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

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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-SItI) 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 specifcity 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% (Diederen et al., 2008; Benitez and

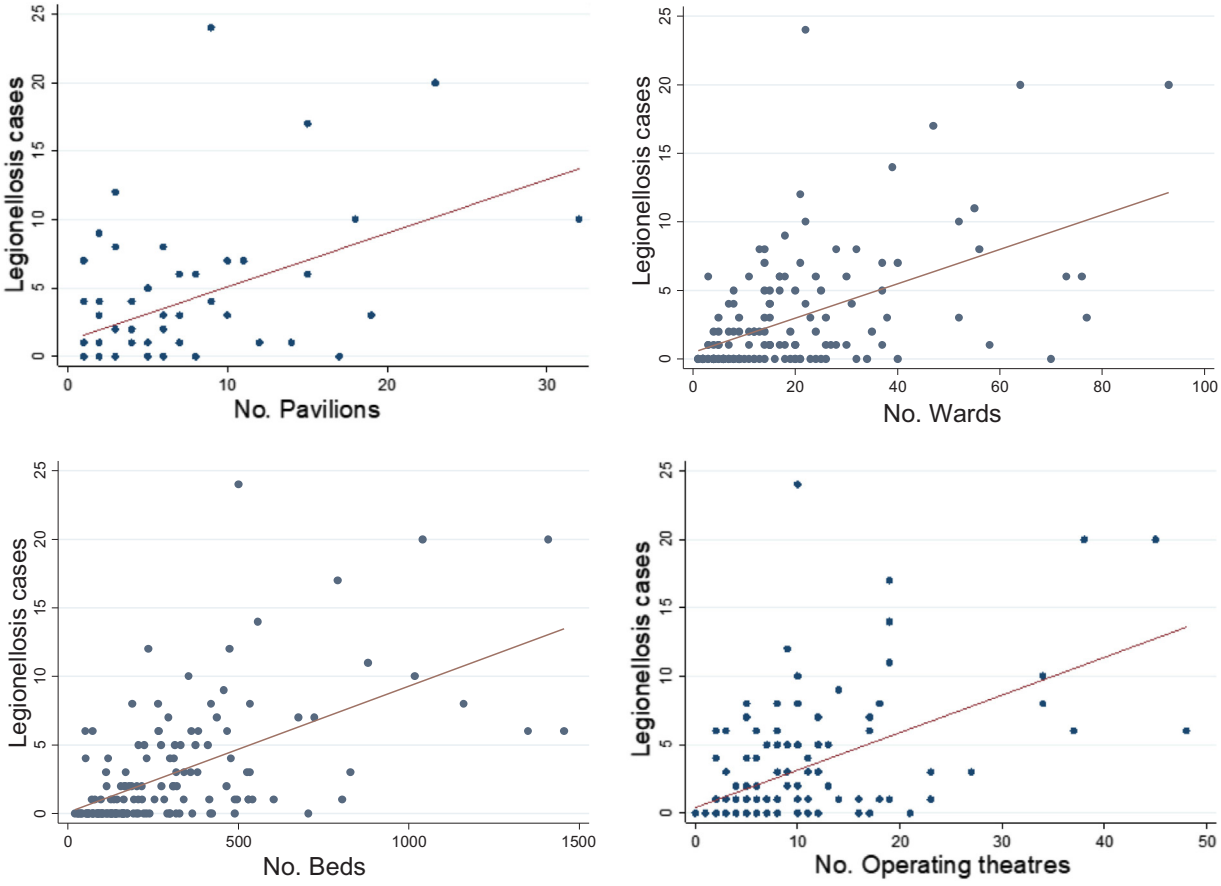

Fig. 1. Correlation between legionellosis cases in 2015 and hospitals' general characteristics.

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^3) (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.

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