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Colonization by *Legionella* spp. of water networks in residential buildings of the Province of Pisa, Italy

A. Baggiani*, B. Casini*, M. Totaro*, F. Aquino*, P. Valentini*, B. Bruni*, A. Porretta*, F. Casalini*, M. Miccoli**, G. Privitera*

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Parole chiave: Legionella, condomini, rischio idrico

Abstract

Background: *Despite the increase of community acquired cases of legionellosis in Italy over the last years, the Italian guidelines do not give indications for prevention and control of Legionella in the hot water networks (or centralized conditioning systems) of residential buildings. We performed a survey on eight medium sized apartment buildings in the Pisa district to assess the prevalence of Legionella spp. in the water network and the respondance to drinking water requisites at the point of use, according to the Italian norms.*
Methods: *For each building two hot water and three cold water samples (located at water entrance from the aqueduct network into the building pipework, at the exit from pressure autoclave, and at a remote tap) were collected.*
Results: *Legionella was detected in 20% of residential buildings, mostly in those with a central hot water production system.*
Conclusions: *The study highlights a condition of potential risk for susceptible population subgroups and supports the need for measures of risk assessment and control.*

Introduction

The genus *Legionella* includes more than 50 species of Gram negative aerobic rods, which are widely present in freshwater and soil and tend to contaminate man-made water systems and replicates between 25°C and 42°C. *Legionella* can survive at higher temperatures: several hours at 50°C and up to a maximum of 57°C-63°C (1, 2). *Legionella* is killed almost instantaneously at 70°C, with the exception of some situations involving symbiosis with other microorganisms (3).

Legionella infection may cause two distinct clinical diseases: Legionnaires'

* *Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy*
** *Department of Clinical and Experimental Medicine, University of Pisa, Italy*

Disease and Pontiac fever. Old age, tobacco smoking, chronic diseases and immunocompromission are predisposing factors for the development of disease. Infection is acquired by inhalation, aspiration or micro-aspiration of *Legionella* carrying aerosols. Droplets carrying the pathogen can originate by water spraying or by gurgling air through contaminated water (4).

The risk of developing the disease results from a combination of individual susceptibility and degree of exposition, related to bacterial load and duration of exposition (5).

Legionella pneumophila is the species most commonly associated with the disease (6, 7), with 16 known serogroups, of which *Legionella pneumophila* serogroup 1 was associated to the first recognized epidemic of Legionellosis in Philadelphia 1976 and is still the cause of 95% of *Legionella* infections in Europe and 85% worldwide (8).

The need for appropriate control of legionellosis has prompted the development in Europe of dedicated networks and working groups. The European Working Group for Legionella Infections (EWGLI) was established in 1986 with the aim to improve and share knowledge of *Legionella* control, legionellosis epidemiology and microbiology. More recently the activities related to the surveillance have been transferred to the European Centre for Disease Prevention and Control (ECDC).

The surveillance of legionellosis in Italy is coordinated by Istituto Superiore di Sanità, which maintains a National Legionellosis registry and issues an annual report. The last available data, related to 2013, give 1347 new cases of legionellosis in the Italian population, corresponding to an incidence rate of 22,6 cases per million inhabitants, a figure not different from the previous year. However it must be noticed that 2012 has seen an increase of 13% in the incidence when compared with the previous years. Of the 1347 cases notified in 2013, 62 (4,6%) were hospital acquired, 21 (1,5%) were acquired in long term care facilities and 1123 (83,4%) were community acquired infections (9, 10), underscoring the relevance of *Legionella* contamination of community environment and to the subsequent need for control.

Literature data on environmental Legionella contamination (especially in warm water networks and air conditioning systems) are available for hospitals (11-13) long term care facilities (14, 15) and commercial accommodation sites (2, 16).

Regulations have been adopted for health care and hotel facilities, requiring periodic monitoring and disinfection of water systems (5, 17). A guideline for legionellosis control in hotels has been issued in Italy in 2005, defining goals and standards.

It should be noted however that there are very few papers in the literature about the presence of *Legionella* in small residential buildings and on the factors associated to contamination of water systems.

In a survey performed in 6 Italian cities *Legionella pneumophila* was found in 22.6% of water samples, most of the isolates belonging to group 2-14 (18).

The Italian regulations on Legionella control are quite specific about the responsibilities for hospitals and hotels, but little, if any, is said about residential housing.

However the Drinking Water Directive (Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption) adopted in Italy with the D.Lgs. 31/2001 (19) and D.Lgs. 27/2002 (20), identifies the building administrator as the person in charge of assuring water quality at the points of use.

Aim of this study is to assess the quality of drinking water in residential buildings in the Pisa district, Italy, by testing samples from water systems not only for drinking water regulatory parameters , but also for the presence of *Legionella*.

Materials and methods

Sampling

In agreement with some Building Administrators of the Pisa district, from April 2014 to November 2014, 81residential buildings were selected for the survay. The buildings were mainly of small size with an average of eight flats per building. Only 5 had central water heating, while in the others each flat had independent hot water production system. In each building 3 cold water samples (one at water entrance from aqueduct network into the building pipework, one at the exit from pressure autoclave and one at the most remote tap from the autoclave) and two hot water samples (from taps at the first and last floor of the building, respectively "point A" and "point B") were taken.

243 samples of cold water for the determination of potability requirements and 162 hot water samples for detection of *Legionella* were collected.

Hot water sampling were performed in accordance to the Italian guidelines for legionellosis control (5) while cold water samples were collected in accordance to ISO 19458:2006 (21). Water temperature and chlorine concentration (free and residual) of the samples were also determined.

Legionella testing

The samples were tested for presence of *Legionella* in accordance to Italian guidelines (5) and the ISO 11731:1998 norm (22). One liter samples were filtered through a 0.2 μm membrane (Millipore, Billerica, MA). The membrane was immersed in 10 ml of water and sonicated for 5 minutes to allow the bacterial cells to separate from the membrane. The resulting suspension was brought to 50°C to select *Legionella* spp. over other non heat-resistant bacterial species. After the thermal inactivation phase 0,1 ml of the sample was plated on GVPC Agar plates (Oxoid, UK), and incubated at 37°C for 7-10 days in jars under an atmosphere containing 2.5% CO_2. The suspect Legionella colonies were tested for species and serogroup by polyvalent agglutination latex test (Legionella latex test – Oxoid, UK).

Drinking water parameters

In accordance to D.Lgs. 31/2001 (19) the total microbial count at 22°C, and the counts of faecal coliforms and enterococci were determined. Total microbial count was performed by inclusion in Plate Count Agar (Oxoid, UK) according to ISO6222:1999 (23), while faecal coliforms and enterococci detection was performed by filtration of 100ml of water through 0,45 μm membranes (Nalgene, USA). The membranes were layered on m-FC Agar plates (Biolife, Italy) for coliform growth according to ISO 9308-1:2014 (24) and on Slanetz Bartley Agar plates (Biolife, Italy) for enterococcal growth according to ISO 7899-2:2003 (25). Species confirmation of suspect colonies was obtained by Mini API galleries (bioMeriéux, France).

Data analysis

The Shapiro-Wilk test was performed to verify normality of distributions. The Mann-Whitney test was used to compare the total microbial counts at 22°C, adjusted for water temperature and residual chlorine concentration. The Kruskall-Wallis test and the Dunn's test were performed to compare the total microbial counts at 22°C detected in different sampling points. The power tests were used to estimate the sample sizes, the 1-β values of the significant variables were > 0.8, assuring a low risk of type II error and appropriate sample sizes. The statistical analysis was carried out using the IBM SPSS software package, version 17.0.1.

Results

Water temperature and residual chlorine

The temperature of the hot water samples ranged between 26.8°C and 56.4°C (mean value 43.2 ± 8,7°C), while for cold water ranged between 16.1°C and 24.1°C (mean value 20.1 ± 2.9°C). Residual chlorine concentration in hot water samples ranged between 0 and 0.3 ppm (mean value 0.09 ± 0.05ppm), while in cold water samples ranged between 0 and 2 ppm (mean value 0.11 ± 0.05ppm).

Cold water samples with a temperature >20.1°C showed a mean value of total microbial count at 22°C higher than samples with a temperature <20.1°C (p = 0.00014). Moreover, cold water samples showing a residual chlorine <0.1 ppm revealed a mean value of total microbial count at 22°C higher than samples with a residual chlorine >0.01 ppm (p<0.0001) (Table 1).

Table 1 - Mean values of total microbial count at 22°C in cold water samples at different temperatures and residual chlorine concentrations.

Physical and chemical parameters	Total microbial counts (mean CFU/mL ±DS)
T>20,1°C	39,2(±68) CFU/mL
T<20,1°C	20,5(±40) CFU/mL
Free Chlorine >0,1 ppm	21(±62) CFU/mL
Free Chlorine <0,1 ppm	35,1(±60)CFU/mL

Legionella detection

Legionella spp. was detected in 21/162 (13%) hot water samples examined. However 7/21 positive samples were collected from residential buildings with central hot water system, with a positivity ratio of 7/10 (70%), while in buildings with independent hot water production system only 14/152 (9%) samples were positive for *Legionella*.

Overall, about 20% (16/81) of the buildings examined had at least one sample positive for *Legionella*. Particularly, 4/5 (80%) of central hot water systems and 12/76 (16%) of independent hot water systems were colonized by *Legionella*.

Legionella positive samples showed the following counts: in the range of 10^2-10^3 CFU/L in 6/21 (28%) of samples; 10^3-10^4 CFU/L in 9/21 (43%) of samples; >10^4 CFU/L in 6/21 (29%) of samples. The higher counts were detected in samples with residual chlorine and temperature below 0,1 ppm and 46°C respectively (Table 2 and Figure 1).

Legionella was isolated in 20% of water samples with residual chlorine <0.1 ppm and in 11% of water samples with residual chlorine >0.1 ppm (Table 2).

Legionella pneumophila sg1, *Legionella pneumophila* sg2-14 and *Legionella* spp. were respectively isolated in 12/21 (57%), 3/21 (14%) and 6/21 (29%) of the tested samples (Figure 2).

Drinking water parameters tests

Cold water resulted free from microbiological hazards. Entercococci were not isolated in any sample, but *Serratia liquefaciens* (3 CFU/100mL) was detected at the exit from one pressure autoclave.

Table 2 - Percentage of *Legionella pneumophila* sg 1, *Legionella pneumophila* sg 2-14 and *Legionella* spp. in hot water samples at different residual chlorine concentrations.

Hot water residual free chlorine	% of positive samples for Legionella	% positive samples for *Legionella pneumophila* sg 1	% positive samples for *Legionella pneumophila* sg 2-14	% positive samples for *Legionella* spp.
>0,1 ppm	11% (13/122)	62% (8/13)	8% (1/13)	30% (4/13)
<0,1 ppm	20% (8/40)	50% (4/8)	25% (2/8)	25% (2/8)

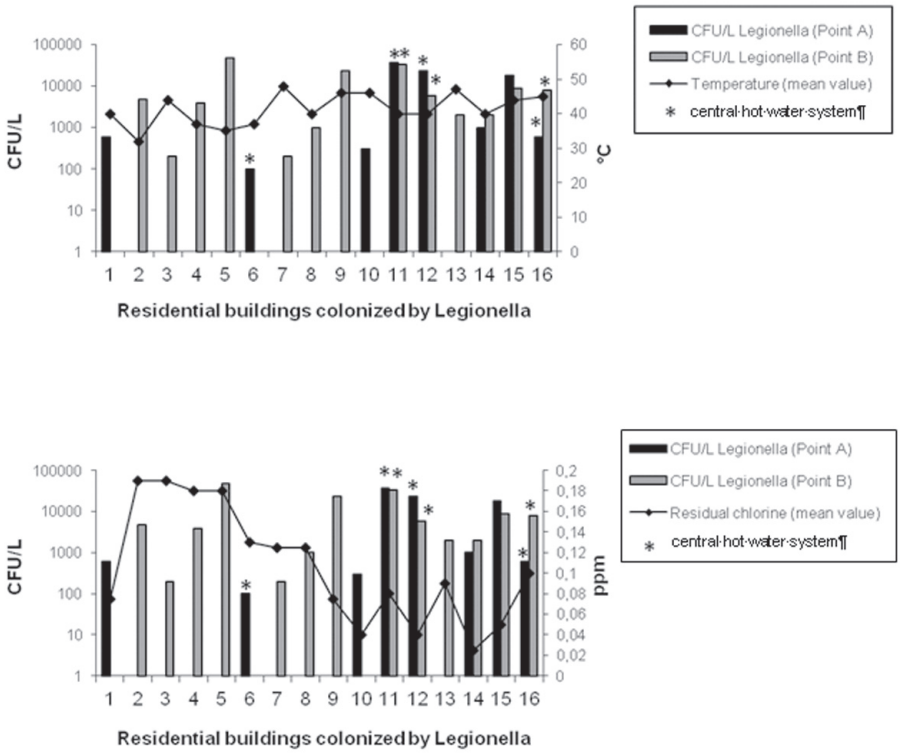

Figure 1 - Legionella concentrations and mean values of temperature at the sampling points (a). Legionella concentrations and mean values of residual chlorine at the sampling points (b).

A remarkable variability in microbial growth at 22°C was observed among the cold water samples.

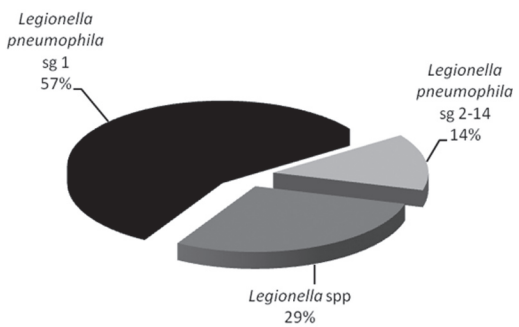

Figure 2 - Results of polyvalent agglutination latex tests.

The bacterial counts at the entrance from the aqueduct were significantly ($p<0.0001$) lower than those recorded in samples obtained in the buildings' pipework (Table 3). A total microbial count ranging between 1 and 400 CFU/ml was observed.

Bacterial counts $>10^2$CFU/ml were detected in 32/243 (13%) of the samples, mostly at the exit from the autoclave.

Table 3 - Mean values of total microbial counts at 22°C (CFU/ml ± SD) at different points of sampling.

At entrance from the aqueduct	At the exit from autoclave	At remote tap
10,9 CFU/ml (±28)	44,7 CFU/ml (±77)	41,2 CFU/ml (±59)

Discussion and conclusions

The present study shows that *Legionella* is present in 20% of warm water networks, especially in residential buildings provided with a centralized hot water production system.

During recent years, water risk management focused on *Legionella* and on evaluation of disinfection systems employed to control colonization in hospitals and tourist accommodation facilities water networks. However, epidemiological data show that more than 80% of Legionnaire's disease cases diagnosed are acquired in community, outside hospitals or hotels (9). Moreover, community cases may be underestimated due to underdiagnosis by clinicians who fail to require the specific diagnostic tests prior to prescribe broad-spectrum antibiotics. These evidences confirm the importance of extending prevention initiatives and water risk control to residential buildings.

Besides showering, which favors aerosolization of potentially contaminated water and consequent inhalation, many other household activities may expose to a prolonged contact with splashing water favoring infections (26).

Home networks contamination outline a real and impacting risk for health, in particular for susceptible categories as immunocompromised, elderly and people affected with chronic diseases. Furthermore, despite *Legionella* infections are generally more frequent in advanced age, at least 50% occurs in subjects in under 65, with incidence rates that gradually rise from the age of 40 years.

Our data, according with other studies (2, 12), demonstrate the presence of *Legionella*, especially *Legionella pneumophila* sg 1, even with water temperatures far from the optimum for growth (<40 ° C), although with counts not exceeding 10^2CFU/L.

Water networks, especially in the Italian residential buildings context, with a high rate of old blocks of flats distributed on several floors, often have dead legs where water stagnation promotes biofilm formation, creating the ideal habitat for *Legionella*. Our results also show a higher frequence of positive sites in distal points of the installations, probably due to the longer path of the water column in the pipeline and consequently to low concentrations of residual chlorine.

Another issue evidenced by this study concerns the status of residential building autoclaves. An increase in total microbial counts at 22°C between the samples collected at the water entry point compared to the ones collected at the exit of autoclave and at terminal points of use in apartments was observed. This was attributed to debris deposition and sediment formation on the bottom, confirmed by turbidity at visual inspection, which favored microbial growth.

D.Lgs. 27/2002 (20), updating of D.Lgs. 31/2001 (19), extends to building administrators the responsibility of water hygienic control in the building, from point of delivery by the water supplier up to the points of use. It defines a clear obligation for Building Administrators to make sure that water introduced into residential buildings pipelines meets the quality requirements of the D.Lgs. 31/2001 (19). Inspections and controls of water quality must be careful, timely and should use a regular monitoring of water systems to ensure the health safety of buildings.

A proper periodic maintenance of buildings water systems is essential. Reported data on autoclaves total microbial counts are indicative of how a systematic tank cleaning is recommended and required to assure water quality. Consequently, proper training of all workers involved in residential water systems management (building administrators, plumbers), is advisable in order to provide the knowledges needed for the implementation of prevention measures and control.

Riassunto

Colonizzazione da* Legionella *spp. delle reti idriche di edifici a destinazione residenziale della Provincia di Pisa

Introduzione: Sebbene negli ultimi anni in Italia sia stato documentato un incremento di casi comunitari di legionellosi, le linee guida per la prevenzione e il controllo della legionellosi non individuano specificatamente ruoli e responsabilità in merito al controllo di Legionella in edifici a destinazione residenziale provvisti di acqua calda e/o impianti di condizionamento centralizzati. Il presente studio riporta i risultati preliminari di una indagine sulla colonizzazione da *Legionella* spp. in impianti idrici di edifici residenziali della Provincia di Pisa e l'idoneità al punto d'uso dell'acqua secondo quanto previsto dal D.Lgs. 31/2001.

Metodi: In ogni edificio sono stati prelevati due campioni di acqua calda per la ricerca di *Legionella* spp., e tre campioni di acqua fredda per la valutazione dei parametri microbiologici del controllo di routine, rispettivamente al punto di entrata dall'acquedotto, all'uscita dall'autoclave e al punto più distale dell'impianto.

Risultati: La presenza di Legionella è stata rilevata nel 20% delle strutture esaminate, percentuale che sale fino all'80% fra i condomini con impianto di riscaldamento centralizzato.

Conclusioni: Ciò può rappresentare una situazione di rischio per le categorie di soggetti più vulnerabili e indica la necessità di interventi di controllo.

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Corresponding author: Prof. Angelo Baggiani, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa., Via S. Zeno 35-39, 56127 Pisa, Italy
e-mail: angelo.baggiani@med.unipi.it