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Legionella pneumophila: An Environmental Organism and Accidental Pathogen

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ABSTRACT

Ecological studies on *Legionella spp.* are essential to better understand their sources in the natural environments, the mechanism of their entry into man-made water systems and the factors enabling their survival and growth in aquatic habitats. The *Legionella pneumophila* specie is the principal etiologic agent of legionnaires'disease, a form of lobar pneumonia. Ubiquitous in aquatic environments, the gram-negative *Legionella* organism is a facultative, intracellular parasite of protozoa. This bacteria exhibits peculiar and multiple strategies to adapt to stressful environment conditions which normally impair other germ survival. These strategies include the ability to enter in a viable but non-cultivable state, to multiply intracellularly within a variety of protozoa, such as amoebae, to survive as free organisms within biofilms and to be enhanced/ inhibited by the presence of other aquatic bacteria. The infection with *Legionella pneumophila* ranks among the most common causes of severe pneumonia in the community setting. There are no clinical features unique to Legionnaires' disease. The availability of a good diagnostic constitutes the basis for the early recognition and treatment of the individual patient as well as for effective measures for prevention against Legionnaires diseases. This review summarizes the available information regarding the ecology and points out important areas which require further study. Lastly, new perspectives in controlling *Legionella pneumophila* contamination can arise from investigations from our laboratory in measures to prevent the contamination of water intended for human consumption and prevent the infection by Legionnaires' diseases.

Keywords: *Biofilms, environmental exposure, Legionella pneumophila, legionnaire's disease, water microbiology, water supply.*

1. INTRODUCTION

Legionella pneumophila was identified first approximately a quarter century ago after an outbreak of a serious pneumonia occurred in individuals attending an annual convention of the Pennsylvania Legionnaires in the Belleview Stratford Hotel in Philadelphia, Pennsylvania, in June 1976. Of the approximately 3000 conventioneers attending the meeting, approximately 200 developed pneumonia within the first few days after the convention. Approximately 2 dozen succumbed to respiratory failure. This outbreak of pneumonia in the hotel initially was speculated to be caused by a toxic substance or some other environmental problem. After a concerted analysis was conducted by investigators from Centers for Disease Control and Prevention (CDC), the causative agent was identified as a Gram-negative bacillus that was subsequently termed *L. pneumophila*, its name reflecting both its victims and the newly described Legionnaires' disease [1-2-3-4]. In the relatively short period since *L. pneumophila* was first identified as a human pathogen, more than 50 species of *Legionella* have been recognized, and at least 24 of these have been associated with human disease. The great majority of Legionnaires' disease, approximately 90%, is caused by *L. pneumophila*, and despite the description of at least 15 serogroups, *L. pneumophila* serogroup 1 is responsible for over 84% of cases worldwide [5-6]. The bias toward *L. pneumophila* as the most prevalent *Legionella* species to infect humans does not reflect the environmental distribution of the genus. A large study comparing clinical and environmental *Legionella* isolates in France showed that *L. pneumophila* serogroup 1 accounted for 28% of environmental *Legionella* isolates compared to 95% of clinical isolates [7]**.** Similar studies provided strong evidence that *L. pneumophila* is more pathogenic to humans than other *Legionella* species. The public health impact of *L. pneumophila* is therefore reflected in the greater research focus on this organism.

L. pneumophila is an inhabitant of natural and man-made aquatic environments, surviving free, in biofilms, and as an intracellular parasite of protozoa [8]. However, colonization of potable hot and cold water systems in large buildings, such as hospitals and hotels, with *L. pneumophila* is well recognized and has been responsible for major outbreaks and isolated cases of nosocomial legionellosis [9-10].

L. pneumophila was found to have many characteristics distinguishing it from other intracellular bacteria in terms of interactions with cells of the immune response system. In the lung, the bacterium grows within the macrophages that line the alveoli. For example, infection of macrophage cultures with *Legionella* results in rapid multiplication of the bacteria. Usually, within 48 to 72 hours, a hundred- to a thousand-fold increase in the number of these bacteria are present in infected macrophages [11].

Intensive efforts in subtyping and virulence testing revealed differences in infectivity even for different strains of the same serotype. Results from epidemiological studies showed that infection control is only possible by interference with the transmission at many points of the infection route [12]. Therefore, an integrated view of *Legionella* ecology together with clinical and genetic aspects appears to be necessary to establish effective prevention measures.

2. TAXONOMY

Although some phenotypic characteristics (i.e., gram stain, cell membrane fatty acid and ubiquinone content, morphology, and growth on specific media) can be used to recognize *Legionella* bacteria at the genus level, more specific diagnostic techniques are required to differentiate individual species [13]. Currently, the best methods to classify *Legionella* species are DNA analysis and antigenic analysis of various proteins and peptides.

Some investigators have proposed placing the *Legionellae* in three separate genera: *Legionella*, *Fluoribacter*, and *Tatlockia* [14-15]. However, recent studies using 16S rRNA analysis confirm the family *Legionellaceae* as a single monophyletic subgroup within the gamma-2 subdivision of the *Proteobacteria* [16-17]. The number of recognized species and serogroups of the genus *Legionella* continues to increase. There are currently, the *Legionella* genus includes 52 species [18-19], and more than 70 different serogroups. However, more than 20 species have been proven to be causative agents of Legionnaires' disease on the basis of their isolation from clinical material [20-21]. The specie *L. pneumophila* comprises at least 16 different serogroups and accounts for approximately 90% of confirmed cases of legionellosis, and *L. pneumophila* serogroup 1 has been recognized as the most important agent in this regard, as that specific strain was initially implicated as the pathogen causative of Legionnaires' disease in 1977 [20].

3. MICROBIOLOGY

3.1 General Microbiology

Generally the *Legionellae* are gram-negative rods that range from 0.3 to 0.9 µm in width and 2 to over 20 µm in length [22]. Unlike most gram-negative bacteria, *Legionellae* cell walls contain high amounts of branched-chain cellular fatty acids and ubiquinones with side chains of 9-14 isoprene units that make cell staining difficult. *Legionellae* are urease-negative, catalase-positive, heterotrophic, aerobic, chemo-organotrophic, and transitionally motile. When motile, they have one or more straight or curved polar or lateral flagella. *Legionellae* utilize amino acids for energy and carbon, do not oxidize or ferment carbohydrates, and require L-cysteine-HCl and iron salts for growth amongst other nutrients [22].

3.2 Life Cycle

Life cycles tell us a great deal about the natural history of a living organism. They are a synthesis of years of evolution, adaptation, specialization, and survival in nature. From an infectious disease standpoint, life cycles may provide clues to understand the mechanisms of pathogenesis of a particular parasite. The origin of pathogens and the evolution of virulence lie within the dominion of microbial ecology, and the life cycle of *Legionella* surely is no exception.

Legionella has a two phase life cycle, and is infectious only when the bacterium is a short, thick rod that is flagellated, stress resistant, sodium sensitive, and does not have the ability to replicate [23]. During this phase *Legionella* search for a host to infect. Once the bacteria find a host, they enter it through coiling phagocytosis and reside within the phagosome [24]. *Legionella* inhibit phagosome-lysosome fusion, thereby avoiding bacterial lysis. Living freely within the host, the bacterium enters a replicative (exponential) phase, which is characterized by a long, filamentous rod structure that is non-flagellated, sodium resistant, stress sensitive, and has the ability to replicate [23]. *Legionella* multiply within the host and overpopulate the phagosome, completely overwhelming it in as little as 48 hours [24]. The bacteria then return to the infectious phase and the protist undergoes necrosis, bursting and releasing *Legionella* into the environment to find another host. Without a host, growth of *Legionella* has only been observed on laboratory media, which may better simulate the environment present inside the host organism relative to normal aquatic environments [25].

4. OCCURRENCE IN NATURAL AND MAN-MADE HABITATS

Because routine environmental monitoring for *Legionella* is not a common practice, the occurrence of these bacteria is often indicated by outbreaks or sporadic cases of legionellosis. Therefore, this section considers the worldwide occurrence or incidence of legionellosis and outbreaks of legionellosis as well as the occurrence of *Legionella*, and especially *L. pneumophila* bacteria in water, soil and air.

Environmental factors influencing *Legionella* survival also are discussed. The members of the genus *Legionella* are found worldwide in freshwater environments, such as stagnant lakes, rivers, springs, or mud streams [26-27], but their concentration in these natural habitats is usually low. Furthermore, *Legionella* has also been shown to survive in marine waters [28]. Ocean waters receiving treated sewage have been found to contain *Legionella* species, they are also present in all phases of sewage treatment, and their population numbers do not decline significantly during the treatment process [29]. The bacteria have also been isolated from damp or wet soil, but isolation from dry soil has not been reported. Since *Legionella* is ubiquitous in aquatic habitats, it appears impossible to prevent its entry into man-made water systems [23]. Thus, Legionella is largely present in hot water distribution systems, but it has also been isolated from decorative fountains, spa pools [23], whirlpools, dental devices [30], as well as in air conditioning and cooling towers, where *L. pneumophila* was the main isolate [31]. Indeed, Borella has detected *Legionella spp* in 22.6% out of 146 domestic hot water samples and 89% of them were *L. pneumophila* [32]. In a study of hot water systems in Morocco, *L. pneumophila* were isolated from 36.5 % of hotels, 40% of gyms and 12.5% of factories, which 45% of the results are concentrations more than 10^4 CFU/ml [33].

In order to link occurrence of Legionnaires' disease with a water source, the same serogroup and subtype of *Legionella* that is found in the patient's sputum must be found in the water [34].However, Legionnaires' disease is most strongly associated with human-made aquatic environments that contain water at elevated temperatures. In particular, many disease outbreaks are linked to air-conditioning cooling towers and evaporative condensers, which can produce contaminated water droplets that are inhaled by passers by. The increased presence of these large-scale, human-made aquatic reservoirs has likely led to the increased human exposure to *Legionella* and subsequently an increased incidence of *Legionella* infection in the latter half of the 20th century [35].

5. ENVIRONMENTAL FACTORS

5.1 Physical Factors

The genus of *Legionella* can survive in a wide range of temperatures and *L. pneumophila* is more frequent in warm water systems [36]. Water temperatures in the range of 20°C to 45°C favor growth. The optimum laboratory temperature for the growth of the bacterium is 37°C. Many studies of potable water supplies have found that temperatures below a certain level are positively associated with *Legionella spp and L. pneumophila* colonization, however this level varies between 50 and 60ºC [27-37]. Kusnetsov has found that growth of all strains tested decreased at temperatures above 44-45 ºC, with the growth-limiting temperature being between 48.4 ºC and 50 ºC [38]. The *Legionella* strains studied produced carbon dioxide up to 51.6 ºC, suggesting that some respiratory enzymes survive at this temperature [39]. Therefore, to prevent *Legionella* infection, the recommended temperature for storage and distribution of cold water is below 25 °C, and ideally below 20 °C. In addition, *L. pneumophila* specie is thermotolerant and able to withstand temperatures of 50 °C for several hours. The identification of this bacterium in hot-water tanks or in thermally polluted rivers emphasizes that water temperature is a crucial factor in the colonization of water distribution systems [40]. However the maintaining the temperature of hot and cold-water systems within buildings to prevent or minimize the growth of *Legionella* is an important control measure to prevent the risk of *Legionella* infection.

Nutrients supporting the survival of *Legionella* may come from the water, from dirt entering the system or from the construction materials [41]. In man-made systems, the presence of dead-end loops, stagnation in plumbing and periods of non-use has been shown to be technical risk factors [30]. Also the material of the piping system has been shown to influence the occurrence of high bacterial concentrations. It has been found that copper pipes limit *Legionella* colonization [42-43]. However, Zeybek and Çotuk were isolated *L. pneumophila* from 22.8% of water systems where iron and plastic were used together but no bacteria were isolated from those made of plastic only [44]. Rubber and silicone within the distribution system also can support *Legionella* growth, and perhaps *Legionella* can attach to its surface easier than other distribution system components [45]. Recently, it was found that among the buildings with *L. pneumophila* growth, 84% had plastic and 15% had steel piping [46]. Stagnation and interruptions in water service increase the prevalence of *Legionella*, possibly due to lower disinfectant levels [37-47]. Turbulent conditions can cause large clusters of *Legionella* to become detached and move into the bulk water [47].

5.2 Chemical Factors

Studies have attempted to correlate *Legionella* occurrence in potable water systems with presence in environmental samples. For example, copper in potable water is believed to exert an inhibiting action on *Legionella* [48-49-50]. In two surveys in Italy, copper levels above 50 ppb were correlated with lower *Legionella* colonization [27-32].

One survey noted that six times less *Legionella* were present in water samples containing greater than 50 ppb copper. The effect of iron on *Legionella* is less clearly defined. Some studies have found that iron is positively correlated with *Legionella*, while others have found that it is negatively correlated [32-49-50]. However, it is known that *Legionella* need iron to grow in culture media, and virulence is significantly reduced in iron-limited conditions [24-51]. States has found that very high levels of iron (>50 ppm) had a toxic effect [50]. Zinc concentrations both above 200 ppb and below 100 ppb have been correlated with lower *Legionella* levels, indicating that the optimal amount of zinc for *Legionella* growth may be between 100 and 200 ppb [27-32]. Manganese levels below 3μg/l are associated with lower *Legionella* levels, however above 10 μg/l there seems to be a negative correlation [32-52]. Although a study of Italian hotels demonstrated that magnesium has a negative correlation with *Legionella*, a study of Pittsburgh water supplies indicated that there was no correlation [27-53]. Conflicting results have been found regarding the association of calcium and *Legionella*, with two studies showing positive, one negative, and another no correlation [27-49-52-53]. While one study found there was a positive correlation between nitrate and *Legionella*, another found a negative correlation between nitrate + nitrite and *Legionella* [52-53]. Negative correlations have also been found with sodium, barium, chloride, and hardness, and positive correlations with potassium, phosphate, sulphate, and TOC [50-53-54].

Legionella are believed to survive in dissolved oxygen concentrations between 0.3 and 9.6 ppm, however in one study naturally occurring *L. pneumophila* grew between 6.0 to 6.7 mg/l and not 1.7 to 2.2 mg/l [55]. At lower temperatures (4 to 20ºC) *Legionella* can survive at up to 3% sodium chloride, while at higher temperatures (30 and 37ºC) sodium chloride concentrations above 1.5% resulted in a greater than 2 log reduction of *Legionella*. Levels of sodium chloride between 0.1 and 0.5% have been shown to enhance *Legionella* survival [56]. Another study demonstrated that while L. pneumophila was noncultivable in hot spring water with salt concentrations almost the same as seawater, the bacterium maintained metabolic activity [57]. Since amino acids serve as the carbon source for *Legionella*, several studies have sought to determine which amino acids are required for growth. George has found that *L. pneumophila Bloomington-2* and *Los Angeles-1* strains required arginine, cysteine, isoleucine, threonine, valine, methionine, serine, and phenylalanine or tyrosine for growth. Tesh and Miller had similar results, except instead of phyenylalanine or tyrosine they found that glutamic acid was required [58]. Tesh was also found in another study that the only amino acids used for energy were glutamate, serine, threonine, and tyrosine [59]. All of these amino acids are present in the BCYE growth medium, however they are present only at low concentrations, if at all, in potable water [60-61].

The effect of pH on *Legionella* survival was also investigated. Katz and Hammel demonstrated that *L. pneumophila* showed a 2-log decline after incubation for 1 month in tap water varying in pH from 4 to 7 but a 6-log decline at pH 8 [62]. In contrast, the study of Ohno et al, demonstrated that the best survival of *L. pneumophila* was evident at pH 8 which is the most frequent pH value in hot spring water [57]. In previous study conducted by Sheehan in Yellowstone National Park, showed that *Legionella* were able to survive in very acidic pH of about 2.7 but the bacteria were in fact protected within protozoa as Naegleria or Euglena, which could be a Fuel tank for potentially pathogenic *Legionella* [63].

5.3 Survival in Aerosols

Since *Legionella* is an aquatic pathogen, and humidity is also an important factor in determining the persistence and suspension of aerosols, humidity is an important consideration relative to human exposure. Hambleton et al, has found that *L. pneumophila* aerosols stayed in the air and survived better at 65% relative humidity relative to 30%, 55%, or 90% [64]. Fisman et al, showed that the key factor of the occurrence of sporadic infections with *Legionella* was the humidity, they indeed found very close association with rainfall and increased humidity 6 to 10 days before the event, the time of incubation of legionellosis [65]. The metabolic state of *L. pneumophila* affects its survival in air, since germs in the low metabolic activity phase have the greatest resistance [27]. Humidity also has a profound effect on aerosol stability. Survival of *L. pneumophila* improves with increased relative humidity in the range from 30 to 90% [27-66]. It was also observed that strains of *L. pneumophila* serogroup 1 monoclonal subtype Pontiac, the most commonly associated with disease, survived better than other subtypes, with 30% of the population remaining viable after 30 min at 20°C and 60% of relative humidity [66]. During an outbreak of legionellosis in France, the atmospheric dispersion of the microdroplets of water from a lagoon was studied and *L. pneumophila* was found over long distances. Deloge-Arbarkan et al, were found that the concentration of *Legionella* in the water was not correlated with their airborne abundance, which obviously provides a challenge relative to setting hazard guidelines because ultimately exposure depends on specifics of the local environmental and other factors such as shower nozzle design [67].

5.4 Potential Symbiotic and Competitive Interactions with other Bacteria

The microbial flora of aquatic environment is generally made up of several species of bacteria, fungi and protozoa. Within this complex community, a large number of interactions occur, in particular among different bacteria. Thus, *Legionella* survival may be positively or negatively influenced by other aquatic germs. Stout and his coworkers found that environmental bacteria and sediment both improved the survival of *L. pneumophila* in environmental samples [68]. Although they can compete for nutrients, non-*Legionella* bacteria have not been found to compete with *Legionella* for uptake by protist hosts [69]. Algae, Pseudomonas and flavobacteria are noted by OSHA for their ability to supply essential nutrients for *Legionella* growth, and *Pseudomonas aeruginosa* has been shown to support the replication of *L. pneumophila* in *Naegleria lovaniensis* and *Acanthamoeba castellanii* [34-69]. In contrast, it has also been hypothesized that *Pseudomonas* can compete with *Legionella* for growth. In two surveys in Italy, Leoni et al, found a negative correlation between *Legionella* and *P. aeruginosa* in swimming pools in Italy, and Borella et al. found *L. pneumophila* serogroup 1 to be associated with lower *Pseudomonas* prevalence in private homes in Italy [70-32].

6. LEGIONNAIRES' DISEASES

Legionnaires' Disease (LD) classically presents as two distinct clinical entities, pneumonia with severe multisystem disease, and Pontiac fever, a self-limited flu-like illness [3-21]. This infection results from inhaling airborne water droplets or mist containing viable *L. pneumophila*, which are small enough to pass deep into the lungs and be deposited in the alveoli, the small pockets in the lungs. The dose of *L. pneumophila* required to infect humans is not definitively known. Ingesting *L. pneumophila* has not been shown to cause illness.

Cases of LD may be sporadic or occur as part of an outbreak. Sporadic cases are reported throughout the year, but most cases of epidemic infection occur in the summer and autumn, presumably because warmer weather encourages proliferation of the bacteria in water [21]. Most reported cases have occurred in the 40 to 70 year old age group. Although healthy individuals may develop LD, people thought to be at increased risk of infection include smokers, patients with cancer, chronic respiratory diseases, kidney disease, and any immuno-suppressed condition. The fatality rate is estimated at 10 to 20% of those who contract the disease; but in immuno-suppressed persons or those with other underlying diseases, this figure can be much higher [71].

The incubation time of LD is between 2 and 10 days. It is not possible to clinically distinguish patients with LD from patients with pneumococcal pneumonia. Several prospective studies have shown that the two diseases have nearly identical clinical and radiological findings, and that non-specific laboratory test results cannot differentiate between the two diseases [25-72]. Non-specific features of LD include fever, non-productive cough, myalgias, rigors, dyspnea and diarrhea [21]. Neurological symptoms range from headache and lethargy to encephalopathy. Change in mental status is the most common neurologic abnormality [73].Suspicion should be raised in cases of pneumonia and the presence of headache, confusion, hyponatremia, elevated creatine kinase [74]. Also, the diagnosis becomes more likely if an acute consolidating pneumonia fails to respond to several days of b-lactam antimicrobial therapy, or if the pneumonia is severe enough to require intensive care unit hospitalization [25].

Epidemiologic clues might include use of a hot tub or recreational spa; recent pneumonia of a co-worker, relative, or fellow traveler; and recent plumbing work done at home or work. The non-specific presentation of LD makes clinical diagnosis very difficult and mandates empiric therapy for this disease in most patients with community-acquired pneumonia (CAP) of uncertain etiology. The key to diagnosis is performing appropriate microbiologic testing [25].

7. PREVENTION OF LEGIONNAIRES' DISEASE

7.1 Environmental Monitoring and Risk

The role of environmental monitoring in *L. pneumophila* prevention has been the source of debate for many years [75]. However several studies exist that provide evidence for the use of monitoring in the prevention of hospital-acquired LD. Two studies from Spain show that *Legionella* colonization was extensive in Barcelona hospitals, and that environmental monitoring followed by intensive clinical surveillance identified previously unrecognized cases of hospital-acquired LD [76]. The Allegheny County Health Department in Pennsylvania recommends periodic environmental monitoring of acute care facilities as part of their recommended prevention plan. The effect of this approach recently was evaluated and the results showed a significant decrease in the number of health care-associated cases of LD after the preventive guideline was in place [77]. Based on these and other results, the Center for Diseases Control recommendations now state that monitoring for *Legionella* in transplant units can be performed as part of a prevention strategy. The New York Department of Health's went further and mandates quarterly monitoring for *Legionella* in transplant units. Routine periodic environmental monitoring for *Legionella* in hospital water systems is now recommended in several countries namely France, Italy, Spain, Germany, and the Netherlands. There continues to be confusion regarding the interpretation of *Legionella* monitoring results. It has been shown that there is an increased risk of hospital transmission if a high proportion of water sites are positive for *Legionella* species (particularly *L. pneumophila*), and that the proportion is more predictive of risk than the concentration (CFU/ml). This has been validated by several studies [78-79-80]. Conversely, a relationship with a predetermined concentration of *Legionella* from a given site to the risk of illness has not been scientifically validated. Furthermore, complete elimination of *Legionella* from a hospital water supply has not been necessary to reduce or eliminate hospital acquired LD [81-82].

7.2 Detection and Disinfection

The conventional plate culture method described in the standard AFNOR NF T90-431 is a very important technique for the detection of *Legionella* and *L. pneumophila*, because it is the currently used standard method and it provides strains for epidemiological studies. However a number of factors, including other bacteria, can interfere with growth of *L. pneumophila*, even on selective media [23-83]. Also serology-based methods are not regarded to be the gold standard anymore since the progressive characterization of new species has shown that antigen cross-reactivity limits specificity [84]. Further routine methods rely on pulsed field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), arbitrarily primed and nested PCR [85]. Additionally, gas chromatographic mass spectrometry based on the unique 3-hydroxy and 2,3-dihydroxy fatty acids of the *Legionella* LPS has been described for complex microbial consortia [86]. After detection of unacceptable high levels of *Legionella* effective decontamination and maintenance of water are critical for prevention of outbreaks of legionellosis. In general, actions need to be taken when the concentration of *Legionella* exceeds 10^3 CFU/l. More restrictive standards apply for high-risk areas, including intensive care and transplantation units [87]. In the recent years a number of methods for controlling the growth of *Legionella* in drinking water supply systems (heat flushing, ultraviolet light irradiation, ozonation, metal ionization, chlorination) and cooling towers (biocides) have been described [88]. Unfortunately, the decreased heat transfer and biocide penetration into biofilms as well as unused pipes of the water system often interfere with disinfection attempts. Also the interaction of *Legionella* with amoebae hampers the disinfection in man-made water systems. This is complicated by the fact that protozoa may adapt to biocides [23-89-90].

8. CONCLUSION

L. pneumophila per se is not adapted to the human host, however novel man-made environmental niches and changes in human behavior have led to legionellosis as a new public health risk that can be associated with serious morbidity and mortality, especially when the infection is not rapidly diagnosed and treated. Therefore, *Legionella* can be viewed as an aquatic microbe that goes astray. The broad protozoal host spectrum in the environment and the exploitation of very basic cellular mechanisms of eukaryotes obviously allow *Legionella* to infect human cells. Consequently, it has been suggested that protozoa are the driving force in the evolution of the pathogenicity of *Legionella* [23-91]. Additional information is needed to institute optimal prevention and control measures and to minimize the infection associated with *Legionella*. However, research and studies into this area are not exhausted and hygienists, microbiologists, clinicians and all the other interested persons are called to contribute to the construction of an integrated network of knowledge on *Legionella* in order to substantially improve environmental control of the pathogen and prevent the appearance of new cases, with indisputable advantages for the collective health of the population.

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