CONTROL AND PREVENTION MEASURES FOR LEGIONELLOSIS IN HEALTHCARE FACILITIES: A MULTICENTER SURVEY IN ITALY

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Introduction

The genus *Legionella* includes Gram-negative microorganisms living in natural and artificial water systems, able to grow at 25-45°C, especially if the water is stagnant. An infected source (e.g. taps, showerheads, cooling towers) can disseminate sprays or droplets of water containing *Legionella*, leaving airborne particles of less than 5 µm in diameter which can be deeply inhaled.

The microorganism is responsible of a severe pneumonia, known as Legionnaires' disease (LD), or a flu-like illness, the Pontiac fever, normally acquired by inhalation or aspiration of contaminated aerosol (Montagna 2006, Rota 2013, Montagna 2014; Montagna 2017 n.14). To date, about 60 species of *Legionella* are known: *Legionella pneumophila* (Lpn) is the species most frequently associated to human disease and includes 16 serogroups (sg). Though the literature states that Lpn sg 1 is the most common isolate in humans, more and more cases are being attributed to other species and serogroups (Napoli 2010; ECDC 2015).

The first evidence of the association between potable water and nosocomial legionellosis was described approximately 40 years ago (Tobin 1980). The complexity of healthcare facility (HF) water systems and the vulnerability of the hospitalized patient increase the risk for *Legionella* transmission and severe outcomes. A review of 27 LD outbreaks investigated by the CDC during 2000–2014 indicates that healthcare–associated

LD accounted for 33% of the outbreaks, 57% of outbreak-associated cases, and 85% of outbreak-associated deaths (Garrison 2016; Soda 2017). Only one case of probable person-to-person transmission has been reported (Correia 2016); so, currently, the hot water system is still thought to be the most frequent source of cases or outbreaks within a hospital (Borella 2008; Napoli 2010; Montagna 2017 n.15).

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 in 2016. The majority of cases are community-acquired, followed by travel-associated and healthcare-associated cases (5.3% in 2015). Clinical outcome is affected by comorbidities; mortality ranges from 40 to 80% in untreated immunocompromised patients, from 5 to 30% in case of appropriate therapy. Overall, in Italy the case fatality rate ranges from 8 to 17% (Rota 2013; ISS 2016).

In 2000, the Italian Institute of Health (ISS) issued the first Guidelines on the prevention and control of legionellosis, followed, in 2005, by instructions for laboratories with a role in microbiological diagnosis, environmental control and for tourist accommodation and Spa. In 2015, all national recommendations, including those for HF, were incorporated in a single updated document [Linee guida 2015]. The instructions report the risk assessment evaluation as one of the most effective prevention measures to manage *Legionella* spp. contamination in water systems. This is particularly relevant in HF because, a part from the water system, medical equipment can also be a potential source of infection (endoscopes, devices for artificial respiration and oxygen therapy, dental tools, etc.) (Castiglia 2008; Pasquarella 2010; Pasquarella 2012). Nevertheless, the control and prevention of legionellosis remains a critical issue in Italian healthcare settings, since the guidelines are considered reference documents and not compulsory protocol of prevention; therefore, nothing is known about their implementation.

In light of this background, 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 Prevention of Healthcare Associated Infections (SIMPIOS) promoted a national survey to collect information about specific measures for legionellosis control and prevention adopted in Italian HF, in order to understand the critical aspects of LD prevention and implement targeted correction measures.

Methods

Study design

Overall, 163 HF from all Italian regions took part on a voluntary basis and without remuneration for their contribution. After having given the consent (as required by Italian privacy law), the participants were asked to complete an anonymous questionnaire divided into 7 sections comprising 71 questions about HF location and general characteristics, data on clinical and environmental surveillance for legionellosis detected in 2015 and related control and preventive measures. The survey was performed using SurveyMonkey® software that allows archiving and data processing.

This study followed the principles of the World Medical Association Declaration of Helsinki and did not report any experiment on humans or human samples, nor research on identifiable human material and data.

Statistical analysis

Since Gaussian distributions could not be assumed, continuous variables were summarized using median and interquartile range; non-parametric Mann-Whitney U and Kruskal-Wallis tests were used in order to compare legionellosis cases in the different groups of the collected variables. A correlation among the cases of legionellosis and the collected information was carried out 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.

Results

Healthcare Facility general characteristics

The 163 enrolled HF were located in the North (60.7%), Center (12.9%) and South Italy (26.4%). The sample included both public (79.8%) and private hospitals (20.2%).

The HF buildings were over 60 (23.9%), between 20 and 60 (55.2%), under 20 (20.9%) years old. They were generally structured in monobloc (44.7%), pavilions (21.4%) or both (34%), mostly on several floors up to 14.

The 20.9% HF had more than 30 wards (range 1-93), and 36.8% had more than 10 operating theatres (range 0-48). Overall, HF had less than 300 beds (60.8%), from 300 to 500 beds (23.9%), more than 500 beds (15.3%) (range 21-1455). Cooling tower were in 46.9% HF, maternity bathtub in 42.9% HF, and ornamental fountains in 20.3% HF.

Clinical surveillance

In 2015, 63.2% HF reported at least one case of legionellosis, of which 28.2% was of nosocomial origin. The reported cases showed a geographical gradient, with the highest number notified in the North and the lowest in the South Italy ($p<0.01$). The HF built before 1950 reported more legionellosis cases than those built later ($p<0.05$).

The HF with a pavilion structure registered a higher number of cases than HF with mixed or monobloc structures ($p<0.05$) (Figure 1). In particular, among the HF with pavilions, a correlation between the number of pavilions and the number of registered cases was found ($\rho= 0.44$, $p<0.001$). The higher number of cases was also associated to the number of beds, wards and operating theatres ($\rho= 0.59, 0.51, 0.56$, respectively; $p<0.001$), and to the presence of cooling tower ($p<0.01$) and maternity bathtub ($p<0.001$).

Laboratory diagnosis was performed by only urinary antigen (31.9%), both urinary antigen and single antibody titer (17.8%), added with seroconversion [defined as a four-fold or greater increase in titer, after at least 20-30 days] (21.5%). Culture-based or molecular investigations from clinical specimens were performed in 28.8% and 22.1%, respectively. The 16% HF did not report any laboratory investigation.

Environmental surveillance

Legionella contamination was routinely tested in the water systems of 97.4% HF, mostly with a six months frequency (51.7%), and 62% of them detected at least a positive result (>1000 cfu/L). The methods used were culture-based investigations (97.3%), molecular investigations (1.3%) or both (1.3%). The most frequent strains resulted Lpn sg 2-15 (58.4%), followed by Lpn sg 1 (31.5%) and *Legionella* species (*L. micdadei, L. longbeachae, L. bozemanii*) (10.1%).

The investigation for *Legionella* in air was conducted in 5.4% HF, usually with annual (43.8%) and biannual (43.8%) frequency, but no positive results appeared. Methods used were active sampling (43.8%), settle plates (43.7%) or both (12.5%).

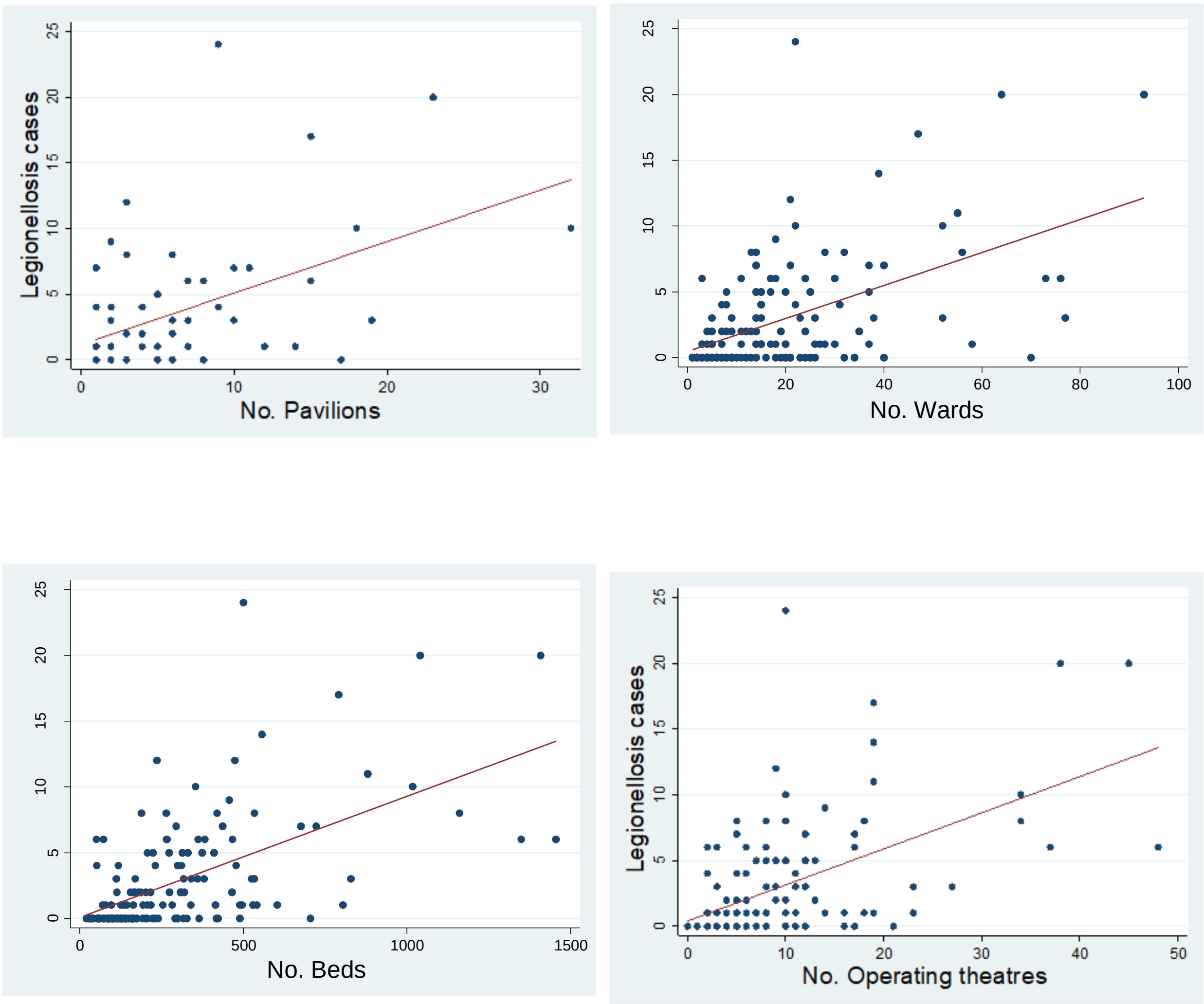

Figure 1. Correlation between legionellosis cases in the 2015 and the HF general characteristics

Control and preventive measures

The 55.2% HF referred to the 2015 National Guidelines for the control and prevention of legionellosis (LG 2015). The risk assessment was performed in 73% HF; a multidisciplinary team formally dedicated was present in 52.8% HF, mainly acted for hygienists (49.1%) and engineers (49.1%). A manager of prevention and control interventions was provided for 68.7% HF, with a register (79.8%), a calendar (77.3%) and a checklist (75.5%) for the service works of water or aerial systems.

The measures to reduce the legionellosis risk were adopted in 93.3% HF, mostly by water system disinfection procedures (73.7%), cleaning (70.4%) or substitution (69.7%) of the tap and showerhead. The most used water system disinfection procedures were thermal shock (37.4%), a constant temperature between 55 and 60°C (34.4%) and chlorine dioxide (34.4 %). Cooling tower were treated with antibacterial substances in 81.3% HF, mostly using chlorine-based products (37.5 %).

Training courses dedicated to the control and prevention of LD were planned in 36.5% HF, once per year (50.9%), twice per year (16.9%), every two-year or more (32.2%) frequency, involving nurses (30.7%), physicians (28.8%), biologists (21.5%), technicians (26.4%) and cleaners (11%).

Discussion

Healthcare facilities 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, use of respiratory therapy equipment have been identified as the main risk factors for nosocomial legionellosis [WHO 2016].

According to national report (Rota 2013), our study showed the highest number of cases of legionellosis in northern Italy. The reason for this geographical difference might be partly due to the clinicians' greater awareness about legionellosis risk or greater attention in notifying cases of disease or a more accurate laboratory diagnosis. It should be also noted that geographical variation in LD incidence rate could partly be related to the climate and meteorological conditions, as suggested for other acute respiratory infections (du Prel JB 2009)

Overall, the patients were tested to only one of the investigations recommended by the Italian Guidelines (LG 2015), generally urinary antigen. The diagnosis of legionellosis shows some difficulties (often it is not a routine laboratory practice, urine antigen emission is not constant, the antibody response is slow, etc); therefore, a great attention to the laboratory tests is necessary. All specific tests should ideally be performed for each patient with pneumonia, including those 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-titre serological test [Montagna 2006; Montagna 2014; De Giglio 2015; Montagna 2016]. Tests for LD should also be performed for patients displaying symptoms that do not match any other diagnosis, and particularly on ill patients who are older than 40 years, immunosuppressed, or unresponsive to beta-lactam antibiotics (WHO 2016). It should not be forget that prevention measures are targeted to the prevention of the diseases in both patients and healthcare personnel: scientific evidences show how HF workers have an increased risk of legionellosis (Napoli 2007; Borella 2008).

Though the literature states that Lpn sg 1 is the most common isolate in humans, the most frequently isolated species resulted *Legionella* non-*pneumophila* 1, referred to as Lpn sg 2-15. A large European study on 1,335 strains isolated from human cases showed that 33.9% of hospital-acquired infection were caused by *Legionella* non-*pneumophila* 1 [Helbig 2002]. We think that monovalent serotyping of the isolates need to turn into a standard procedure because the pool of specific antisera for typing Lpn sg 2-15 is too large to obtain a rigorous epidemiological data, to reach the source of infection and to program the necessary disinfection measures. Moreover, the isolation and identification of the etiological agent is fundamental to plan a proper antimicrobial therapy, particularly in severe case. Moreover, more accurate analysis, including antibiotic susceptibility, should be performed also on environmental strains isolated during environmental routine surveillance; as a matter of fact, it has been demonstrated that susceptibility testing of Legionella strains to appropriate antibiotics may be useful to evaluate the possible emergence of resistance, to improve the outcomes of patients, and to reduce the direct costs associated with hospitalization (De Giglio 2015).

Regarding environmental surveillance, Italian HF demonstrate a high level of interest about control and prevention measures for legionellosis: the water systems was routinely tested, mostly with a six months frequency, but the most frequently adopted survey resulted only culture-based investigations. This data is not completely satisfying: scientific evidences show that the simple measurement of colony forming units does not give the real estimate of the infection risk and not reveal the presence of all forms of *Legionella* present in water systems (alive, viable but not culturable). In fact, on one side the concentration of *Legionella* spp. from the water system is not necessarily constant over time [Napoli 2009], on the other it is important to evaluate the presence of *Legionella* regardless of *viable* and *not viable* (Montagna 2017 n.15). 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 PCR, have some important advantages such as the ability to provide results in a few hours and to detect all forms of Legionella [Lee 2011]. Moreover, in our study the detection of *Legionella* in air is reported to be conducted only in 5.4% HF. Although a previous study shows that *Legionella* air detection cannot replace water sampling because the absence of microorganisms from the air does not necessarily represent their absence from water, air sampling may provide useful information for risk assessment (Montagna 2016, Montagna 2017).

The risk assessment - in our study performed only by 73% HF - could be useful to predict *Legionella* spp. contamination in water systems. In fact, a validated and standardized procedure of risk assessment was demonstrated to be useful for a rapid evaluation of the principal environmental risk factors and achieve a positive result in environmental analysis for *Legionella* spp (Hadjichristodoulou 2006; Napoli 2010).

Conclusions

The present study identifies the principal features of the Legionella prevention actions in Italian HF. As previously known, the greater attention must be payed to HF with a pavilion structure and cooling tower, especially with higher number of beds, wards and operating theatres, which resulted associated to an higher number of cases. Risk analysis and environmental microbiological surveillance should be considered as starting point for any prevention procedure, especially in Italian Central and Southern regions. Moreover, the laboratory tests, for both diagnosis and environmental purposes, should include molecular and antimicrobial assessments in order to complete the risk assessment. Finally, our study highlighted that, although the availability of national documents on the prevention of LD, almost the 50% of the investigated HF do not refer to those documents, suggesting the risk of inappropriate risk management. Therefore, training courses for health professionals targeted to the implementation of the national guidelines are still necessary at both central and local level.

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