Article

Legionella monitoring in building's water distribution systems: the case study of a sparse University campus

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Abstract: We have monitored the presence of Legionella in the building's plumbing of the University of Perugia (Italy) and identified the Legionella isolates. More than 300 water samples collected from 100 taps throughout the university campus were analyzed. Legionella was absent in the great majority of the samples, while it was found in only five buildings. Molecular analysis indicated that the contaminations were ascribed to *L. pneumophila* (sg 1, sg 8 and sg 10) as well as to other species (*L. taurinensis* and *L. anisa*). In only three cases the levels of contamination were above the limit at which, according to international guidelines, remedial actions are required. In particular, a thermal disinfection, i.e., raising the water temperature above the level at which Legionella cells do not survive, was applied to the hot water supply systems where high temperature could be maintained throughout. On the contrary, in a building in which Legionella contamination originated inside the heat exchanger, a chemical disinfection with silver hydrogen peroxide was carried out. This case study indicates how a multidisciplinary approach is necessary for an effective definition of Legionella prevention and control strategies.

Keywords: building's plumbing system; Legionella contamination; disinfection; *16S rRNA*; *mip* gene.

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1. Introduction

The occurrence of Legionella contaminations in building's tap water represents a serious health threat to end-users [1]. Indeed, the genus *Legionella* comprises many different bacterial species and serogroups (sg), of which *L. pneumophila* sg1 is the most often associated to the human lung infection that causes legionellosis, often referred as Legionnaires' disease, a form of atypical pneumonia [2,3]. Legionella is found naturally in fresh water environments, like lakes and streams, but can proliferate in human-made water systems such as building's plumbing and hot water networks [1,4,5]. Consequently, monitoring the presence of Legionella bacteria and preventing their colonization of water distribution mains and building's plumbing systems are both technical and environmental challenges and are therefore considered crucial aspects by international and national guidelines that regulate the quality of drinking water [4-6].

This paper reports a case study regarding a one year-long survey of the presence of Legionella in the water distribution systems of the buildings that compose the campus of the University of Perugia (Italy). The molecular characterization of the retrieved Legionella isolates is also shown.

2. Experimentals

More than 300 water samples were collected from 100 taps in 42 different buildings throughout the university campus. Cold and hot water samples were taken, before and after flushing water for 5 minutes, the latter in order to account for possible contaminations inside the plumbing system. The presence of Legionella was assessed by standard methods as indicated by Italian guidelines [6]. Briefly, 1 litre water samples were concentrated by filtration and spread onto GVPC agar plates to allow the culture and enumeration *Legionella* spp. bacteria. The identification of Legionella isolates was conducted first by selective growth on BCYE agar plates with and without cysteine and then confirmed by by agglutination test and sequencing of *16S rRNA* and *mip* genes [7].

3. Results and discussion

The University of Perugia features a sparse campus comprising many different buildings distributed in eight different locations within the city of Perugia (Figure 1) and in two locations in the city of Terni, in Central Italy. The buildings span from very old ones, dating back to the 13th Century, to modern ones and so feature very different water distribution networks and hot water production systems.

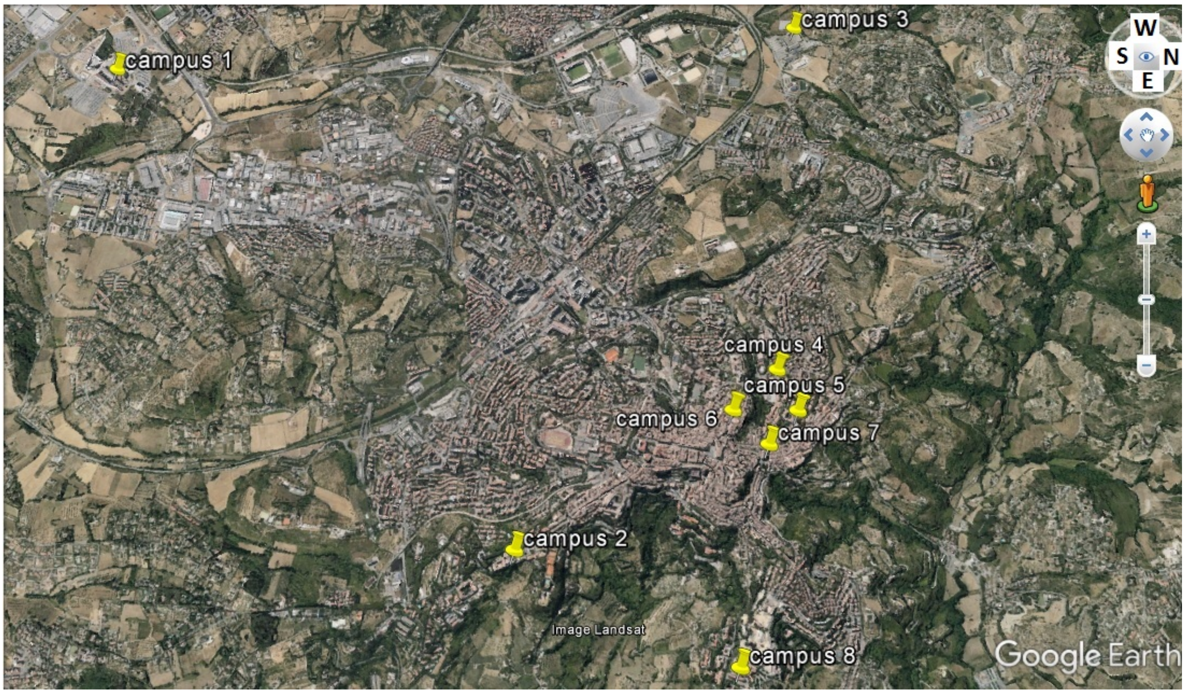

Figure 1. University of Perugia campus locations within the city of Perugia (Central Italy).

In most of the campus buildings, no Legionella contaminations were found. Despite the fact that these buildings included also those with very old plumbing systems, this evidence was not surprising considering that in most of them there was no hot water production system. Indeed, Legionella is rarely found in cold water as this bacteria are known to proliferate between 25 and 42 °C [1,6].

Legionella was found in only five buildings of the entire campus and in only three cases the levels of contamination were above the limit (> 10^3 cfu/litre) at which, according to the Italian national guidelines in the absence of human infection cases [6], remedial actions are required. Further, molecular analysis indicated that the contaminations were only partially ascribed to *L. pneumophila* sg1, the bacteria mostly responsible for causing legionellosis [2,3]. In fact, other serogroups (sg8 and sg10) as well as other species (*L. taurinensis* and *L. anisa*) were also found [7].

Among the contaminated buildings, two showed the presence of Legionella in the hot water samples with concentrations ranging from 10^2 to 10^4 cfu/litre. These buildings were of recent construction and featured hot water supply systems based either on centralized or single heaters (boilers), where high water temperatures could be achieved and maintained throughout. In this cases, a thermal disinfection, i.e. raising and maintaining the water temperature above the level at which Legionella cells do not survive (> 60 °C), was implemented [1,6,8]. This approach proved to be effective as Legionella was absent in the water samples taken after 48 hours and in the following periodical monitoring.

One case proved to be more complicated. Legionella contaminations, up to 10^4 cfu/litre and identified as *L. pneumophila* sg 1, were found in the samples taken from the hot water distribution taps and in the hot water production unit (heat exchanger), despite this was off at the time of sampling. Indeed, the sampling was performed in the summer period and the temperature of these water samples ranged from 23 to 25°C, a level above the limit (< 20°C) under which Legionella proliferation is inhibited [1]. On the contrary, Legionella was absent in cold water samples taken after flushing, as well as in the common part of the water distribution system (network inlet, reservoir tank, water softener), indicating that the contamination was limited to the hot water plumbing system and likely originated inside the hot water production unit. In this building the production of hot water was granted by a heat exchanger with the circulating heating system that doesn't allow to reach high water temperatures. Thus, a chemical disinfection with silver hydrogen peroxide was carried out [6,9]. One week after disinfection and turning on hot water production, Legionella was absent in all samples. After one month, Legionella was found again in hot water samples, though at lower levels than before treatment (10^2-10^3 cfu/litre), while after four months the contamination raised above 10^5 cfu/litre, overreaching the levels found before the disinfection. Noticeably, the water temperature inside this heat exchanger ranged, during the day, between 22 and 48°C suggesting that, even after disinfection, the re-growth of Legionella may have been favored by the conditions inside the hot water production unit [1,6]. Further, the treatment with silver hydrogen peroxide showed to be effective in controlling Legionella growth only in the short-term.

4. Conclusions

A one year-long survey of the presence of Legionella in the water distribution systems within the sparse campus of the University of Perugia indicated that contaminations were limited to few buildings. Thermal disinfection was effective in those cases where the hot water supply systems allowed to maintain the water temperature above the level at which Legionella cells do not survive (> 60 °C). On the contrary, in a building where Legionella contamination originated in the heat exchanger, a chemical disinfection with silver hydrogen peroxide was carried out but proved to be effective only in the short-term.

Taken together, the results obtained in the case study herein reported indicate how a multidisciplinary approach that integrates microbiological analysis with the survey of building's plumbing systems is necessary for the definition of effective strategies for Legionella prevention and control.

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Conflicts of Interest: The authors declare no conflict of interest.

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