGLI database. *L. pneumophila* strain ATCC 33153 was included as control. The AFLP profiles were phylogenetically analyzed by the software GelCompare II (Applied Maths, Belgium) using as a cluster analysis, the Dice coefficient and the method "unweighted pair group method with averages" (UPGMA). In agreement with Fry et al. [12], the isolates with homology greater than 90% have been considered homologous strains.

The results are summarized in Table I. Our isolates can be classified into 3 distinct STs, by using the SBT method: five isolates (71.4% of all isolates) belong to the ST42, one to the ST23 (14.3%), and one to the ST1 (14.3%). The alphabetic designations (A, B and C) based on AFLP electrophoresis pattern confirm the SBT results.

The detected STs were submitted to the EWGLI SBT-database and compared to those already in. According to the EWGLI database, the ST42 profile was found ubiquitously across the Netherlands (n = 49), France (n = 29) and UK (n = 24). In Italy, a part from our 5 ST42, other seven ST42 strains were isolated: 5 of clinical origin (1 nosocomial acquired) and two from environment.

With regard to ST1, the EWGLI database shows that its geographic distribution is mainly in France (n = 225) and UK (n = 162). In Italy, a part from our unique ST1, 26 isolates were identified: 16 were of clinical origin, of which 9 associated with nosocomial infections, and 10 were environment strains. Finally, genotype ST23 was largely present in France (n = 364) followed by the Netherlands (n = 31). In Italy, a part from our unique ST23, 19 isolates were identified: 13 were clinical strains (all community acquired or travel associated cases) and 6 environmental isolates. In addition, recently a cluster of travel-associated LD caused by ST23 in a small town located in North of Italy was documented by the Italian Institute of Health (ISS) [13].

Our molecular analysis carried out on *L. pneumophila* sg 1 clinical isolates associated with nosocomial infections, demonstrate perfectly overlapping results by both molecular methods, AFLP and SBT, as already shown by other Authors [14, 15]. In addition, these results confirm previous data on the distribution of STs in our country; as a matter of fact, the three STs identified are the most frequent in Italy. In particular according to the EWGLI records the ST1 is the most frequent, followed by ST23 and ST42. These STs strains are frequently isolated also in Europe [16, 17] and in other large geographic area such as Canada, China and Japan [7, 18, 19]. These findings support the hypothesis that some specific *L. pn.* sg 1 clones have gained widespread dissemination probably due to a common ecological niche. The Apulia region can be consider a well-integrated part of European ecosystem where the most common sequence types of *L. pn*. sg 1 appears to be not only ubiquitous and characterized by common epidemiological behaviors but also easily spreading.

Since Legionnaires' disease occurs both in sporadic and epidemic forms, a rapid molecular identification of *L. pn*. is strongly suggested to control epidemics and to identify the source of infection.

In conclusions, further researches are required to investigate the potential changing incidence of STs and the fitness of emerging strains or clonal groups in environmental strains too.

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