



## Control and prevention measures for legionellosis in hospitals: A cross-sectional survey in Italy

Maria Teresa Montagna<sup>a,\*</sup>, Osvalda De Giglio<sup>a</sup>, Christian Napoli<sup>b</sup>, Giusy Diella<sup>a</sup>, Serafina Rutigliano<sup>a</sup>, Antonella Agodi<sup>c</sup>, Francesco Auxilia<sup>d</sup>, Tatjana Baldovin<sup>e</sup>, Francesco Bisetto<sup>f</sup>, Luca Arnoldo<sup>g</sup>, Silvio Brusaferrò<sup>g</sup>, Marina Busetti<sup>h</sup>, Gioia Calagreti<sup>i</sup>, Beatrice Casini<sup>j</sup>, Maria Luisa Cristina<sup>k</sup>, Rossano Di Luzio<sup>l</sup>, Maurizio Fiorio<sup>m</sup>, Maurizio Formoso<sup>n</sup>, Giorgio Liguori<sup>o</sup>, Enrica Martini<sup>p</sup>, Andrea Molino<sup>q</sup>, Placido Mondello<sup>r</sup>, Ida Mura<sup>s</sup>, Roberto Novati<sup>t</sup>, Giovanni Battista Orsi<sup>u</sup>, Andrea Patroni<sup>v</sup>, Anna Poli<sup>w</sup>, Gaetano Privitera<sup>j</sup>, Giancarlo Ripabelli<sup>x</sup>, Andrea Rocchetti<sup>y</sup>, Francesco Rose<sup>z</sup>, Mario Sarti<sup>aa</sup>, Sandra Savini<sup>p</sup>, Antonio Silvestri<sup>ab</sup>, Luisa Sodano<sup>ab</sup>, Anna Maria Spagnolo<sup>k</sup>, Stefano Tardivo<sup>ac</sup>, Valeria Teti<sup>ad</sup>, Maria Valeria Torregrossa<sup>ae</sup>, Emanuele Torri<sup>af</sup>, Licia Veronesi<sup>ag</sup>, Raffaele Zarrilli<sup>ah</sup>, Claudia Pacifico<sup>ai</sup>, Antonio Goglio<sup>aj</sup>, Matteo Moro<sup>ak</sup>, Cesira Pasquarella<sup>ag</sup>

<sup>a</sup> Department of Biomedical Science and Human Oncology, University of Bari Aldo Moro, Square G. Cesare 11, 70124 Bari, Italy

<sup>b</sup> Department of Medical and Surgical Sciences and Translational Medicine, Sapienza University of Roma, Square A. Moro 5, 00185 Roma, Italy

<sup>c</sup> Department of Medical and Surgical Sciences and Advanced Technologies "GF Ingrassia", University of Catania, Str. S. Sofia, 87, Comparto 10 Edificio C, 95123 Catania, Italy

<sup>d</sup> Department of Biomedical Sciences for Health, University of Milano, Str. Pascal 36, 20133 Milano, Italy

<sup>e</sup> Department of Cardiac, Thoracic and Vascular Sciences, University of Padova, Hygiene and Public Health Unit, Str. Loredan, 18.35131 Padova, Italy

<sup>f</sup> Presidio Ospedaliero di Camposampiero, AULSS6 Euganea Str. P. Cosma 1, 35012 Camposampiero, PD, Italy

<sup>g</sup> Department of Medicine, University of Udine, Str. Colugna 50, 33100 Udine, Italy

<sup>h</sup> University Hospital ASUTS, Microbiology Unit, Strada di Fiume, 447, 34149 Trieste, Italy

<sup>i</sup> Hospital "Alto Tevere", AUSL Umbria 1, Città di Castello, PG, Italy

<sup>j</sup> Department of Translational Research, N.T.M.S. - Hygiene and Epidemiology Unit, University of Pisa, Str. S. Zeno 35-39, 56127 Pisa, Italy

<sup>k</sup> Department of Health Sciences, University of Genova, Str. A. Pastore 1, 16132 Genova, Italy

<sup>l</sup> Hospital Santo Spirito, AUSL di Pescara, Pescara, Italy

<sup>m</sup> AOU Perugia, Italy

<sup>n</sup> Hospital "Miulli", Str. 127 km 4.1, Santeramo-Acquaviva delle Fonti, BA, Italy

<sup>o</sup> Department of Movement Sciences and Wellbeing, University "Parthenope", Napoli, Italy

<sup>p</sup> AOU "Ospedali Riuniti", Str. Conca, 71, 60126 Ancona, Italy

<sup>q</sup> Hospital "Madonna delle Grazie", Contrada Cattedra Ambulante s.n.c. 75100 Matera, Italy

<sup>r</sup> Hospital "G. Martino", Messina, Italy

<sup>s</sup> Department of Biomedical Science, University of Sassari, Sassari, Italy

<sup>t</sup> Regional Hospital, Aosta, Italy

<sup>u</sup> Department of Public Health and Infectious Disease, Sapienza University of Roma, Roma, Italy

<sup>v</sup> ASST Valcamonica, Str. Manzoni 142, 25040 Esine, BS, Italy

<sup>w</sup> Hospital "San Giovanni di Dio", Str. Torre Galli 3, 50143 Firenze, Italy

<sup>x</sup> Department of Medicine and Health Sciences "Vincenzo Tiberio", University of Molise, Campobasso, Italy

<sup>y</sup> ASO "SS. Antonio, Biagio and C. Arrigo", Str. Venezia 17, 15121 Alessandria, Italy

<sup>z</sup> Hospital of Cosenza, Italy

<sup>aa</sup> Hospital "OCSAE", Str. Giardini 1355, Baggiovara, MO, Italy

<sup>ab</sup> Hospital "San Camillo Forlanini", Circonvallazione Gianicolense 87, 00152 Roma, Italy

<sup>ac</sup> Department of Diagnostic and Public Health, University of Verona, Str. Le Grazie 8, 37134 Verona, Italy

<sup>ad</sup> Azienda Sanitaria Provinciale, Catanzaro, Italy

<sup>ae</sup> Department of Sciences for Health Promotion and Mother-Child Care "G. D'Alessandro", University of Palermo, Str. Vespro, 133, 90127 Palermo, Italy

<sup>af</sup> Department of Health and Social Policy, Str. Gilli 4, 38123 Trento, Italy

<sup>ag</sup> Department of Medicine and Surgery, University of Parma, Str. Volturmo 39, Parma, Italy

<sup>ah</sup> Department of Public Health, University of Napoli "Federico II", Str. S. Pansini, 5, Napoli, Italy

<sup>ai</sup> Centre of Biostatistics for Clinical Epidemiology, School of Medicine and Surgery, University of Milano-Bicocca, Milano, Italy

<sup>aj</sup> SIMPIOS, board of directors, Bergamo, Italy

<sup>ak</sup> Hospital "San Raffaele", Str. Olgettina 60, 20132 Milano, Italy

\* Corresponding author.

E-mail address: [mariateresa.montagna@uniba.it](mailto:mariateresa.montagna@uniba.it) (M.T. Montagna).

## ARTICLE INFO

**Keywords:**  
 Legionellosis  
 Hospital  
 Control measures  
 Prevention  
 National survey

## ABSTRACT

Risk assessment, environmental monitoring, and the disinfection of water systems are the key elements in preventing legionellosis risk.

The Italian Study Group of Hospital Hygiene of the Italian Society of Hygiene, Preventive Medicine, and Public Health and the Italian Multidisciplinary Society for the Prevention of Health Care-Associated Infections carried out a national cross-sectional survey to investigate the measures taken to prevent and control legionellosis in Italian hospitals.

A multiple-choice questionnaire was developed, comprising 71 questions regarding hospital location, general characteristics, clinical and environmental surveillance, and control and preventive measures for legionellosis in 2015. Overall, 739 hospitals were enrolled from February to June 2017, and 178 anonymous questionnaires were correctly completed and evaluated (response rate: 24.1%). The survey was conducted using the SurveyMonkey® platform, and the data were analyzed using Stata 12 software.

Of the participating hospitals, 63.2% reported at least one case of legionellosis, of which 28.2% were of proven nosocomial origin. The highest case numbers were reported in the Northern Italy, in hospitals with a pavilion structure or cooling towers, and in hospitals with higher numbers of beds, wards and operating theaters. Laboratory diagnosis was performed using urinary antigen testing alone (31.9%), both urinary antigen testing and single antibody titer (17.8%), or with seroconversion also added (21.5%). Culture-based or molecular investigations were performed in 28.8% and 22.1% of the clinical specimens, respectively.

The water systems were routinely tested for *Legionella* in 97.4% of the hospitals, 62% of which detected a positive result (> 1000 cfu/L). *Legionella pneumophila* serogroup 2–15 was the most frequently isolated species (58.4%). The most common control measures were the disinfection of the water system (73.7%), mostly through thermal shock (37.4%) and chlorine dioxide (34.4%), and the replacement (69.7%) or cleaning (70.4%) of faucets and showerheads.

A dedicated multidisciplinary team was present in 52.8% of the hospitals, and 73% of the hospitals performed risk assessment. Targeted training courses were organized in 36.5% of the hospitals, involving nurses (30.7%), physicians (28.8%), biologists (21.5%), technicians (26.4%), and cleaners (11%).

Control and prevention measures for legionellosis are present in Italian hospitals, but some critical aspects should be improved. More appropriate risk assessment is necessary, especially in large facilities with a high number of hospitalizations. Moreover, more sensitive diagnostic tests should be used, and dedicated training courses should be implemented.

## 1. Introduction

The genus *Legionella* includes Gram-negative microorganisms living in natural and artificial water systems. These microorganisms are able to grow at 25–50°C, especially in backwater systems. Infected sources (e.g., faucets, showerheads, or cooling towers) can spread spray or droplets of water containing *Legionella*, leaving airborne particles of less than 5 µm in diameter that can be deeply inhaled.

These microorganisms can cause a severe form of pneumonia, known as Legionnaires' disease (LD), or a flu-like illness, the Pontiac fever, which is normally acquired by inhaling contaminated particles suspended in air (Montagna et al., 2006, 2014, 2017a; Rota et al., 2013). To date, about 60 species of *Legionella* are known. *Legionella pneumophila* (Lpn) is the species most frequently associated with human disease and includes 16 serogroups (sg). Though the literature states that Lpn sg 1 is the most common isolate in humans, an increasing number of cases are being attributed to other *Legionella* species and serogroups (ECDC, 2015; Napoli et al., 2010).

The association between potable water and nosocomial legionellosis was described for the first time approximately 40 years ago (Tobin et al., 1980). The complexity of hospitals' water systems and the vulnerability of hospitalized patients increase the risk for *Legionella* transmission and severe outcomes. A review of 27 LD outbreaks investigated by the Centers for Disease Control and Prevention from 2000 to 2014 indicated that health care-associated LD accounted for 33% of the outbreaks, 57% of the outbreak-associated cases, and 85% of the outbreak-associated deaths (Garrison et al., 2016; Soda et al., 2017). Only one case of probable person-to-person transmission has been reported (Correia et al., 2016). Currently, the hot water system is thought to be the most frequent source of cases or outbreaks of LD in hospitals (Borella et al., 2008; Montagna et al., 2017b; Napoli et al., 2010).

In Italy, according to the National Surveillance System for LD, the

number of cases has been steadily increasing, from 192 cases in 2000 to 1710 cases in 2016. Most of these cases are community-acquired, followed by those that are travel-associated and then those that are associated with health care (5.3% in 2015). Overall, the case fatality rate in Italy ranges from 8% to 17% (ISS, 2016; Rota et al., 2013). Clinical outcomes are affected by comorbidities, with mortality ranging from 40% to 80% among untreated immunocompromised patients, and from 5% to 30% with appropriate therapy (ISS, 2016; Rota et al., 2013).

In 2000, the Italian Institute of Health issued its first guidelines for the prevention and control of legionellosis. These were followed by instructions for laboratories involved in microbiological diagnosis, environmental control, tourist accommodation, and spas in 2005. In 2015, all of the national recommendations, including those for hospitals, were incorporated in a single updated document (Linee, 2015). These instructions list risk assessment evaluation as one of the most effective prevention measures to manage *Legionella* spp. contamination in water systems. This is particularly relevant in hospitals because, in addition to the water system, health practices concerning the airways (e.g., ventilation, aspiration, devices for artificial respiration and oxygen therapy, and dental tools) can increase the risk of infection (Castiglia et al., 2008; Pasquarella et al., 2010, 2012; Montagna and De Giglio, 2018). Nevertheless, the control and prevention of legionellosis remain critical issues in Italian health care settings. These guidelines are considered reference documents and not compulsory protocol for prevention, and very little is known about their implementation.

In light of this situation, the Italian Study Group of Hospital Hygiene of the Italian Society of Hygiene, Preventive Medicine, and Public Health (GISIO-SIH) and the Italian Multidisciplinary Society for the Prevention of Health Care-Associated Infections (SIMPIOS) conducted a national survey to i) collect information about specific measures for legionellosis control and prevention adopted in Italian hospitals; ii) identify the critical aspects of LD control and prevention; and iii) plan

targeted corrective measures, where necessary.

## 2. Material and methods

### 2.1. Study design

After consulting the members of the GISIO-SItI and SIMPIOS working groups in 2016, each working group developed a list of hospitals in its own region and contacted the medical management of each hospital by e-mail to request their participation in the study. Hospitals in all regions of Italy were invited to take part in the study on a voluntary basis and without remuneration. After having given their verbal informed consent (as required by Italian privacy law), each participant was asked to complete an anonymous questionnaire. The 71 item questionnaire was divided into six sections, collecting data on location and general structural characteristics of hospital, clinical and environmental surveillance, and control and preventive measures for legionellosis in 2015. To assess the accuracy of the questionnaire, an internal pre-validation procedure was carried out at the University of Bari Aldo Moro, involving 10 regional hospitals (Cronbach's  $\alpha=0.71$ , indicating good internal consistency).

The survey was conducted from February to June 2017, using SurveyMonkey® software to facilitate archiving and data processing. This study followed the principles of the World Medical Association Declaration of Helsinki and does not report the results of any experiment on humans or human samples, or research on identifiable human material or data.

### 2.2. Clinical and environmental surveillance for Legionella

A brief description of the main tests investigated in the

questionnaire and regarding the clinical and environmental surveillance of legionellosis is shown below.

#### 2.2.1. Urine antigen test

This test uses monoclonal antibodies that recognize most Lpn sg 1 lipopolysaccharide antigens. It, however, fails to detect disease caused by other serogroups of Lpn or other species of *Legionella* (Pierre et al., 2017). Approximately 8% of patients with LD do not excrete antigen (Munoz et al., 2009). The sensitivity and specificity range from 69% to 100% and 99–100% respectively (Shimada et al., 2009).

#### 2.2.2. Culture of respiratory tract

Culture can identify all of the known *Legionella* species and serogroups. The sensitivity and specificity of culture on selective media is 81% and 99%, respectively (Lindsay et al., 2004). A positive result usually appears within 3–5 days, although 2 weeks may be required because additional treatment, necessary to reduce background flora that can inhibit the growth of *Legionella*.

#### 2.2.3. Serological tests

Of the various antibody detection methods, indirect immunofluorescence assays (IFA) and enzyme-linked immunosorbent assays (ELISA) are the most commonly used ones. A fourfold or greater increase in the titer of antibody is considered diagnostic. The reported sensitivities of serological assays vary substantially, from 41% to 94% (Boshuizen et al., 2003; den Boer and Yzerman, 2004).

#### 2.2.4. Molecular tests

Commercially-available kits for PCR/RT-PCR for respiratory tract specimens have sensitivities ranging from 17% to 100% and specificities ranging from 95% to 100% (Diederer et al., 2008; Benitez and

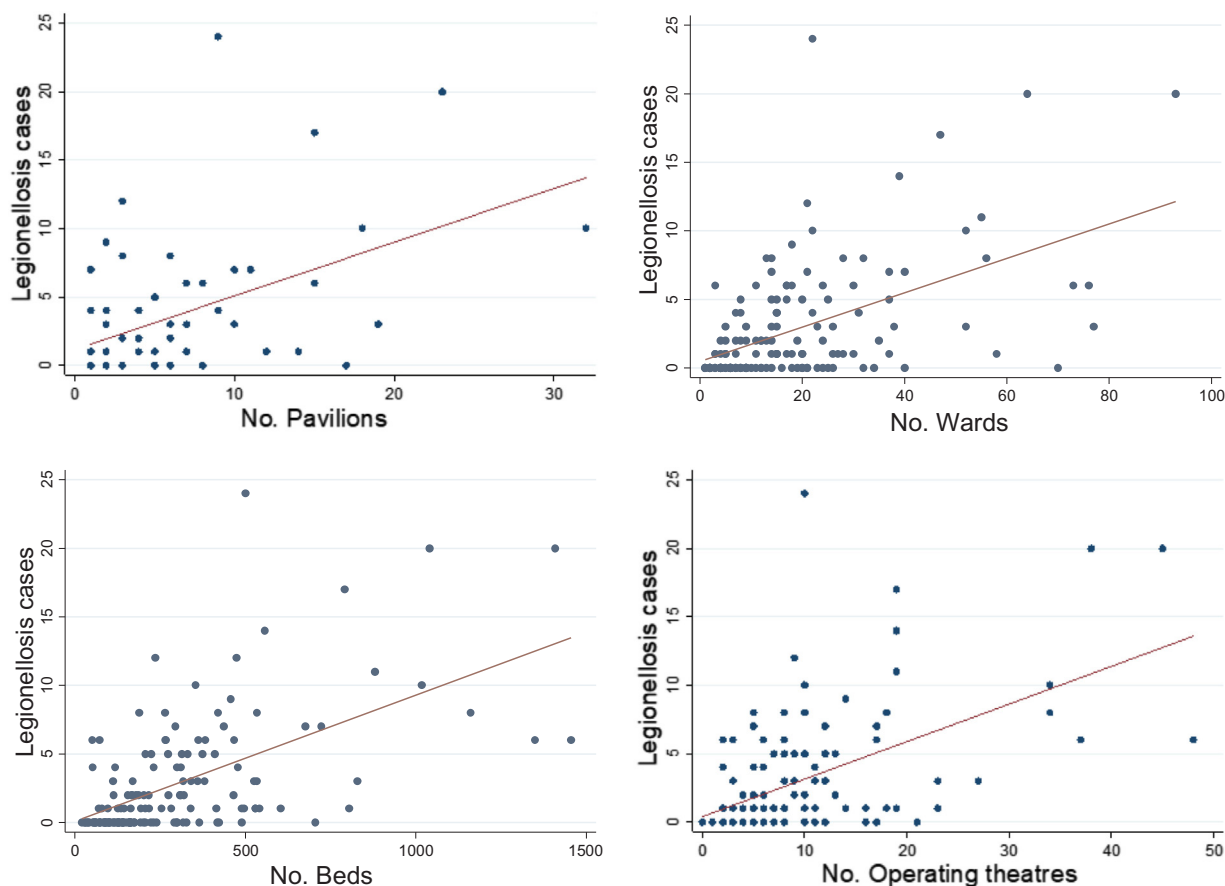


Fig. 1. Correlation between legionellosis cases in 2015 and hospitals' general characteristics.

Winchell, 2013). Genus probes and *Lpn* probes have been developed, but results rarely identify specific species or serogroups. PCR can be performed in a few hours, but laboratory expertise is required.

### 2.2.5. Environmental surveillance

Water contamination was monitored according to the procedures reported in the Italian Guidelines for the Prevention and Control of Legionellosis (Linee, 2015).

Air contamination was assessed by the active and passive sampling. Active sampling was performed by Surface Air System (SAS, PBI International, Milan, Italy). The number of colony forming units was adjusted using the conversion table provided by the manufacturer, and was expressed in colony forming units per cubic meter (cfu/m<sup>3</sup>) (Montagna et al., 2017a). Passive sampling was performed to determine the Index of Microbial Air Contamination (IMA) (Pasquarella et al., 2000), corresponding to the number of CFU counted on a Petri dish with a diameter of 9 cm.

### 2.3. Statistical analysis

Because Gaussian distributions could not be assumed, continuous variables were summarized using medians and interquartile ranges. Non-parametric Mann–Whitney *U* and Kruskal–Wallis tests were used to compare legionellosis cases by different categories of the collected variables. Correlations between the cases of legionellosis and the collected information were calculated using the Spearman correlation coefficient. A *p*-value < 0.05 was regarded as statistically significant. All analyses were conducted using the statistical software Stata 12.

## 3. Results

The study involved 739 Italian hospitals who were invited to participate. A total sample of 195 anonymous questionnaires from 195 different hospitals was collected (response rate: 26.4%). Only the correctly completed questionnaires (*n* = 178) were included in the analysis. No data were available on the non-participating hospitals. The professional figures who replied to the questionnaire were health management doctors (67.4%), nurses (17.8%), microbiologists (4.4%), risk managers (3.7%), infectious diseases doctors (3.7%) and health workers (3%).

### 3.1. General characteristics of the health care facilities

The 178 hospitals included in the study were located in the Northern (60.7%), Central (12.9%), and Southern (26.4%) regions of Italy. The sample included both public (79.8%) and private (20.2%) hospitals. The age of the hospital buildings was as follows: over 60 years (23.9%), from 20 to 60 years (55.2%), under 20 years (20.9%). The hospitals were generally structured as a single-building structure (44.7%), a pavilion (21.4%), or both (34%), and most occupied multiple floors (up to 14).

More than 30 wards were found in 20.9% of the hospitals (range: 1–93 wards), and 36.8% had more than 10 operating theaters (range: 0–48). Most of the hospitals (60.8%) had fewer than 300 beds, 23.9% had from 300 to 500 beds, and 15.4% had more than 500 beds (range: 21–1455). Cooling towers were present at 46.9% of the hospitals, 42.9% had maternity bathtubs, and 20.3% had ornamental fountains.

### 3.2. Clinical surveillance

In 2015, 63.2% of the hospitals reported at least one case of legionellosis, of which 28.2% were of nosocomial origin. The reported cases showed a geographical gradient, with the highest number found in the North and the lowest number found in the South (*p* < 0.01). Hospitals that were built before 1950 reported more legionellosis cases than did those that were built later (*p* < 0.05).

Hospitals with a pavilion structure registered a higher number of cases than did those with mixed or single-building structures (*p* < 0.05) (Fig. 1). In particular, among the hospitals with pavilions, a correlation was found between the number of pavilions and the number of registered cases ( $\rho = 0.44$ , *p* < 0.001). Higher numbers of cases were also associated with the number of beds, wards, and operating theaters ( $\rho = 0.59$ , 0.51, 0.56, respectively; *p* < 0.001), and with the presence of a cooling tower (*p* < 0.01) and maternity bathtubs (*p* < 0.001).

Laboratory diagnosis was performed using urinary antigen testing alone in 31.9% of the hospitals and using both urinary antigen testing and single antibody titer in 17.8% of the hospitals. Seroconversion [defined as a four-fold or greater increase in titer, after at least 20–30 days] was added in 21.5% of the hospitals. Culture-based or molecular investigations were performed on 28.8% and 22.1% of the clinical specimens, respectively. No laboratory investigation was reported by 16% of the hospitals, all with positive environmental cultures < 1000 cfu/L.

### 3.3. Environmental surveillance

The water systems were routinely tested for *Legionella* contamination in 97.4% of the hospitals, mostly with a 6-month frequency (51.7%), and 62% of them detected a positive result (> 1000 cfu/L). Each hospital established the number of water samples to be analyzed considering the number of beds, according to Italian Guidelines (Linee, 2015). The methods used were culture-based investigation (97.3%), molecular investigation (1.3%), or both (1.3%). The most frequent strains resulted from *Lpn* sg 2–15 (58.4%), followed by *Lpn* sg 1 (31.5%) and *Legionella* species (*L. micdadei*, *L. longbeachae*, *L. bozemanii*) (10.1%).

Investigations for airborne *Legionella* were conducted in 5.4% of the hospitals, usually with annual (43.8%) or biannual (43.8%) frequency, but no positive results appeared. The methods used were active sampling (43.8%), settle plates (43.7%), or both (12.5%).

### 3.4. Control and preventive measures

Referrals were made to the 2015 national guidelines for the control and prevention of legionellosis (Linee, 2015) by 55.2% of the hospitals. Risk assessments were performed in 73% of the hospitals, and formally dedicated multidisciplinary teams were present in 52.8% of the hospitals. These teams mainly comprised hygienists (49.1%) and engineers (49.1%). A prevention and control interventions manager was present in 68.7% of the hospitals, and most hospitals had a register (79.8%), a calendar (77.3%), and a checklist (75.5%) for the service work on the water or air systems.

Measures to reduce the risk of legionellosis had been adopted in 93.3% of the hospitals, mostly through water system disinfection procedures (73.7%), cleaning (70.4%), or the replacement of taps and showerheads (69.7%). The most commonly used water system disinfection procedures were thermal shock (37.4%), maintaining a constant temperature from 55 °C to 60 °C (34.4%), and chlorine dioxide (34.4%). Cooling towers were treated with antibacterial substances in 81.3% of the hospitals, mostly using chlorine-based products (37.5%).

Training courses dedicated to the control and prevention of LD were planned in 36.5% of the hospitals. Of these hospitals, 50.9% held these courses once per year, 16.9% held them twice per year, and 32.2% held them more than twice per year. These courses involved nurses (30.7%), physicians (28.8%), biologists (21.5%), technicians (26.4%), and cleaners (11%).

## 4. Discussion

Hospitals represent a high-risk environment for LD transmission because they frequently have old plumbing systems and medical



devices used by hospitalized patients. In particular, recent surgery (especially head and neck), nasogastric intubation, mechanical ventilation, and the use of respiratory therapy equipment have been identified as the main risk factors for nosocomial legionellosis (WHO, 2007).

In accordance with a national report (Rota et al., 2013), our study showed the highest number of cases of legionellosis in Northern Italy. Part of the reason for this geographic difference might be a greater awareness about the risk of legionellosis among clinicians, greater attention given to reporting cases of disease, and/or a more accurate laboratory diagnosis in the North. It should be also noted that geographical variation in the LD incidence rate could be, in part, related to the climate and meteorological conditions, as has been suggested for other acute respiratory infections (Du Prel et al., 2009).

Overall, the patients were generally tested using only one of the investigations recommended by the Italian guidelines (Linee, 2015), usually the urinary antigen test, that unfortunately is sensitive only for Lpn sg 1. For the other neglected serogroups and species, the application of *Legionella* culture of sputum becomes most important even if it is not widely performed (Lin et al., 2011; Pierre et al., 2017). Really, there are some difficulties with diagnosing legionellosis (e.g., it is often not a routine laboratory practice, urine antigen emission is not constant, the antibody response is slow, in the early phase, the illness is often accompanied by a dry cough with little sputum). Therefore, it is necessary to pay a great deal of attention to the laboratory tests. All specific tests should ideally be performed for each patient with pneumonia, including those who are seriously ill, whether or not they have clinical features suggesting legionellosis. In fact, it has been shown that LD cannot be excluded by a negative urine antigen or by a single low-titer serological test (De Giglio et al., 2015; Montagna et al., 2006, 2014, 2016). It is important to underline that the recovery of the isolate from the culture of clinical samples, if compared with environmental strains through molecular investigations, allows to identify the source of the infection (Yu and Stout, 2009).

Tests for LD should also be performed for patients displaying symptoms that do not match any other diagnosis, particularly for ill patients who are aged over 40 years, immunosuppressed, or unresponsive to beta-lactam antibiotics (WHO, 2007). It should be remembered that prevention measures are targeted to the prevention of disease in both patients and health care personnel. Scientific evidence has shown that hospital workers have an increased risk of contracting legionellosis (Borella et al., 2008; Napoli et al., 2007).

Although previous work has found that Lpn sg 1 is the most common isolate in humans, the present study found that the most frequently isolated species was *Legionella non-pneumophila* 1, referred to as Lpn sg 2–15. A large European study on 1335 strains isolated from human cases showed that 33.9% of hospital-acquired infections were caused by *Legionella non-pneumophila* 1 (Helbig et al., 2002). We think that monovalent serotyping of the isolates should become a standard procedure, because the pool of specific antisera for typing Lpn sg 2–15 is too large to obtain rigorous epidemiological data making it possible to identify the source of the infection and to program the necessary disinfection measures. Moreover, the isolation and identification of the etiological agent is fundamental in planning a proper antimicrobial therapy, particularly in severe cases. A more accurate analysis, including antibiotic susceptibility, should be performed also on environmental strains isolated during routine environmental surveillance. Past work has demonstrated the importance of antimicrobial susceptibility analysis of *Legionella* isolates for patients' therapy and positive outcomes and for reducing the direct costs associated with increasing hospitalizations (De Giglio et al., 2015).

In terms of environmental surveillance, Italian hospitals demonstrate a high level of attention to measures for the control and prevention of legionellosis. The water systems are routinely tested, usually with a 6-month frequency. However, culture-based investigations are frequently the only action performed for surveillance. This finding is

not completely satisfactory. Scientific evidence has shown that the simple measurement of colony-forming units does not give a real indication of the infection risk or reveal the presence of all forms of *Legionella* present in water systems (cells that are alive and viable, but not culturable). The concentration of *Legionella* spp. in water systems is not necessarily constant over time (Napoli et al., 2009), and it is important to evaluate the presence of *Legionella* regardless of the viable or not viable status of the cells (Montagna et al., 2017b). Currently, rapid and alternative molecular techniques can be used in combination with culture-based techniques to specify and quantify *Legionella* in environmental samples. Molecular methods, especially those based on Polymerase Chain Reaction, have important advantages, such as the ability to provide results in a few hours, to detect all forms of *Legionella* (Lee et al., 2011), and to perform epidemiological investigations (Yu and Stout, 2009).

We also found that the detection of airborne *Legionella* was reported to be conducted in only 5.4% of the participating hospitals. Although a previous study showed that the detection of airborne *Legionella* cannot replace water sampling because the absence of microorganisms in the air does not necessarily mean that they are not present in the water, air sampling may provide useful information for risk assessment (Montagna et al., 2016, 2017b).

Regarding preventive and control measures, our study has demonstrated that the adopted disinfection procedures for the water system are mainly traditional methods (thermal shock and chlorination). Following a recent study (Borella et al., 2016), we think that remediation systems must be selected and adapted to the structural characteristics of the hospital (e.g., age and type of construction, material used for the water system), also considering the choice of an appropriate cost-effective measure, to obtain the best effect with the least damage to the pipes. Moreover, risk assessment—performed by 73% of the hospitals in our study—could be useful for predicting *Legionella* spp. contamination in water systems. In fact, a validated and standardized procedure of risk assessment has been demonstrated to be useful for the rapid evaluation of the principal environmental risk factors and for detecting *Legionella* spp. when it is present (Hadjichristodoulou et al., 2006; Napoli et al., 2010). In this regard, according to many countries legislation or guidelines, it might be useful to incorporate the strategy to use the percentage of positive points to define the risk of legionellosis in hospitals: if the percent of positive environmental cultures at the distal sites is equal to or greater than 30% of the total number sampled, than the disinfection of the water distribution system is appropriate (Allegheny County Health Department, 1997; Linee, 2015).

Our study has the limitation of being based on the voluntary participation of a number of hospitals, and it is not sufficiently representative of Italy as a whole. For this reason, a further study needs to be planned with larger participation, including different specialists, particularly those who are new to the topic or who are collaborating in a multidisciplinary team and need targeted knowledge.

## 5. Conclusions

The present study identifies the main features of actions taken for legionellosis risk control and prevention in Italian hospitals. Greater attention must be paid to hospitals with a pavilion structure and with cooling towers, especially when these hospitals also have a larger number of beds, wards and operating theaters, which are associated with high numbers of cases of legionellosis. Risk analysis and environmental microbiological surveillance should be considered a starting point for any prevention procedure. Moreover, laboratory testing, both for diagnosis and for environmental purposes, should include molecular and antimicrobial assessments, as part of the risk assessment. Finally, although national documents on the prevention of LD are available, almost 50% of the investigated hospitals did not refer to those documents, reflecting an underestimation of the importance of

adequate risk analysis and management. Therefore, training courses for health professionals targeted to the implementation of the existing guidelines are still necessary at central and local levels.

### Conflict of interest

The authors declare they have no actual or potential competing financial interests.

### Acknowledgments

The authors thank all the people and the hospitals who participated in the study. We thank Jennifer Barrett, PhD, from Edanz Group ([www.edanzediting.com/ac](http://www.edanzediting.com/ac)) for editing a draft of this manuscript.

### References

- Allegheny County Health Department, 1997. January. Approaches to prevention and control of Legionella infection in Allegheny County Health Care Facilities.
- Benitez, A.J., Winchell, J.M., 2013. Clinical application of a multiplex real-time PCR assay for simultaneous detection of Legionella species, Legionella pneumophila, and Legionella pneumophila serogroup 1. *J. Clin. Microbiol.* 51 (1), 348–351.
- Borella, P., Bargellini, A., Marchegiano, P., Vecchi, E., Marchesi, I., 2016. Hospital-acquired Legionella infections: an update on the procedures for controlling environmental contamination. *Ann. Ig.* 28, 98–108.
- Borella, P., Bargellini, A., Marchesi, I., Rovesti, S., Stancanelli, G., Scaltriti, S., Moro, M., Montagna, M.T., Tatò, D., Napoli, C., Triassi, M., Montegrosso, S., Pennino, F., Zotti, C.M., Ditommaso, S., Giacomuzzi, M., 2008. Prevalence of anti-Legionella antibodies among Italian hospital workers. *J. Hosp. Infect.* 69, 148–155.
- Boshuizen, H.C., Den Boer, J.W., de Melker, H., Schellekens, J.F., Peeters, M.F., van Vliet, J.A., Conyn-van Spaendonck, M.A., 2003. Reference values for the SERION classic ELISA for detecting Legionella pneumophila antibodies. *Eur. J. Clin. Microbiol. Infect. Dis.* 22, 706–708.
- Castiglia, P., Liguori, G., Montagna, M.T., Napoli, C., Pasquarella, C., Bergomi, M., Fabiani, L., Monarca, S., Petti, S., SItI Working Group Hygiene in Dentistry, 2008. Italian multicenter study on infection hazards during dental practice: control of environmental microbial contamination in public dental surgeries. *BMC Public Health* 8, 187.
- Correia, A.M., Ferreira, J.S., Borges, V., Nunes, A., Gomes, B., Capucho, R., Gonçalves, J., Antunes, D.M., Almeida, S., Mendes, A., Guerreiro, M., Sampaio, D.A., Vieira, L., Machado, J., Simões, M.J., Gonçalves, P., Gomes, J.P., 2016. Probable person-to-person transmission of Legionnaires' disease. *N. Engl. J. Med.* 374, 497–498.
- den Boer, J.W., Yzerman, E.W., 2004. Diagnosis of Legionella infection in Legionnaires' disease. *Eur. J. Clin. Microbiol. Infect. Dis.* 23, 871–878.
- De Giglio, O., Napoli, C., Lovero, G., Diella, G., Rutigliano, S., Caggiano, G., Montagna, M.T., 2015. Antibiotic susceptibility of Legionella pneumophila strains isolated from hospital water systems in Southern Italy. *Environ. Res.* 142, 586–590.
- Diederer, B.M., Kluytmans, J.A., Vandenbroucke-Grauls, C.M., Peeters, M.F., 2008. Utility of real-time PCR for diagnosis of Legionnaires' disease in routine clinical practice. *J. Clin. Microbiol.* 46, 671–677.
- Du Prel, J.B., Puppe, W., Gröndahl, B., Knuf, M., Weigl, J.A., Schaaff, F., Schmitt, H.J., 2009. Are meteorological parameters associated with acute respiratory tract infections? *Clin. Infect. Dis.* 49, 861–868.
- European Centre for Disease Prevention and Control, 2015. Legionnaires' Disease in Europe, 2013. ECDC, Stockholm, Sweden.
- Garrison, L.E., Kunz, J.M., Cooley, L.A., Moore, M.R., Lucas, C., Schrag, S., Sarisky, J., Whitney, C.G., 2016. Vital signs: deficiencies in environmental control identified in outbreaks of Legionnaires' disease—North America, 2000–2014. *MMWR* 65, 576–584.
- Hadjichristodoulou, C.H., Goutziana, G., Mouchtouri, V., Kapoula, C.H., Konstantinidis, A., Velonakis, E., Vatopoulos, A., Kremastinou, J., 2006. Evaluation of standardized scored inspections for Legionnaires' disease prevention, during the Athens 2004 Olympics. *Epidemiol. Infect.* 134, 1074–1081.
- Helbig, J.H., Bernander, S., Castellani Pastoris, M., Etienne, J., Gaia, V., Lauwers, S., Lindsay, D., Lück, P.C., Marques, T., Mentula, S., Peeters, M.F., Pelaz, C., Struelens, M., Uldum, S.A., Wewalka, G., Harrison, T.G., 2002. Pan-European study on culture-proven Legionnaires' disease: distribution of Legionella pneumophila serogroups and monoclonal subgroups. *Eur. J. Clin. Microbiol. Infect. Dis.* 21, 710–716.
- Istituto Superiore di Sanità (ISS), 2016. Rapporto Annuale sulla Legionellosi in Italia nel 2015. 29. Notiziario ISS, Rome, Italy, pp. 3–10.
- Lee, J.V., Lai, S., Exner, M., Lenz, J., Gaia, V., Casati, S., Hartemann, P., Lück, C., Pangon, B., Ricci, M.L., Scaturro, M., Fontana, S., Sabria, M., Sánchez, I., Assaf, S., Surman-Lee, S., 2011. An international trial of quantitative PCR for monitoring Legionella in artificial water systems. *J. Appl. Microbiol.* 110, 1032–1044.
- Lin, Y.E., Stout, J.E., Yu, V.L., 2011. Prevention of hospital-acquired legionellosis. *Curr. Opin. Infect. Dis.* 24 (4), 350–356.
- Lindsay, D.S., Abraham, W.H., Findlay, W., Christie, P., Johnston, F., Edwards, G.F., 2004. Laboratory diagnosis of legionnaires' disease due to Legionella pneumophila serogroup 1: comparison of phenotypic and genotypic methods. *J. Med. Microbiol.* 53 (Pt 3), 183–187.
- Linee Guida per la Prevenzione ed il Controllo della Legionellosi, 2015. Available online: [http://www.salute.gov.it/imgs/C\\_17\\_pubblicazioni\\_2362\\_allegato.pdf](http://www.salute.gov.it/imgs/C_17_pubblicazioni_2362_allegato.pdf) (Accessed 8 May 2018).
- Montagna, M.T., Cristina, M.L., De Giglio, O., Spagnolo, A.M., Napoli, C., Cannova, L., Deriu, M.G., Delia, S.A., Giuliano, A., Guida, M., Laganà, P., Liguori, G., Mura, I., Pennino, F., Rossini, A., Tardivo, S., Torre, I., Torregrossa, M.V., Villafrate, M.R., Albertini, R., Pasquarella, C., 2016. Serological and molecular identification of Legionella spp. isolated from water and surrounding air samples in Italian hospitals. *Environ. Res.* 146, 47–50.
- Montagna, M.T., De Giglio, O., 2018. La gestione dei sistemi idrici nelle strutture collettive e sanitarie per la tutela della salute pubblica (1° Ed.). In: Moscato, U. (Ed.), *Acqua e Salute: governance e qualità dei sistemi idrici complessi*. Università Cattolica del Sacro Cuore, Roma, pp. 147–153.
- Montagna, M.T., De Giglio, O., Cristina, M.L., Albertini, R., Pasquarella, C.G.I.S.I.O., AIA and SIMPIOS Working Groups, 2017a. Legionella indoor air contamination in health care environments. In: Capolongo, S., Settimo, G., Gola, M. (Eds.), *Indoor Air Quality in Healthcare Facilities*. Springer Briefs in Public Health Ed., Cham, pp. 63–71.
- Montagna, M.T., De Giglio, O., Cristina, M.L., Napoli, C., Pacifico, C., Agodi, A., Baldovin, T., Casini, B., Coniglio, M.A., D'Errico, M.M., Delia, S.A., Deriu, M.G., Guida, M., Laganà, P., Liguori, G., Moro, M., Mura, I., Pennino, F., Privitera, G., Romano Spica, V., Sembeni, S., Spagnolo, A.M., Tardivo, S., Torre, I., Valeriani, F., Albertini, R., Pasquarella, C., 2017b. Evaluation of Legionella air contamination in hospitals by different sampling methods: an Italian multicenter study. *Int. J. Environ. Res. Public Health* 14, E670.
- Montagna, M.T., De Giglio, O., Napoli, C., Cannova, L., Cristina, M.L., Deriu, M.G., Delia, S.A., Giuliano, A., Guida, M., Laganà, P., Liguori, G., Mura, I., Pennino, F., Rossini, A., Tardivo, S., Torre, I., Torregrossa, M.V., Villafrate, M.R., Albertini, R., Pasquarella, C., 2014. Legionella spp. contamination in indoor air: preliminary results of an Italian multicenter study. *Epidemiol. Prev.* 38, 62–65.
- Montagna, M.T., Napoli, C., Tatò, D., Spilotros, G., Barbuti, G., Barbuti, S., 2006. Clinical-environmental surveillance of legionellosis: an experience in Southern Italy. *Eur. J. Epidemiol.* 21, 325–331.
- Munoz, M., Martinez Toldos, M.C., Yague, G., 2009. Evaluation of three immunochemical assays for detection of Legionella pneumophila serogroup 1 antigen in urine sample. *Rev. Esp. Quimioter.* 22 (4), 207–209.
- Napoli, C., Fasano, F., Iatta, R., Barbuti, G., Cuna, T., Montagna, M.T., 2010. Legionella spp. and legionellosis in southeastern Italy: disease epidemiology and environmental surveillance in community and health care facilities. *BMC Public Health* 10, 660.
- Napoli, C., Iatta, R., Fasano, F., Marsico, T., Montagna, M.T., 2009. Variable bacterial load of Legionella spp. in a hospital water system. *Sci. Total Environ.* 408, 242–244.
- Napoli, C., Tatò, D., Iatta, R., Montagna, M.T., 2007. Assessment of occupational risk of Legionella spp. infection among dental healthcare personnel. *Ig. Sanita Pubbl.* 63, 683–689.
- Pasquarella, C., Pitzurra, O., Savino, A., 2000. The index of microbial air contamination. *J. Hosp. Infect.* 46, 241–256.
- Pasquarella, C., Veronesi, L., Castiglia, P., Liguori, G., Montagna, M.T., Napoli, C., Rizzetto, R., Torre, I., Masia, M.D., Di Onofrio, V., Colucci, M.E., Tinteri, C., Tanzi, M., SItI Working Group "Hygiene in Dentistry", 2010. Italian multicentre study on microbial environmental contamination in dental clinics: a pilot study. *Sci. Total Environ.* 408, 4045–4051.
- Pasquarella, C., Veronesi, L., Napoli, C., Castiglia, P., Liguori, G., Rizzetto, R., Torre, I., Righi, E., Farruggia, P., Tesaro, M., Torregrossa, M.V., Montagna, M.T., Colucci, M.E., Gallè, F., Masia, M.D., Strohmenger, L., Bergomi, M., Tinteri, C., Panico, M., Pennino, F., Cannova, L., Tanzi, M., SItI Working Group Hygiene in Dentistry, 2012. Microbial environmental contamination in Italian dental clinics: a multicenter study yielding recommendations for standardized sampling methods and threshold values. *Sci. Total Environ.* 420, 289–299.
- Pierre, D.M., Baron, J., Yu, V.L., Stout, J.E., 2017. Diagnostic testing for Legionnaires' disease. *Ann. Clin. Microbiol. Antimicrob.* 16 (1), 59.
- Rota, M.C., Caporali, M.G., Bella, A., Ricci, M.L., Napoli, C., 2013. Legionnaires' disease in Italy: results of the epidemiological surveillance from 2000 to 2011. *Eur. Surveill.* 18, 20497.
- Shimada, T., Noguchi, Y., Jackson, J.L., Miyashita, J., Hayashino, Y., Kamiya, T., Yamazaki, S., Matsumura, T., Fukuhara, S., 2009. Systematic review and meta-analysis urinary antigen tests for Legionellosis. *Chest* 136, 1576–1585.
- Soda, E.A., Barskey, A.E., Shah, P.P., Schrag, S., Whitney, C.G., Arduino, M.J., Reddy, S.C., Kunz, J.M., Hunter, C.M., Raphael, B.H., Cooley, L.A., 2017. Vital signs: healthcare-associated Legionnaires' disease surveillance data from 20 states and a large metropolitan area—United States, 2015. *MMWR* 66, 584–589.
- Tobin, J.O., Beare, J., Dunnill, M.S., Fisher-Hoch, S., French, M., Mitchell, R.G., Morris, P.J., Muers, M.F., 1980. Legionnaires' disease in a transplant unit: isolation of the causative agent from shower baths. *Lancet* 2, 118–121.
- Yu, V.L., Stout, J.E., 2009. Rapid diagnostic testing for community-acquired pneumonia: can innovative technology for clinical microbiology be exploited? *Chest* 136 (6), 1618–1621.
- World Health Organization, 2007. Legionella and the Prevention of Legionellosis. World Health Organization, Geneva, Switzerland.