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Legionella contamination in hot water systems of hospitals, nursing homes, hotels, factories and spas in Tuscany-Italy

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Abstract

Following the report of many cases of Legionnaires' disease associated with accommodation facilities such as hotels, spas, workplaces, hospitals and nursing homes, we verified if *Legionella pneumophila* and *Legionella* spp. were present in some of those structures in Tuscany, in order to estimate the species and serogroups in circulation. *Legionella pneumophila* serogroup 1 (30.9%) was the most frequently isolated species along with serogroups 3 (16.1%) and 6 (13.3%); these three serogroups are identified, in literature, as those most responsible for Legionnaires' disease (LD). Studying all analyzed structures, we found some parts of the water system where *Legionella* concentration was higher than 10^3CFU/L, indicated, in Italy, as the maximum admitted concentration value above which a decontamination treatment is necessary when one or more cases of healthcare-acquired Legionnaires' disease are observed. Moreover disinfection is recommended in any case when counts exceed 10^4CFU/L.

Consequently, in order to prevent cases of Legionnaires' disease, a continuous surveillance of the water systems of all accommodation facilities is necessary, with particular attention to hospitals and nursing homes where immunocompromised patients lodge, so as to promptly estimate the presence of the pathogen and consequently plan the most suitable intervention activities. We concluded that, in any structure, a continuous surveillance and disinfecting treatment of water systems is necessary. Moreover, after any disinfection treatment the temperature of the hot water flowing in the system must be necessarily maintained near 51°C in order to minimize the probability of recontamination from *Legionella* and limit the risk of LD in consumers.

Key words: Legionella, legionellosis, contamination, hotel, hospital, nursing home, spa

Introduction

Legionella is a Gram-negative aerobic bacillus which can be inhaled with aerosolized water containing the bacteria. *Legionella* can reach the lungs and cause an infection which can be asymptomatic, can result in Pontiac fever with flu-like symptoms, or in an atypical pneumonia (Legionnaires' disease) with high lethality in community cases (5-15%) [1,2] and in nosocomial ones (70-80%) [3].

Although more than 50 different species of *Legionella* have been described [3], not all are linked to community-acquired and nosocomial legionellosis in humans [4,5]. *Legionella pneumophila* is the most commonly isolated species in the diagnosed cases, *Legionella pneumophila* serogroup 1 is the most pathogenic one, causing about 85-90% of cases, while *Legionella micdadei* and *Legionella longbeachae* are isolated in only about 10-15% of cases [4,6,7]. This is, primarily, due to the fact that *Legionella pneumophila* serogroup 1 is the most frequent bacterium of the species circulating in water systems [8,9] when chlorine is adopted in *Legionella* risk control. *Legionella* is capable of entry and intracellular multiplication in aquatic amoebae and, in response to environmental stress, amoebae containing internalized bacteria can form cysts capable of withstanding attempted killing by water purification treatments [10]. *Legionella pneumophila* sg. 1 is more virulent than other serogroups of *L. pneumophila* [11] and since there are striking similarities in the processes by which *Legionella* infect protozoa and mammalian phagocytic cells, *L. pneumophila* sg. 1 seems to have more opportunities to invade host cells and become resistant to chemical disinfectants. Secondly, *L. pneumophila* serogroup 1 is more pathogenic than the other species and serogroups, because when laboratory diagnosis is carried out,

in most of the cases when detecting the *Legionella* antigen in urine samples, only *L. pneumophila* sg 1 is distinguished [3,12]. Although the urinary antigen specific for *L. pneumophila* sg 1 can cross react with other serogroups of *L. pneumophila*, cases of pneumonia, due to other species or serogroups different from *L. pneumophila* sg 1, are not diagnosed and consequently are not present in epidemiological data. The incidence of Legionnaires' disease (LD) reported in Italy in 2007 was in the nature of 14.46 inhabitants per million. Of these, 10% were nosocomial cases with a fatality rate of 37.5%, and 38.27% were tourists (Italian and not) [12]. The incidence of legionellosis can be underestimated because of poor clinical awareness, wrong diagnosis, delayed sero-conversion, non classical symptoms or non sufficiently specific tests used in diagnostic microbiology laboratories.

Legionella spp. can survive chlorination and thus colonize all water systems. In all Countries every year epidemic clusters of LD occur in structures such as hospitals [7,13], nursing homes, holiday villages or hotels and cruise-ships [14] where people lodge for several days, and sporadic episodes occur in thermal springs, swimming pools, or airplanes where the users stay only for few hours. Cases of *Legionella* infection are also found in factories, where stress and hard work can represent a cause of temporary immunosuppression [15,16]. All cases are due to bacteria colonizing the hot water systems in those buildings because of their complex structure providing optimal conditions for the growth of these microorganisms [17,18].

To limit the risk of LD in Italy and in other countries, (France, Denmark, Germany, The Netherlands, Spain, Norway, Portugal and Switzerland) national guidelines and recommendations are adopted [19-23] indicating the maximum admitted *Legionella* concentration in water systems (10^3CFU/l) above which a decontamination is necessary, when one or more cases of healthcare-acquired Legionnaires' disease are observed, and in any case where counts exceed 10^4CFU/L, describing the decontamination techniques to be used.

Even if the exact minimum infective dose for humans is not known, the risk for LD acquisition is mainly correlated to the exposed subjects' susceptibility to *Legionella* concentration in water (CFU/l), and also to the virulence of the strain.

During the last few years, community-acquired LD has become very important, because of the high percentage of old people in industrialized countries. In fact, the incremented incidence of immune system pathologies and the wide use of drugs and immunosuppressant treatments, has determined a remarkable increase in immuno-depressed people easily hit by infectious opportunistic pathologies [6,24-26].

This study was done in the course of surveillance in order to control for Legionellosis in some structures in Tuscany with the aim of taking into account the circulation of *Legionella* in the centrally heated water systems of some facilities such as hospitals, nursing homes, factories and spas in Tuscany.

Materials and methods

Sampling

The research was carried out in some structures of Tuscany that asked us to plan a surveillance of the presence of *Legionella* in their water systems. In a three year survey, a total of 493 environmental water samples were obtained from 14 buildings: 3 hotels, 3 hospitals, 3 nursing homes, 2 factories and 3 spas with thermal springs.

From each structure, we took more than one sample, collecting water samples in the more distal taps, from the hot water tanks and near end-plate pipes, were stagnation of water can occur. These are hazard points having optimal conditions for the growth of *Legionella* in the water system. In the three analyzed hospitals we also took three samples, respectively, from three taps of the bathrooms used by the patients hospitalized for kidney transplant, spinal cord injury and liver cirrhosis in a heavy drinker, that had been affected by LD during hospitalization. In Factory A we also took samples from the showers of the dressing rooms, where the worker who had been affected by LD showered every day at the end of his work shift. These points probably represented the originating point of the infection for the LD cases that had occurred in the hospitals (taps) and in the factory (showers) under surveillance.

Each sample of 1l of water was collected in a sterile bottle containing 1ml of a 10mg/ml solution of sodium thiosulphate; before collecting the samples, the water was allowed to flow for 10sec. The water temperature and residual free chlorine were determined immediately after collection (DPD method, colorimeter La Motte, Model DC 1100, Chestertown, MD, USA).

Culture method and serotyping

For sample processing, storage conditions and isolation, we used the methods described in the Italian Guidelines for Legionellosis control and prevention [19,27]. Samples were concentrated by filtration of 1l of water through 0.22µm

pore size cellulose acetate membrane filters (Millipore S.p.A., Milan, Italy) and re-suspended into 10ml of the filtrate. To decontaminate the suspension from bacteria other than *Legionella*, the suspension was then treated at 50°C for 30 minutes. Aliquots (500µL) of the suspension were plated on to BCYE and MWY selective media (Oxoid, Basingstoke, UK). Plates were incubated at 36°C in a humidified environment in 6% CO_2 for seven days. Suspected colonies were subcultured on BCYE and CYE media (Oxoid, Basingstoke, UK) for three days. Colonies grown on BCYE and not on CYE media were identified through anti-sera for agglutination test for *Legionella pneumophila* sg 1 to 14 and for *Legionella* species for *L. dumoffii*, *L. micdadei* and *L. bozemanii* (Legionella antisera, Denka Seiken CO.,LTD). Colonies not identified through agglutination test were classified as *Legionella* spp.. The results were expressed as CFU/L.

The detection limit of the procedure was 20 CFU/L.

The concentrated suspensions were incubated at 44°C for seven days, to allow multiplication of bacteria and to enable isolation of *Legionella* species below the detection limit of the procedure. After that, the suspension was plated as described before and the colonies were identified through specific anti-sera as previously described.

Results

Residual free chlorine, determined immediately after collection in all tested points of all structures, showed values of 0.1-0.2mg/L.

Hotels

The water temperature of all analyzed points, determined immediately after collection, had low values, near to 45°C, so allowing *Legionella* multiplication and out of the 30 samples collected in the 3 analyzed Hotels, 11 (36.7%) were contaminated by *Legionella* spp.. From the positive water samples we isolated 13 strains, 46.1% of which were *Legionella pneumophila* serogroup 1 (Table 1). Four of them had concentrations higher than 10^3CFU/l: one from Hotel 1 and three from Hotel 3 where, and before the first sampling, two LD cases had occurred, one affecting a tourist and one a cleaner. The second sampling was effected in all the analyzed Hotels one month after the disinfection with hyperchlorination of the water system at 50ml/l for an hour; all samples were not contaminated thus showing the efficiency of this disinfection method.

Hospitals

After three nosocomial LD cases in three hospitals respectively, we began surveillance in all hospital departments of those structures. Out of the total of 155 samples, 104 (67.1%) were positive for *Legionella*, which was present in 17 (36.9%) of the 46 samples from Hospital 1, in 62 (83.8%) of the 74 samples from Hospital 2, and in 25 (71.4%) of the 35 samples collected from Hospital 3 (Table 2). The water temperature, measured in the collecting points, was always below 48°C.

From all analyzed hospitals, we isolated 39

Table 1. Legionella contamination of hotels.

Hotels	*Sampl. N°*	*Pos. Sampl. N° (%)*	*N° Isolat. Strains*	*L. pn 1*	*L. pn 2*	*L. pn 3*	*L. pn 4*	*L. pn 5*	*L. pn 6*	*L. pn 7/14*	*L. micd*	*L. boz*	*L. dum*	*L. spp.*	*N°Sp./ Sg. ≥10³ UFC/l*
A-1	5	3(60.0)	4	3	-	-	-	-	-	-	-	-	-	1	1L.pn1
A-2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B-1	6	5(83.3)	6	-	3	-	-	-	-	2	-	-	-	1	2 L.pn.9
B-2	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C-1	4	3(75.0)	3	3	-	-	-	-	-	-	-	-	-	-	3 L.pn1
C-2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tot. n	30	11	13	6	3	-	-	-	-	2	-	-	-	2	6
Tot. %		36.7		46.1	23.1	-	-	-	-	15,4	-	-	-	15,4	46.1

L. micd = L. micdadei; L. boz = L. bozemanii; L. dum = L. dumoffii.
The numbers near the letters mean subsequent samplings.

strains of *L. pneumophila* sg. 1 representing 40.0% of the total strains (8) of hospital 1, 40.0% (30 strains) of hospital 2 and 2.63% (1 strain) of hospital 3. In each hospital we found samples with concentrations higher than 10^3CFU/l (Table 2), a value representing the limit above which, in Italy, a reclamation of the water system is necessary [19] when one or more cases of LD are observed as in the scenario here. In the water systems of the three hospitals we found also *L. pneumophila* non sg. 1 and *Legionella non pneumophila:* 19.5% of the strains were *L. pneumophila* sg. 3, 18.0% were *Legionella micdadei*, and 12.0% were *L. pneumophila* sg. 7/14, plus other species (Tab. 2).

Nursing homes

Out of the total 164 samples collected from three nursing homes, 39 (23.8%) were positive for *Legionella*, allowing 44 strains to be isolated, with a high prevalence of *L. pneumophila* sg 6 (40.9%) and sg 1 (38.5%). Other serogroups and species were isolated with lower frequency. The surveillance of structure A (Table 3) showed that, before the first disinfection cycle carried out with hyperchlorination at 50mg/l and occurring between samplings 3 and 4, there was a massive colonization by *Legionella*. We isolated *L. pneumophila* sg. 1, sg. 2, sg. 6 and *Legionella bozemanii.* In the sampling of A-1 and A-3 we found one and two points, respectively, in which *Legionella* concentration was greater than 10^3CFU/l. From the results shown in Table 3 we can also see that, six months after the disinfection, a re-colonization by *L. pneumophila* sg. 1 and sg. 6 had taken place, although at low concentrations, and was eliminated through a new hyperchlorination disinfection cycle.

From the results shown in Table 3, we can see a similar trend for the other two structures also.

Furthermore, in these structures the water temperature, measured in the collecting points, was always below 48°C.

Factories

The first sampling in Factory A was carried out after a case of LD in a healthy 50 year old worker had been communicated. It was thought that the worker had possibly inhaled the microorganism when showering every day at the end of his work shift. In the water system we found 10 positive points (50.0%), four of them were contaminated by *Legionella pneumophila* sg 1, and the *Legionella* concentration was higher than 10^3CFU/l (Table 4). Because of the presence of high levels of contamination in half of the analyzed points, a thermal disinfection of the industry was carried out through thermal shock at 80°C for three

Table 2. Legionella contamination in hospitals.

Hospitals	*Sampl. N°*	*Pos. Sampl. N° (%)*	*N° Isolat. Strains*	*L. pn 1*	*L. pn 2*	*L. pn 3*	*L. pn 4*	*L. pn 5*	*L. pn 6*	*L. pn 7/14*	*L. micd*	*L. boz*	*L. dum*	*L. spp.*	*N°Sp./Sg. ≥10^3 UFC/l*
A	46	17(36.9)	20	8	3	-	-	-	7	1	-	-	-	1	7L.pn1 6L.pn6
B	74	62(83.8)	75	30	-	1	2	-	2	12	21	1	-	6	10L.pn1 5L.mcd
C	35	25(71.4)	38	1	-	25	-	-	-	3	3	-	-	6	1L.pn1 20L.pn3 3L.micd
Tot. n	155	104	133	39	3	26	2	-	9	16	24	1	-	13	52
Tot. %		67.1		29.3	2.2	19.5	1.5	-	6.8	12.0	18.0	0.7	-	10.0	39.1

L. micd = L. micdadei; L. boz = L. bozemanii; L. dum = L. dumoffii.

days, as recommended by Italian guidelines for the prevention of legionellosis [19]. One month and six months after the thermal disinfection we carried out another two samplings (A-2 and A-3 respectively), in those sites positive at first sampling, and they resulted not contaminated. The measured temperature in each point was higher than 60°C. After one year we repeated the sampling, in the same and in new points, and we found re-colonization of the water system by *Legionella pneumophila* sg 6 and by *Legionella* spp. The water temperature was below 50°C in all analyzed points.

In Factory B (Table 4) we can observe the same trend of Factory A: in the first sampling we isolated *L. pneumophila* sg. 3 and *Legionella* unidentifiable through the sera used in the investigation and in the second sampling, carried out after the thermal disinfection, we did not isolate any strain. The third sampling was carried out one year after the second one and we found that a re-colonization of the water system had taken place. We also isolated serogroups of *Legionella pneumophila* which had not been isolated before. The water temperature was below 50°C thus permitting the active multiplication of *Legionella*. Seven sampled points had a high level of contamination by *L. pneumophila* sg. 1 and 3, representing a high risk of contracting legionellosis for the workers.

Table 3. Legionella contamination in nursing homes.

Nursing Homes	*Sampl. N°*	*Pos. Sampl. N° (%)*	*N° Isolat. Strains*	*L. pn 1*	*L. pn 2*	*L. pn 3*	*L. pn 4*	*L. pn 5*	*L. pn 6*	*L. pn 7/14*	*L. micd*	*L. boz*	*L. dum*	*L. spp.*	*N°Sp./ Sg. ≥10³ UFC/l*
A-1	11	4(36.4)	4	2	-	-	-	-	1	-	-	1	-	-	1Lpn.1
A-2	11	6(54.5)	6	1	2	-	-	-	1	-	-	2	-	-	-
A-3	12	6(50.0)	9	3	5	-	-	-	-	-	-	1	-	-	1L.pn.1 1L.pn.2
A-4	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-5	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-6	12	6(50.0)	7	3	-	-	-	-	4	-	-	-	-	-	-
A-7	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B-1	18	1(5.55)	2	1			-	-	1	-	-	-	-	-	-
B-2	12	4(33.3)	4	2	-	-	-	-	2	-	-	-	-	-	2Lpn1
B-3	12	-	-	-	-	-	-	-		-	-	-	-	-	-
B-4	12	3(25.0)	3	-	-	-	-	-	3	-	-	-	-	-	-
C-1	18	6(33.3)	6	3	-	-	-	-	3	-	-	-	-	-	3L.pn1
C-2	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C-3	18	3(33.3)	3	-	-	-	-	-	3	-	-	-	-	-	-
Tot. n	164	39	44	15	7	-	-	-	18	-	-	4	-	-	8
Tot. %		23.8		38.5	15.9	-	-	-	40.9	-	-	9.09	-	-	18.2

L. micd = L. micdadei; L. boz = L. bozemanii; L. dum = L. dumoffii.
The numbers near the letters mean subsequent samplings.

Thermal springs

We decided to analyze the water of three thermal springs in order to evaluate the risk to those frequenting these spas where people can receive thermal therapies, mineral water cures, inhalation therapies, creotherapies and mud-therapies. The water temperature, measured in the collecting points, was always below 45°C and in some points it was at environmental temperature.

In these structures we isolated *L. pneumophila* sg 5 and *L. dumoffii*, that had always been absent in all of the other analyzed establishments. In thermal springs A and B we isolated *L. pneumophila* sg 1, representing 25.0% of the total isolated strains and in thermal spring C we isolated *L. micdadei* (6.25%) (Table 5). In Table 5 we can observe how real the risk of contracting *Legionella* is by inhaling this type of water, both from different therapies and while waiting for drinking water.

Results synthesis

In the Table and the Figure summarising our total isolated strains (Table 6, Figure 1), we can observe that the most frequently isolated species were *L. pneumophila* sg 1 (30.9%), *L. pneumophila* sg 3 (16.1%) and *L. pneumophila* sg 6 (13.3%); these three serogroups are identified, in literature, as the strains most responsible of Legionnaires' disease. *L. pneumophila* sg 1 was found in all analyzed structures, while *L. pneumophila* sg 2 was found everywhere but in thermal springs water. Other *L. pneumophila* serogroups and *Legionella* species were variously distributed.

Discussion

We investigated the circulation of *Legionella* in some establishments with centralised hot water systems because the colonization is favoured in this type of water system, more than in independent ones [28]. The analysed water systems of the considered structures were all contaminated, before any treatment, by various species and serogroups of *Legionella*. 100% of the establishments were contaminated by *L. pneumophila*, 43.6% by *Legionella* spp. and 64.3% by species or serogroups not identifiable with our sera. 13 out of 14 (92.8%) analysed structures had at least one sampling with a high

Table 4. Legionella contamination of factories.

Factories	*Sampl. N°*	*Pos. Sampl. N° (%)*	*N° Isolat. Strains*	*L. pn 1*	*L. pn 2*	*L. pn 3*	*L. pn 4*	*L. pn 5*	*L. pn 6*	*L. pn 7/14*	*L. micd*	*L. boz*	*L. dum*	*L. spp.*	*N°Sp./ Sg. ≥10³ UFC/l*
A-1	20	10(50.0)	13	6	-	3	-	-	3	-	-	-	-	1	4L.pn1 3L.pn3 3L.pn6
A-2	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-3	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-4	16	4(25.0)	7	-	-	-	-	-	3	-	-	-	-	4	1L.pn6
B-1	12	6(50.0)	8	-	-	7	-	-	-	-	-	-	-	1	4L.pn.3
B-2	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B-3	23	12(52.2)	15	7	1	4	-	-	-	2	-	-	-	1	4L.pn1 3L.pn.3
Tot. n	103	32	43	13	1	14	-	-	6	2	-	-	-	7	22
Tot. %		31.1		30.2	2.32	32.6	-	-	13.9	4.65	-	-	-	16.3	51.2

L. micd = L. micdadei; L. boz = L. bozemanii; L. dum = L. dumoffii.
The numbers near the letters mean subsequent samplings.

Table 5. Legionella contamination of thermal springs.

Thermal springs	*Sampl. N°*	*Pos. Sampl. N° (%)*	*N° Isolat. Strains*	*L. pn 1*	*L. pn 2*	*L. pn 3*	*L. pn 4*	*L. pn 5*	*L. pn 6*	*L. pn 7/14*	*L. micd*	*L. boz*	*L. dum*	*L. spp.*	*N°Sp./ Sg. ≥10³ UFC/l*
A	14	4	4	2	-	-	-	2	-	-	-	-	-	-	-
B	14	4	5	2	-	-	-	-	-	-	-	-	2	1	1L.spp
C	13	5	7	-	-	-	-	2	-	-	1	-	1	3	2L. pn. 5 1L. micd 1L. dum
Tot. n	41	13	16	4	-	-	-	4	-	-	1	-	3	4	5
Tot. %		31.7		25.0	-	-	-	25.0	-	-	6.25	-	18.7	25.0	31.2

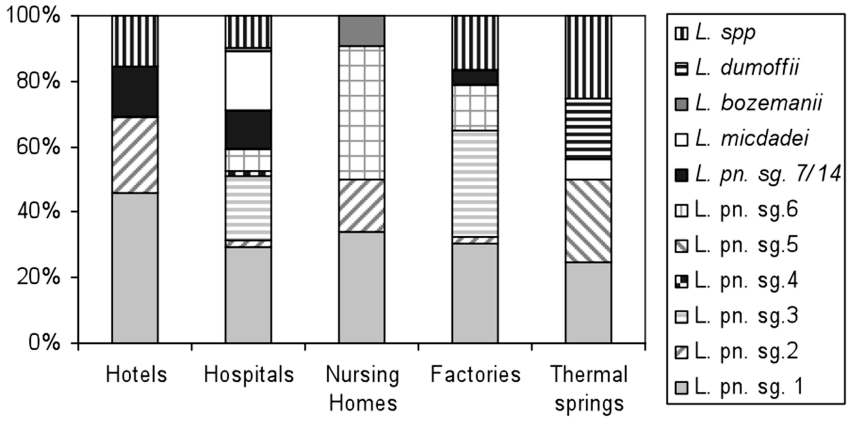
L. micd = L. micdadei; L. boz = L. bozemanii; L. dum = L. dumoffii.

Table 6. Legionella isolated from all samplings.

	Isolat. Strains	*L. pn 1*	*L. pn 2*	*L. pn 3*	*L. pn 4*	*L. pn 5*	*L. pn 6*	*L. pn 7/14*	*L. micd*	*L. boz*	*L. dum*	*L. spp.*
Tot. n	249	77	14	40	2	4	33	20	25	5	3	26
Tot. %	100	30.9	5.62	16.1	0.80	1.61	13.3	8.03	10.0	2.00	1.20	10.4

L. micd = L. micdadei; L. boz = L. bozemanii; L. dum = L. dumoffii.

Figure 1. Isolated serogroups of Legionella pneumophila from the analyzed structures.



level of contamination (≥ 10^3CFU/L). Although there are no scientific data that strictly correlate the concentration level of *Legionella* in water with LD, in our samplings we found many water collecting points with a concentration higher than that recommended by the safety limit established in European guidelines [19,21,22,29]. Particularly *L. pneumophila* sg 1 was the most significant species identified (30.9%) followed by *L. pneumophila* sg 3 and 6 (16.1% and 13.3% respectively) and literature suggests these strains as those most responsible for LD. Many factors can influence the risk of contracting legionellosis. One such factor is host susceptibility, and therefore the presence of *L. pneumophila* sg 1, 3 and 6 in structures such as hospitals and nursing

homes can represent an important Public Health problem because of immunocompromised hosts lodging in those structures [30]. Best and Stout [31] found a correlation between the number of positive samples and the occurrence of nosocomial legionnaires' disease cases: whenever *L. pneumophila* was isolated from more than 30% of the analysed water sites, nosocomial legionellosis cases occurred. Moreover, the analyzed hospitals had very high *Legionella* concentration and at least one LD case was diagnosed in all of them. On the contrary, in the nursing homes, we did not find any LD case, thanks to the poor circulation of microorganisms due to hyper-chlorination disinfection procedures. On the basis of these results, and those regarding the hotels, we can conclude that the disinfection carried out through hyperchlorination is really effective in contrasting the diffusion and the multiplication of *Legionella* only when repeated at regular time intervals of maximum six months. We also need to point out that *Legionella* multiplication is facilitated in the presence of bio film, particularly in complex water systems with many ramifications and end-plate pipes. Consequently, in the presence of such conditions, all hyperchlorination procedures must be necessarily followed by the circulation of hyper-chlorinated water throughout the piping of the whole water system.

Observing the results obtained in the factories, we can conclude that the thermal treatment through thermal shock at 80°C for three days is effective in limiting multiplication and diffusion of *Legionella,* but re-colonization of the water system can be avoided only if the temperature of the hot water is maintained above 65°C [8,32] in the distal points of the water system too. To limit the risk of scalding, it is necessary to maintain water temperature above 60°C only in storage tanks, but the circulating water temperature should be stored at a minimum temperature of 49°C [33,34] because some patients, due to illness, disabilities, advanced age or side effects of medication, could be less sensitive to temperature and could thus be at increased risk for tissue damage caused by extended exposure to hot water.Thermal springs represent the optimum conditions for *Legionella* multiplication because of pH values close to neutrality, high water temperatures and presence of many nutritive factors and symbionts. In this kind of water, besides *Legionella pneumophila* sg. 1, we also isolated *L. pneumophila* sg. 5 and other species such as *L. micdadei*, *L. dumoffii* and other unidentified species, not always correlated to LD cases but showing a probable risk for all hosts and particularly for immunocompromised ones.

Conclusions

We can conclude that, in order to limit the risk of contracting legionellosis, surveillance of all structures must be necessary, especially of those frequented by immunocompromised hosts [7], by searching these water systems for the presence of *Legionella.* Only through surveillance can the necessary actions be undertaken to eliminate these microorganisms from the water systems, especially from those with centralized hot-water systems, because these often have water stagnation in the tanks, and also in water systems having closed pipes, mostly due to restructuring. After the disinfection procedures it would be fundamental to maintain the temperature of the water system above 60°C in the hot water tanks and in all system points, particularly distal ones, to reduce the probability of re-contamination by *Legionella* [35] and bio-film reconstitution in the piping. Instead, to reduce the probability of scald injuries it is necessary to maintain the temperature of the water system near 49°C even if temperatures below 50°C are strictly correlated to the presence of *Legionellae*, which can quickly reproduce between 20° and 40°C and can survive even up to 60°C.

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