

Research on *Legionella pneumophila* in hospital supply networks

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Abstract

The species of *Legionella* (gram-negative aerobic bacillus) are widely distributed in water environments. The *Legionellaceae* family comprises a group of fastidious bacteria that requires special isolation techniques, and of which the *Legionella pneumophila* is the member which is most commonly identified as the cause of human diseases or of the worsening of health conditions. The purpose of this work is to verify conditions in randomly chosen hospitals from the public system for the development of *Legionella pneumophila*, and, thus, contribute to the debate on legislation and technical standardization in the network, with the purpose of controlling and preventing the presence of this bacillus in the Brazilian hospital system. At the same time, some physicochemical and colimetric parameters (total and thermo-tolerant coliforms) were evaluated aiming at defining the total quality of water used. The results show the presence of coliforms and *Legionella sp* in the water used in the monitored hospitals. In this way, it is shown that the maintenance of the quality of water used in hospitals is fundamental, as well as the carrying out of biological tests for the detection of *L. pneumophila* so that the necessary safety of the hospital environment can be achieved.

Keyword

Legionella pneumophila, Legionellosis, pneumonia, water quality, public health, sanitation, hospital environments

Introduction

The species of *Legionella* (gram-negative aerobic bacilli) are commonly found in water environments, and they are essentially related to two diseases: Legionnaires' disease or legionellosis and Pontiac fever. The first one, which is responsible for the most significant clinical manifestation of the infection, evolves into an atypical pneumonia, with an incubation period between 2 and 10 days. It normally appears in the acute form and, in the most serious cases, it may lead to death (BUTLER et al., 1997: p.460; GUTIERREZ et al., 2006: p.170). Moreover, it presents a 28%

fatality rate, and as its source is the water distribution system, it may get into hospital water systems in undetectable amounts (STOUT et al., 2007: p.818).

The *Legionellaceae* family comprises a group of fastidious bacteria that requires special isolation techniques, from which the *Legionella pneumophila* is the member which is most commonly found to be the cause of human diseases or the worsening of health conditions. The *L. pneumophila* is a bacterium that occurs in natural ecosystems, and which may seriously afflict human beings. It is a bacterium that is present everywhere in

natural ecosystems such as lakes, wells, and rivers, and it is capable of opportunistically replicating itself in some artificial environments such as, for example, water supply systems, whenever it finds favorable conditions for its multiplication.

Legionnaires' disease is acquired by inhaling aerosolized water containing *Legionella* or, possibly, by pulmonary aspiration of contaminated water. The *L. pneumophila* capacity to cause the disease depends on its multiplication inside the pulmonary macrophages, causing pulmonary lesions responsible for the appearance of the symptoms, from two to ten days from the beginning of the infection. The bacteria produce cytotoxins, destroy the macrophages and are released in the extracellular medium, restarting the intracellular infectious cycle in another macrophage (SCHULZ et al., 2005: p.250; STOUT et al., 2007: p.822).

According to SCHULZ et al., 2005:252, nosocomial pneumonia, diagnosed according to the criteria from the Centers for Disease Control and Prevention (CDC), is responsible for nearly 15% of all hospital infections, being the second most frequent one. The authors also point out that there are nearly 23,000 cases of legionellosis in the United States every year. *L. pneumophila* is the second major cause of pneumonia, exceeded only by the *Streptococcus pneumoniae*, and it is responsible for several annual outbreaks of pneumonia in hospitals. It still presents a high mortality rate, about 40% in patients with hospital infection, and it may possibly reach 80% in patients with a compromised immune system. The fatality rate varies between 5 and 20% when it is acquired within the community.

Patients with serious chronic diseases or immunodeficiency face high risk of infection caused by *Legionella*. *Diabetes mellitus*, chronic pulmonary disease, non-hematological neoplasia, tabagism, and advanced age represent a moderately increased risk. The presence of underlying diseases and advanced age increase mortality by legionellosis (CDC, 2004: p.23).

In Brazil, pneumonias are the main cause of death among respiratory diseases, and, excluding external causes, they rank fourth place in general mortality. It is estimated that about 1,900,000 cases of pneumonia occur annually and, according to the limited literature in this area, *L. pneumophila* may be the cause of 6% of that morbidity (ROCHA, 1998; PEREIRA et al., 2002).

VERONESI et al. (1984: p.257) did a serologic investigation among blood donors and workers of Intensive Care Units from three hospitals in São Paulo found antibodies in 19% of the samples tested in a universe of 800 patients. LEVIN et al. (1991: p.245) have researched an epidemic outbreak in a renal transplant unit in São Paulo. They identified *L. pneumophila* as the cause and this signaled the necessity for the regular monitoring of this bacillus in hospital environments.

Bearing in mind the fact that the primary function of public health is the control and prevention of health problems, and considering that the presence of this bacterium in the hospital environment can increase the

problems of patients, this research highlights the importance of the operational implementation of preventive rules in this area. According to ROCHA (1998: p.153) its epidemiological behavior in Brazil is similar to that demonstrated in the rest of the world, therefore, if we explore the data from the literature related to the fatalities caused by this bacterium, more than 6,000 deaths per year in Brazil can be expected, as a result of pneumonias caused by *L. pneumophila*, casuistry comparable to that of tuberculosis and greater than that of meningitis.

This study intends to verify the conditions of some hospitals in the State of Rio de Janeiro, in regard to the presence of *L. pneumophila*, and thus contribute to raising awareness of the necessity for having technical standardization in the hospital network, with the purpose of controlling and preventing the occurrence of this bacillus in that environment.

Theoretical grounds: Risk factors of *Legionella* in the water distribution systems

Some natural environmental parameters condition bacterial colonization and multiplication, whereas other artificial parameters influence its increase and dissemination. In water supply systems, the main factors that favor the appearance of the optimal environmental conditions for the development of *Legionella* are:

a) water temperature between 20°C and 50°C (optimum growth between 35°C and 45°C); b) pH conditions between 5 and 8; c) zones of still water (reservoirs, plumbing of building systems, cooling tanks, little used endpoints of the networks, etc.); d) appearance of sediments in the water that bear microbiota, such as algae and protozoa; e) presence of l-cysteine, iron and zinc salts (due to the phenomenon of corrosion) and organic matter; f) presence of biofilms; and g) presence of porous materials and silicone byproducts in the building networks maximizing bacterial growth (BUTLER et al., 1997: p.461; LIN et al., 1998: p.115; BERRY et al., 2006: p.297).

The systems and equipment that offer the greatest risk are those that produce aerosols, through the formation of drops of contaminated water (with the size of 5µm), which can penetrate the respiratory system, reach the pulmonary alveoli and cause the infection (showers, sprinklers, etc.). It should be noted, however, that nearly 48 species of *Legionella* are known and that about 65 serogroups have already been identified, but only 20 of these have been associated with pathological stages in human beings. It is only these last ones that can cause severe problems (pneumonia and surgical incision contamination, for example) in people exposed to contaminated water (GUTIÉRREZ et al., 2006: p.168).

On the other hand, ecology and survival of *Legionellas* in the environment, mainly in water reservoirs, is closely favored by protozoa (*Hartmanella vermiformis*, *Tetrahymena pyriformes*) and amoebas (*Acanthamoeba castellanii*, *Naegleria* spp.) that may provide support for the multiplication of *L. pneumophila*. The infectious mechanism takes place through the invasion of *L. pneumophila* in these hosts, taking possession of their intracellular

macromolecules to carry out an intracellular multiplication. Next comes the intracellular replication stage until the point at which they damage the host cell and then attack new hosts (CIRILLO et al., 1994: p.3257; PHILIPPE et al., 2006: p.198).

Methodology

Type of study: cross- sectional

Collection of samples

The verification of the presence of *L. pneumophila* was carried out in hospitals that allowed the elaboration of the research. Five hospitals in the State of Rio de Janeiro participated in the study, carried out between March and May, 2006. Samples were collected from the water reservoirs of those hospitals, and all of them presented positive results for the presence of *L. pneumophila*. In the course of the study only the two hospitals that presented the worst conditions were researched.

Weekly collection of water was carried out in both chosen hospitals for 10 consecutive weeks¹. The sampling was carried out from a water tap at the first-aid post of those establishments (VICKERS et al., 1987: p.360; STOUT et al., 2007: p.821). 200 analyses were made, with 140 physicochemical analyses, 40 colimetric analyses, and 20 analyses for *Legionella*.

Collection of Samples for the Physicochemical Tests

The 500ml samples were collected in sterilized bottles, and submitted to laboratory tests for: total dissolved solids, iron, residual chlorine, sulphate, chlorides, hardness, and pH. The methodology used was that of the American Public Health Association (APHA, 2001).

Collection of Samples for the Microbiological Tests: Analysis of Total and Thermotolerant Coliforms

For the analyses of total and thermotolerant coliforms sterile bottles with 50 μ l of a sodium thiosulphate solution ($\text{Na}_2\text{S}_2\text{O}_3$) at 1% were used in order to neutralize any residual chlorine. 100 ml was collected for the analyses using the membrane filtration method. After up to six hours all samples were sent to laboratory tests, conditioned in an ice bath and protected from light.

Analysis of the samples

Coliform isolation - Membrane Filtration

The content of the sample was vacuum-filtered with a 0.45 μ m (Millipore HAWG 04700) membrane.

The membrane was placed in a 47 mm (Millipore PD 100 4700) Petri dish over an absorbent pad (Millipore HAWP 04700) soaked with the liquid medium. In the total coliform analyses the absorbent pad was soaked with 20 ml of the Endo Broth cultivation medium (BBL Microbiology Systems, USA). The material was incubated at 37°C for 22 to 24 hours. In the thermotolerant coliform analyses the absorbent pad was soaked with 20 ml of the FC broth cultivation medium (BBL Microbiology Systems, USA). The material was incubated at 44.5°C for 22 to 24 hours.

L. pneumophila analyses

For the analyses of *L. pneumophila* we used sterile bottles with 750 μ l of a sodium thiosulphate solution ($\text{Na}_2\text{S}_2\text{O}_3$) at 1% in order to neutralize any residual chlorine. 1500 ml of *L. pneumophila* were collected in each hospital and, for the isolation and concentration of *L. pneumophila*, we used the membrane filtration method. After up to six hours all samples were sent to laboratory tests, conditioned in an ice bath and protected from light.

Legionella isolation - Membrane filtration

Legionellas are not capable of growing in cultivation media such as agar-blood or any other median normally used in clinical laboratories. The following procedures were used for the isolation of *L. pneumophila*: a) post-filtration, the 0.45 μ m (Millipore HAWG 04700) membrane was aseptically placed in a 30 ml sterile Erlenmeyer flask with a screw cap containing 5 ml of sterile distilled water with 8 glass pearls. The solution was vigorously homogenized; b) it then received the acid treatment (elimination of the competitive microflora). 1 ml of the previous solution was taken out, and 9 ml of HCl-KCl buffer was placed, it was homogenized and the solution was left to rest for 3 minutes; c) after the period of reaction, 0.1 ml was inoculated in a Petri dish with BCYE Selective Agar GVPC (Oxoid). It was incubated at 35°C in an incubator with humidified atmosphere, for 24 to 72 hours; d) the colonies were then biochemically specified (*Legionella* Latex Test - *Legionella* species test reagent DR0803M, *Legionella pneumophila* serogroup 1 DR0801M, *Legionella pneumophila* serogroup 2-14 DR0802M, OXOID).

Results

The results of the physicochemical and colimetric analyses of the water from hospitals A and B can be seen in Tables 1, 2 and 3.

Table 1 - Physicochemical analyses of the water from Hospital A

Samples										
Parameters	1	2	3	4	5	6	7	8	9	10
Total dissolved solids (mg/L)	865	943	1105	1145	997	980	880	910	940	935
Iron (mg/L)	0.22	0.14	0.21	0.23	0.26	0.26	0.27	0.24	0.20	0.13
Residual Chlorine (mg/L)	0.8	1.2	0.3	0.5	1.3	1.5	1.4	1.9	1.1	1.6
Sulphate (mg/L)	188	170	125	222	234	190	201	216	178	165
Chloride (mg/L)	134	122	156	141	198	151	157	149	155	173
Hardness (mg/L)	334	412	417	219	253	214	278	226	197	184
pH	6.7	6.1	5.3	5.5	6.4	5.6	6.3	6.0	5.7	6.1

Table 2 - Physicochemical analyses of the water from Hospital B

Samples										
Parameters	1	2	3	4	5	6	7	8	9	10
Total dissolved solids (mg/L)	680	550	605	588	493	555	603	662	545	679
Iron (mg/L)	0.22	0.31	0.17	0.12	0.16	0.2	0.17	0.28	0.23	0.24
Residual Chlorine (mg/L)	0.5	0.8	0.4	0.2	1.1	0.6	0.0	0.9	1.1	0.4
Sulphate (mg/L)	188	133	145	178	222	210	98	94	123	165
Chloride (mg/L)	110	93	99	82	78	110	102	134	156	169
Hardness (mg/L)	293	228	415	419	398	325	311	229	199	426
pH	6.0	6.2	5.1	6.7	6.3	5.9	5.2	5.6	6.0	5.3

Table 3 - Colimetric analyses for Hospital A and Hospital B

Samples	Hospital A		Hospital B	
	Total coliforms/100 ml	Thermotolerant coliforms/100 ml	Total coliforms/100 ml	Thermotolerant coliforms/100 ml
1	6.7 x 10 ²	1.2 x 10 ²	0	0
2	0	0	1.6 x 10 ²	0
3	0	0	4.2 x 10 ³	2 x 10 ²
4	0	0	2 x 10 ³	0.5 x 10 ²
5	0	0	7 x 10 ³	3 x 10 ²
6	0	0	1.3 x 10 ⁴	7 x 10 ²
7	0.7 x 10 ²	0	0	0
8	0	0	3.2 x 10 ²	0.3 x 10 ²
9	2 x 10 ⁴	0.9 x 10 ²	5 x 10 ⁴	4 x 10 ²
10	0	0	1.8 x 10 ²	0

The results of the physicochemical parameters were compared to the maximum values allowed by Ordinance number 518 (2004), which determines the procedures and responsibilities related to the control and monitoring of the quality of water for human consumption and its potability standards. It should be noted that all samples are in compliance with the ordinance: the total dissolved solids have concentrations below 1000 mg/L, iron concentrations are inferior to 0.3 mg/L, residual chlorine concentrations are inferior to 2.0 mg/L, sulphates are inferior to 250 mg/L, chlorides are inferior to 250 mg/L, hardness was kept below 500 mg/L, and the pH was at a range of between 6.0 and 9.5.

According to Ordinance number 518 (2004), potable water for human consumption and water available at a treatment location must present the following microbiological standard: thermotolerant and total coliforms must be absent in the 100 ml samples. Observing the colimetric analyses from Hospitals A and B, the presence of those bacteria in some samples

is seen to be not in compliance with the provisions of the Ordinance.

By making detailed analyses it was also sought to identify other species of *Legionellas* in an attempt to classify the level of contamination of this species in that hospital circle. Thus other species of *Legionella* were identified. *Legionella micdadei* was isolated in hospital A, and, in hospital B, the following were isolated: *Legionella bozemanii*, *Legionella anisa*, *Legionella micdadei*, and *Legionella pneumophila* serogroups 3 and 4.

Specifically regarding serogroup 1 of *L. pneumophila*, which is the most commonly found serogroup in cases of pneumonia in hospitalized patients (GUTIERREZ et al., 2006: p.170) and, therefore, of greater interest in this study, analyses of this type were made in the hospitals studied. The *L. pneumophila* serogroup 1 was isolated in 60% of the samples collected in hospital A and in 100% of the samples collected in hospital B. Table 4 shows the results of the analyses of the research of isolation of *L. pneumophila* serogroup 1, in hospitals A and B.

Table 4 - Analyses of Legionella pneumophila serogroup 1 in hospitals A and B

Samples	Hospital A	Hospital B
1	0	2.3×10^5
2	0	4.0×10^2
3	1.2×10^3	5.1×10^4
4	0.4×10^2	3.2×10^5
5	0	3.9×10^3
6	1.1×10^3	5.3×10^4
7	2.5×10^4	4.2×10^3
8	0	1.8×10^4
9	0.3×10^2	2.4×10^5
10	2.2×10^4	6.6×10^6

Discussion

The data acquired through the cultivation in water, considering the different results among the samples from the hospitals and the variations in the concentrations of *Legionella* found in the points of collection, reflect different sources of influence for the growth of *L. pneumophila*. In addition, the risk for development after the exposure to a specific source is influenced by other factors, and not only by the presence or concentration of organisms. These factors include the level of contamination of the water (coliforms, parasites), the variations in the physicochemical concentrations, the susceptibility of the host, and the virulent properties of the contaminant strain. This data is sufficient to assign a risk level for

the disease, based on the amount of *Legionellas* detected in the samples of hospitals A and B.

Making a relation between the physicochemical and colimetric parameters found in the water samples from Hospitals A and B and the presence of *L. pneumophila*, the presence of the coliforms (total and thermotolerant) observed can be used as an indicator of contamination, taking into consideration that in hospital A, which presented a smaller presence of these bacteria, there was also a smaller ratio of *L. pneumophila* in the samples. In hospital B, where 100% of the samples demonstrated the presence of *L. pneumophila*, the level of contamination due to coliforms was bigger. Taking that into account, in this study the number of samples was reduced and, therefore, the

relation between the parameters can be considered weak, even though it can be pointed out that the physicochemical parameters, in contrast to the coliforms, may not be used as contamination indicators, bearing in mind that there was no significant alteration in the values.

Personal risk of acquiring legionellosis due to exposure to contaminated water depends on several factors, including the type and intensity of the exposure and the health of the exposed person. People with severe immunodeficiency or diseases with chronic characteristics have a marked increase in the risk of acquiring the disease. The mortality rate is 40% in hospitalized patients that have acquired *Legionella* against 20% for those who acquired the disease, but which were healthy (LIN et al., 1998: p.119). This data demonstrates the permanent importance of water as a reservoir for nosocomial pathogens. The minimization of the risks (involving adequate maintenance procedures, the definition of the regularity of the interventions, the monitoring of parameters, the methodology used for sampling, the study of the most appropriate products for disinfection countermeasures, etc.), must be the main object of legislation and the technical standards to be adopted in our country. That dynamic of bacteriological development conforms to good or excellent conditions in the researched hospitals, bearing in mind the lack of any specific legislation for the monitoring of *Legionella*.

Conclusions

The quality of the water in the researched hospitals showed the presence of contamination caused by coliforms and *Legionella sp.* The absence of coliforms and the presence of physicochemical agents (at the indicated levels) are of crucial importance not only for the potability of the water, but also in order to prevent the conditions for the occurrence of pathogenic contaminants, in particular, those propagated by water.

The prevention measures are known and have already been tested in countries that know and consider the importance of *Legionella sp.* as a human pathogen (ALLEGHENY County Health Department, 2007: p.1-15). In Brazil, however, little or almost nothing is known about the importance of *Legionella sp.* Specific control for this pathogen is not done much, and when it is done, it is anecdotal and not the result of a specific health policy, whether it is carried out in hospitals or water supplies, or in places served by air conditioning systems.

This work showed the presence of *Legionella sp.* in the water used in hospitals. Thus, the harmonization of the methodologies and the elaboration of standards for guidelines and control constitute the regulatory foundation for the construction of an epidemiological control and information center, with the purposes of monitoring and developing diagnostic, maintenance and treatment methodologies. The maintenance of the quality of water used in hospitals is fundamental, as well as the execution of biological control tests for the detection of *L. pneumophila* so that the necessary safety of the hospital environment can be achieved.

Notes

1. Research approved by the Ethics Committee of National School of Public Health Sérgio Arouca (ENSP)/Fiocruz.

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