Environmental Surveillance of *Legionella spp*. in an Italian University Hospital: results of 7 years of analysis

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Keywords

Legionella • Hospital surveillance • Water safety plan

Summary

Introduction. *Nosocomial structures pose a high risk of* Legionella spp. *contamination due to complex water systems with challenging disinfection; moreover, the risk of severe legionellosis as a consequence of nosocomial exposure is very high in settings characterized by vulnerable patient conditions.*

Methods. *In the present work, we described the results of 7 years of environmental surveillance in a reference hospital in Liguria, in which a specific water safety plan (WSP) has been implemented in 2017, including data collected during the COVID-19 pandemic.*

Results. *During the study period, 1190 water samples were collected, of which 277 (23.3%) tested positive for* Legionella spp. *Positive samples with concentration values above 1,000 CFU/l were 184 (66.4%). Based on the new structure categorization contained in the WSP, hospital buildings classified as at "very high" risk resulted the most affected structures over the entire study period; however, the absolute number of positive samples greatly decreased over time, from 61 contaminated water samples in 2017 to only 9 in 2023.*

Conclusions. *Our findings prompted the reinforcement of control and prevention measures, affirming the appropriateness of risk-category classification. Indeed, the majority of contamination cases were associated with the water networks of buildings classified as "very high" risk.*

Introduction

Legionella spp. is a pathogen identified in 1976 during an outbreak of atypical pneumonia at an American Legion conference in Philadelphia and subsequently named *Legionella pneumophila* [1, 2].

Legionella pneumophila, responsible for most human infections, has 16 serogroups, with serogroup 1 being predominant [3]. *Legionella spp*. colonizes natural aquatic environments, artificial water tanks and pipelines, evaporative cooling systems and various facilities like water networks of buildings [1, 3, 4]. Factors contributing to water network contamination involve temperature, amoebae/protozoa presence, stagnant water, low biocide concentration, poor flow, and biofilm/scale presence [1, 3]. Humans can contract *Legionella* infections through aerosol inhalation or contaminated water suction. Risk factors include age over 50, male gender, smoking or intravenous drug use, chronic diseases, and immunosuppression [3, 5-7].

In accordance with Italian guidelines, cultural methods are considered the gold standard for environmental surveillance. The diagnosis of human cases is supported by numerous laboratory methods, such as the isolation of the bacterium by culture, detection of antibodies in sera during the acute and convalescent phases of the disease, detection of urinary antigen, detection of the bacterium in tissues or body fluids by immunofluorescence testing, and detection of bacterial DNA using PCR [3, 8-9].

Nosocomial structures pose a high risk of *Legionella spp*. contamination due to complex water systems with challenging disinfection, including blind-bottomed branches, water stagnation, and biofilms. Moreover, although it is not a problem to achieve high water temperatures at the source of the distribution system, maintaining these high temperatures throughout the entire network, especially in its terminal parts, is challenging [10-13]. The risk of severe legionellosis as a consequence of nosocomial exposure is very high in settings characterized by vulnerable patient conditions and high number of respiratory care practices [3, 7, 14].

In Italy the first national guidelines for legionellosis prevention and control were issued in 2000 and then updated in 2015, with a particular focus on surveillance and control activities in the hospital setting [3].

Current *Legionella* monitoring in hospitals follows 2015 guidelines, considering patient types, high-risk practices, and facility history for risk assessment. The goal is to minimize colonization risk rather than complete elimination, often unachievable in the long term. This does not apply to wards housing profoundly immunocompromised patients; in this case, the inability of the immune system to respond to any exposure makes interventions to ensure the absence of *Legionella*

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(undetectability) necessary. Risk classification mandates various preventive measures and remediation strategies once contamination occurs [3, 14, 15].
In the present work, we described the results of 7 years of environmental surveillance in a reference hospital in Liguria, in which a specific water safety plan (WSP) has been implemented in 2017, including data collected during the COVID-19 pandemic.

Materials and methods

A retrospective study was conducted at the IRCCS Ospedale Policlinico San Martino, a historical university reference hospital for adult care located in Genoa, in the Liguria region of northern Italy. The hospital covers a vast urban area and consists of 31 buildings that host all the nosocomial services and clinical wards, including 1200 beds, built between 1910 and 1923; adult patients at high risk of legionellosis due to their clinical conditions, such as transplant, oncology, haematology, intensive care and infectious disease patients, are hospitalized in six buildings, representing 70% of total beds. Each building is supplied by separate water tanks. Structural and organizational preventive measures against *Legionella* contamination used in the hospital include absolute filters in specific high-risk areas, a chlorine dioxide system in most of buildings hosting patients, a program of environmental sampling involving all the hospital buildings and an alert surveillance system based on laboratory results on human samples.
In 2017, a WSP was adopted, introducing a systematic monitoring of *Legionella* presence in water network and a new classification of risk levels of all buildings. Water Safety Planning is an international framework developed by the World Health Organization in 2004 that utilizes local system knowledge and risk management strategies to continuously improve water quality. The WSP is a cyclical and adaptive framework built on proactive hazard identification, risk mitigation, and operator knowledge [16].
The Hygiene Unit manages and implements the WSP through activities carried out by a multidisciplinary working group. This group includes specialized staff in infection control, biologists, laboratory technicians, health and safety manager, filter suppliers, and plumbers. The main critical issues encountered were related to structural problems, various renovations that have taken place over the years, and the absence of a map of the water network.
The first activity was the introduction of a risk classification for all the hospital buildings: they are categorized in four risk levels (very high, high, intermediate, and low), according to 2015 national guidelines [3].
Buildings hosting transplant centres, oncology, haematology, intensive care and infectious disease wards are considered among very high risk structures (6 buildings): in these buildings the aim of the WSP is to maintaining zero colonization/presence of *Legionella*.
Other 5 buildings host patients considered at risk for *Legionella* infection due to their clinical condition or other individual risk factors: these 5 buildings are classified as high risk structures.
Other 10 buildings are considered at intermediate risk because mainly host outpatient services or clinical wards with a limited patient presence.
The remaining 10 buildings host administrative or other services, with scarce or no presence of patients, presenting minimal risk of legionellosis, comparable to the community.
Sampling is conducted every six months in very high-risk departments and annually in others, with biennial frequency for departments considered at higher risk than general population. Additional sampling can be performed in case of unexpected events. The collected hot water samples, obtained from the points of sampling identified for each department of the Hospital, are transported and processed at the laboratories of the Hygiene Unit, according to national indications. In case of colony growth, agglutination tests are performed for serogroup identification.
During the study period, there were deficiencies of reagents for the execution of the agglutination test for the identification of *Legionella* serogroup 3 and serogroup 6: in this case, after exclusion of serogroup 1, the identified *Legionella* serogroup was generically expressed as "serogroup 2-14".
The environmental samples considered for the purposes of our study included routine sampling and extraordinary sampling following contamination detection.
We calculated the prevalence of *Legionella spp*. contamination for each year investigated and the cumulative incidence in the period between January 2017 and November 2023.
Then, we described in absolute numbers the distribution of isolates for serogroups of *Legionella spp*.; subsequently, we calculated the prevalence of each serogroup annually and over the total period. The results of the sampling were divided into the following ranges of values: < 100 colony-forming units/litre (CFU/l), 100-1,000 CFU/l, 1,001-10,000 CFU/l and more than 10,000 CFU/l.
In addition, we evaluated the distribution of positive samples based on the sampling points and their location in the hospital; therefore, we calculated the prevalence of contamination in individual buildings annually and in the overall period. Chi square for trend was calculated from Epitools - Epidemiological Calculators (Sergeant, ESG, 2018. Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au.).

Results

From January 2017 to November 2023, 1190 water samples were collected and processed, of which 277 (23%) tested positive for *Legionella spp*. Positive samples with concentration values above 1,000 CFU/l were 184 (66%).
Figure 1 shows the prevalence of positive hospital water network samples for *Legionella spp*. from January 2017

Fig. 1. Prevalence of positive sampling found in the sampling of the hospital water network of the IRCCS San Martino Polyclinic Hospital, Genoa (January 2017-November 2023).

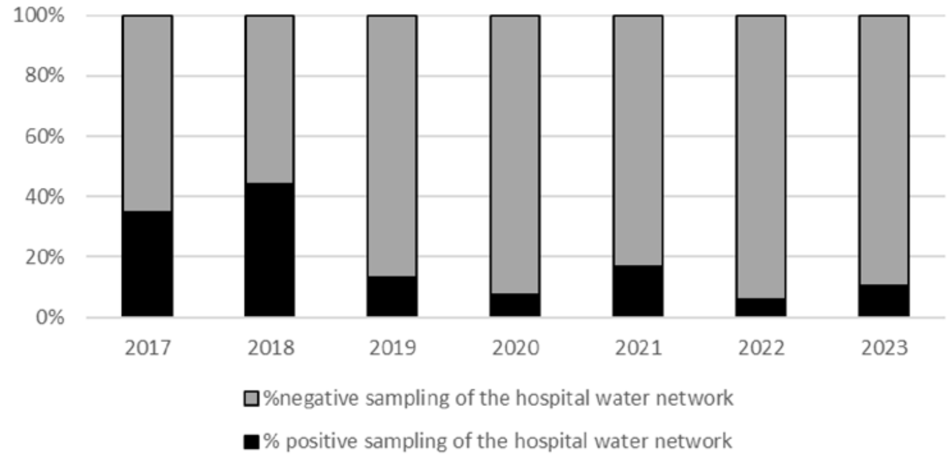
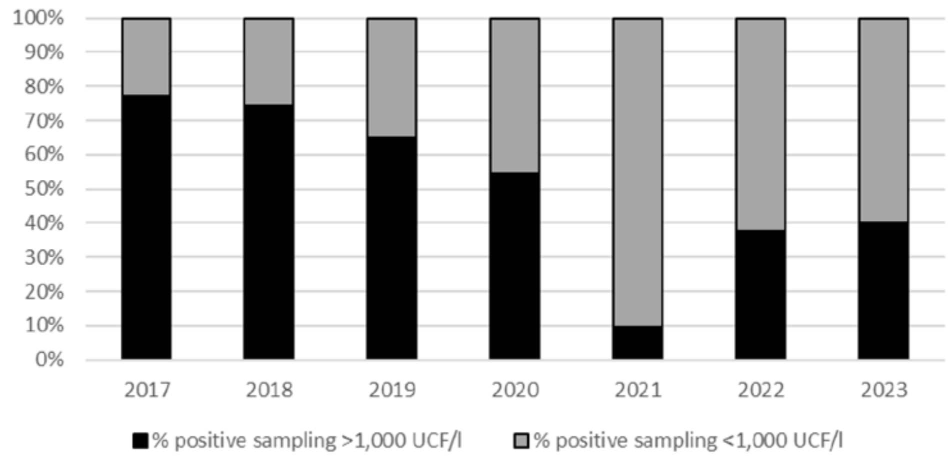


Fig. 2. Prevalence of hospital water network sampling positive results with *Legionella spp.* concentration above 1,000 UCF/l (January 2017-November 2023).



to November 2023; Figure 2 shows the prevalence of positive sampling with *Legionella spp*. concentration above 1,000 UCF/l in the same period. We observed a steadily decreasing trend for both rates during the study period (chi square for trend $p < 0.0001$).

Legionella pneumophila was the only isolated species: serogroup 3 was the most identified serogroup (80% of all positive samples), followed by serogroup 1 (13%) and serogroup 6 (1%); the remaining positive samples (7%) were classified as serogroup 2-14 after exclusion of serogroup 1.

Figure 3 shows the absolute numbers of *Legionella pneumophila* identification by serogroup and year of analysis.

We evaluated the distribution of the contaminated samples according to the sampling points and their location in the hospital. Figure 4 shows the prevalence of *Legionella* contamination of the hospital buildings in relation to the risk category to which they belong in each year of the survey: those classified as "at very high risk" resulted the most affected structures by contamination over the entire study period; however, the absolute number of positive samples greatly decrease over time, from 61 contaminated water samples in 2017 to only 9 in 2023.

Discussion

We observed a dramatic decrease in *Legionella* contamination among water samples collected at IRCCS Ospedale Policlinico San Martino during the study period, after the implementation of a WSP in 2017. One of the main elements of this plan is the scheduling of periodic checks [17]: therefore, a systematic monitoring was introduced with a sampling frequency indicated based on the level of risk. Furthermore, the WSP included mapping of water terminals as part of a larger project to map the water network, which is currently not yet completed.

The decrease was observed for all the *Legionella* serogroup identified, in particular for serogroup 3, that was the most represented serogroup, which is more

Fig. 3. Histogram showing the absolute numbers of *Legionella pneumophila* Ser. 1, Ser. 3, Ser 6 and Ser 2-14 detected in each year of our investigation.

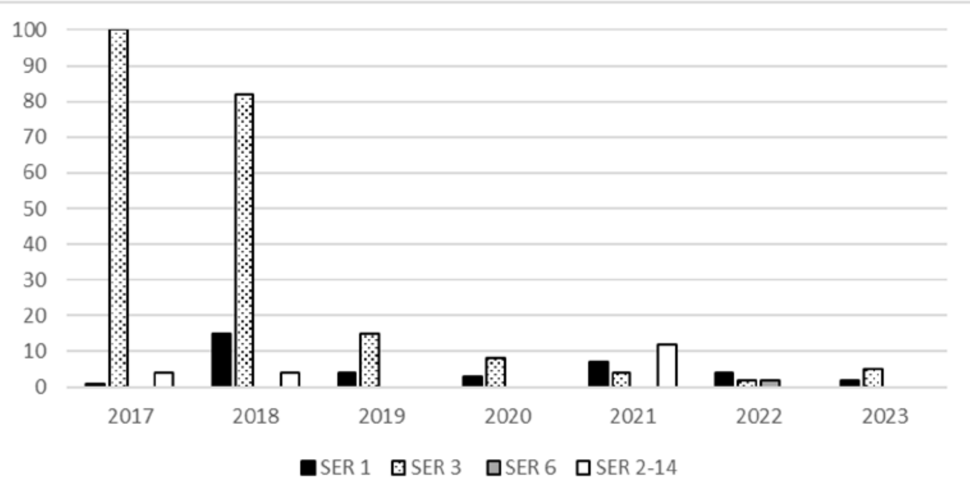
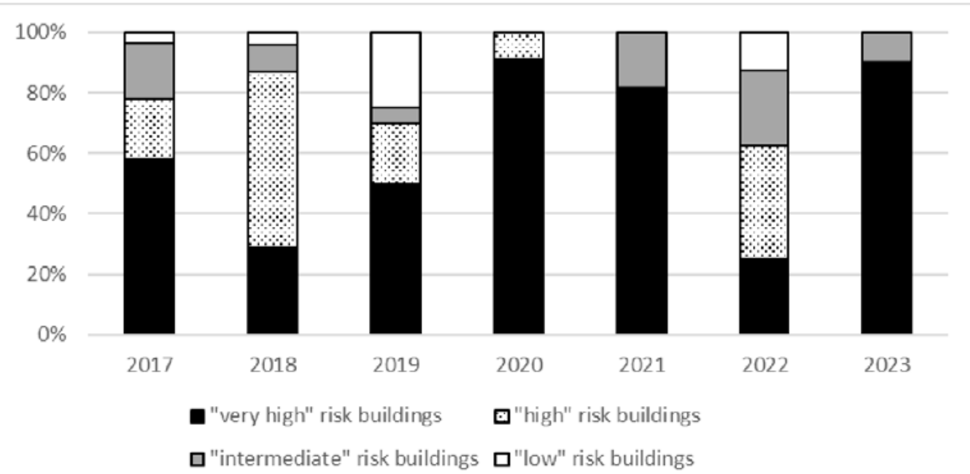


Fig. 4. Prevalence of contamination of the water network of the buildings analyzed in relation to the risk category to which they belong in each year of our survey (January 2017-November 2023).



difficult to diagnose in the case of human contagion since it is not detectable by the search for common urinary antigen tests.

Regarding the risk classification of hospital buildings, structures categorized as "very high" and "high" risk consistently exhibited the highest contamination levels throughout the entire study period. Nevertheless, there was a notable decrease in the absolute number of positive samples in these structures.

Our findings prompted the reinforcement of control and prevention measures, affirming the appropriateness of risk-category classification. Indeed, the majority of contamination cases were associated with the water networks of buildings classified as "very high" risk.

Analyzing the higher presence of positive samples in buildings categorized as high risk, this phenomenon may be partially attributed to a more extensive sampling frequency in these structures. The sustained detection of positivity could also be linked to challenges that can be effectively addressed only through structural interventions, as detailed in [18], because the old water network of the hospital, increases the risk of contamination by *Legionella spp*.

Regarding serogroups in our sample a significant prevalence of serogroups 2-14 has been observed, particularly serogroup 3. These serogroups are less frequently sought, and therefore more challenging to diagnose, as described in other Italian studies such as [19-21]. Consequently, in the fight against hospital-acquired legionellosis, it is essential to ensure correct and effective information and training of the staff to lead to the correct application of the indications for the prevention and control of the infection. Clinicians must be aware of the potential contamination of the hospital water network by *Legionella* and the associated risk of disease occurrence. This is crucial to prevent underdiagnosis and to always consider *Legionella* pneumonia possibly associated with care practices, especially in patients at high risk, using appropriate diagnostic tests. It is therefore essential to train the person responsible for infection control and the person responsible for the management and maintenance of the facilities on the

measures to control legionellosis associated with care practices, to inform the departments of the results of the risk assessment and to ensure the traceability of the activities carried out through appropriate records.
Our study is subject to certain limitations. The surveillance started in 2017, and there are no available systematically collected data for previous years and lack of data on water pipe temperatures hampers the assessment of correlation with *Legionella spp*. contamination.
The COVID-19 pandemic introduces a confounding factor, as it resulted in a decrease in the execution of sampling in the wards. Furthermore, there have been significant changes in the organization of hospital departments, including the conversion of some units into intensive or sub-intensive care facilities for the treatment of severe cases. Consequently, in the years 2020-2021, there was a structural reorganization of the hospital, temporarily modifying the categorization of buildings based on the level of risk.

Conclusions

Our work highlights the importance of an adequate surveillance of legionellosis in a nosocomial environment to implement, effectively and promptly, the planned disinfection and prevention measures, reducing the likelihood of hospital infections. Based on our results, it is important to make all healthcare professionals in the hospital aware of the potential infectious risk in wards, especially those at increased risk of contamination, so that a possible case of legionellosis related to assistance can be timely recognized.

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Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions:

LM, AO: conceptualisation; LM, MO, AO: methodology; MO, AO: formal analysis; MO, PP, OF, RA, EM, DB, RZ: investigation; AM, AO: resources; LM, RA: data analysis; LM, RA: writing – original draft preparation,; MO, AB, AM, AO: writing – review and editing; MO, EM, AO: visualisation; AO: supervision.
All authors have read and agreed to the current version of the manuscript.

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