

# Prevalence of *Legionella pneumophila* in water distribution systems in hospitals and public buildings of the Lublin region of eastern Poland

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## Abstract

**Objective.** The aim of this study was to determine the prevalence of *L. pneumophila* in water supply systems, hospitals and public buildings in the Lublin region of eastern Poland.

**Material and methods.** The study was carried out in 26 different objects in the Lublin region. The number of *Legionella* bacteria in water samples was determined by the membrane filtration method and/or by surface inoculation in accordance with the standards.

**Results.** The study showed the presence of *L. pneumophila* in 166 hot water samples (74.77%). In 34.33% (n=57) of water samples the count of tested bacteria exceeded the acceptable level of >100 CFU/100 ml. Of the samples where an acceptable level of bacteria was exceeded, 49 samples had an average level of *L. pneumophila* (100–1,000 CFU/100 ml), and the level in 8 samples was high (>1,000 CFU/100 ml).

**Conclusions.** The water samples collected from the hot water supply system of hospitals and public buildings showed exceeded counts of *L. pneumophila*, indicating the risk of infection. The constant monitoring of water distribution systems is an important element of the control of infections caused by these organisms.

## Key words

*Legionella pneumophila*, legionnaires' disease, hot water distribution systems, colonization, water temperature

## INTRODUCTION

Bacteria of the genus *Legionella* are etiologic agents of the severe atypical pneumoniae (Legionnaires' disease) and flu-like symptoms (Pontiac fever). The pulmonary form accounts for 1–5% of all cases caused by *Legionella* [1]. The mortality in patients with this form is high and ranges from 15–20%, and in hospitalized patients with compromised immune systems more than 30% [2, 3]. A mild form of infection – Pontiac fever, occurs in more than 90% of the exposed population [3]. In this form of Legionnaires' disease the signs of pneumonia are not observed, which is associated with the lack of proliferation of these bacteria in pulmonary macrophages [4]. *Legionella pneumophila* is responsible for most of the identified cases of legionellosis (80–90%), including 50–75% of cases caused by *L. pneumophila* SG 1. Other species isolated from clinical material are very rare [2].

Water distribution systems are the major reservoirs of the *Legionella* spp. which commonly colonize the water systems in large public buildings, households and industrial systems. The most dangerous is the occurrence of *Legionella* in the water distribution systems in hospitals, medical equipment (e.g. respirators, dialyzers, inhalers, humidifiers, water massage equipment used in balneotherapy) and dental turbines used in dental offices. This poses a risk for human infection with these bacteria, in particular, patients in health centres [2, 3]. The factors influencing the survival

of *Legionella* in the artificial aquatic environments are water temperature, symbiotic microorganisms, and include sediment accumulation and metals content [5].

*Legionella* spp. are generally transmitted through inhalation of contaminated aerosols of water. Microaspiration of contaminated water may be an important mode of transmission, especially in intubated patients. No human-to-human transmission has been recorded.

The probability of infection caused by the *Legionella* depends on the rate of multiplication of bacteria in water installations (degree of contamination of the aquatic reservoir), formation of water-air aerosol and the dose of microorganism (duration and intensity of exposure) [4, 6]. An important aspect contributing to the development of the infection and its clinical form is the interaction between the pathogen and the host. The main role is played by the virulence and number of microorganisms penetrating into the lungs, as well as the immune status of a person exposed to contact with the pathogen [5, 6]. The general population is fairly resistant to infection, although there are reports in the literature of infections among healthy individuals without risk factors. Particularly at the risk of infection are the elderly over 60 years of age, smokers, immunocompromised patients with chronic respiratory diseases, diabetes, chronic severe renal failure, cancer (e.g. lung cancer, haematologic malignancies), patients with rheumatoid arthritis, Crohn's disease, in patients post organ transplantation and patients treated with corticosteroids or anti-TNF  $\alpha$  agents [7]. Other groups at risk of infection are hospital and dental workers, industrial plants workers, car washes workers and frequent travellers (staying in hotels, air-conditioning) [8, 9, 10, 11].

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The site of *Legionella* infection, the *European Legionnaires' Disease Surveillance Network* (ELDSNet), has divided infections into hospital acquired, community-acquired and acquired during a trip [1]. According to data collected by ELDSNet, 4,897 cases were reported in 2011 of which 67% were acquired in the places of residence, 24% were acquired during a trip, and 7% of cases acquired in a hospital or sanatorium. 2% of cases were not classified in any of the groups due to the lack of data on the source of infection. 77% of all cases reported occurred in people over 50 years of age [12].

According to the official data published in the bulletin *Infectious Diseases and Poisoning in Poland*, compiled by the Department of Epidemiology at the National Institute of Public Health/National Institute of Hygiene in Warsaw from 1 January 2003–30 June 2013, there were 244 cases reported in Poland [13]. It should be emphasized that in the opinion of experts the number of legionellosis cases is understated because the majority of etiological agents in the cases of pneumonia are unrecognized.

Registered new cases of legionellosis and epidemic outbreaks tend to monitor potential reservoirs of infection. Many countries have guidelines in place to prevent the growth of the bacteria and reduce the risk of outbreaks and transmission of *Legionella* spp. In Poland, from 1 January 2008 there is an obligation to monitor water systems in large public buildings for the presence of *Legionella* spp. (in accordance with the Act of 29 March 2007 'On the quality of water intended for human consumption'). In the case of detection of an increase above normal of *Legionella* spp. and increase in their number (>100 CFU/100 ml of water), is necessary to eradicate the bacteria from the water. In wards in healthcare facilities where there are immunocompromised patients, including those under immunosuppressive therapy, *Legionella* spp. should be absent in a sample water of 1,000 ml volume. Table 1 presents the minimal frequency of hot water sampling and procedures regarding the results of bacteriological tests in accordance with Polish law.

## OBJECTIVES

Due to the risk of infection by *Legionella* according to the guidelines of the *World Health Organization* (WHO) and the recommendations of the *European Legionnaires' Disease Surveillance Network*, all artificial water reservoirs which could be potentially colonized by these microorganisms should be regularly monitored. Numerous studies worldwide have been carried out to evaluate the prevalence of *Legionella* in water systems in various objects.

In Poland, despite the introduction from 1 January 2008 of the obligation to microbiologically monitor water (in accordance with the Act of 29 March 2007), there are no comprehensive data on the contamination by *Legionella* of water supply systems in hospitals and public buildings. Therefore, the aim of this study was to evaluate the frequency of *L. pneumophila* colonization in the water supply systems in hospitals and public buildings in the Lublin region of eastern Poland.

## MATERIALS AND METHOD

The materials for this study were hot and cold water samples (1,000 ml) collected from the water supply systems of 26 different objects. The water samples were collected from hospitals (12), hotels (5), barracks of a military unit (4), houses for single mothers (2), shopping centres (2) and an industrial plant (1). The study was performed in the Laboratory of the Department of Medical Microbiology at the Medical University in Lublin during the period December 2007 – March 2010. The water samples were collected, transported and stored according to Polish standards: PN-EN ISO 19458:2007, PN-ISO 11731:2002 and PN EN ISO 11731-2:2008 [15, 16, 17].

The water sampling points were: hot water tanks or nearest sites, distal sites from the hot water reservoir, water returning to a boiler (recirculation), and at selected intermediate points, the number of which depended on the size of the system [14]. When there was more than one water circuit at an object, the samples were taken from each circulation (according to

**Table 1.** Minimal frequency of hot water sampling and procedures regarding the results of bacteriological tests<sup>1</sup> (Annex No. 7 to the Regulation of the Polish Minister of Health, The Journal of Laws of 6 April 2007, no. 61, pos. 417) [14]

NUMBER of <i>Legionella</i> spp. in 100 ml of water	ASSESSMENT OF CONTAMINATION	PROCEDURE	TEST
<10 <sup>2</sup>	lack / minimal	system under control – requires no special action	after 1 year and after 3 years <sup>2</sup>
10 <sup>2</sup> -10 <sup>3</sup>	medium	if the majority of the samples is positive, the water system is considered as colonized by bacteria of the genus <i>Legionella</i> , find the cause (review the technical system, check the temperature of the water) and take actions to reduce the number of bacteria further actions (cleaning and disinfection) depend on the result of the next test	after 4 weeks if the test result will not be changed, perform cleaning and disinfection, repeat the test after 1 week, then after 1 year
10 <sup>3</sup> -10 <sup>4</sup>	high	proceed to intervene: find the cause (technically review the system, check the temperature of the water), clean and disinfect the system water is not suitable for showers	after 1 week of cleaning and disinfecting then every 3 months <sup>3</sup>
>10 <sup>4</sup>	very high	equipment and hot water systems immediately excluded from the operation; carry out procedures for cleaning and disinfection	after 1 week of cleaning and disinfecting then every 3 months <sup>3</sup>

### Explanation:

<sup>1</sup> if it is a result of 1-2 water samples, in order to prevent the point contamination more water samples should be collected and examined

<sup>2</sup> if in further studies there were <100 CFU/100 ml annually

<sup>3</sup> if in the following two tests performed at intervals of three months there were <100 CFU/100 ml, then the next test may be done in a year

### Note:

The disinfection procedure (thermal or chemical disinfection) should also be done when:

1. if the water supply is turned off for more than one month
2. if the installation or part of the installation has been replaced or if the maintenance procedures could lead to the contamination
3. in the plumbing system in the place where are people with suspected or confirmed legionellosis

**Table 2.** Numbers of hot and cold water samples collected from the water supply system in tested objects

OBJECTS	TOTAL NUMBER OF WATER SAMPLES	NUMBER OF HOT WATER SYSTEM	NUMBER OF COLD WATER SYSTEM
HOSPITALS	184	168	16
SINGLE MOTHER HOUSES	8	6	2
HOTELS	20	15	5
INDUSTRIAL PLANT	18	18	-
SHOPPING CENTERS	4	4	-
BARRACKS	12	11	1
Total:	246	222	24

the scheme given above). In total, 246 water samples were collected (222 samples from hot water systems, 24 samples from cold water systems) (Tab. 2). The mean temperature for the samples collected from all objects were: for the hot water 45.11 °C (range 24.5 °C – 70 °C) and for cold water 11.85 °C (range 8 °C – 15 °C).

Enumeration of *Legionella* bacteria in the water samples was determined by membrane filtration (FM) and / or by surface inoculation methods according to the standards: PN-ISO 11731: 2002: 'The quality of the water. Detection and enumeration of *Legionella*' and PN-EN ISO 11731-2: 2008: 'Water quality – Detection and enumeration of *Legionella* – Part 2: Methodology of membrane filtration for water with a small number of bacteria' [16, 17].

The membrane filtration method was used for all tested water samples. For samples with an expectedly large number of bacteria of the genus *Legionella* (>10<sup>5</sup> CFU/1,000 ml), the surface inoculation method was also used. The decision for surface inoculation was taken on the basis of the technical condition of the water supply system – 'dead legs' in the water system, installation components covered by calcareous sediment, and the sludge boiler.

**Membrane filtration method.** The method involved sample concentration by filtration of 10 ml, 100 ml and 500 ml water through 0.45 µm cellulose membrane filters. After concentration, the samples concentrates were treated for 10 min. with acid (pH 2.2), to reduce the number of non-legionella bacteria before culture. The buffer was then removed from the filter by washing it with Ringer's solution. The filter was then placed on a selective GVPC agar plate (*Glycine*, *Vancomycin hydrochloride*, *Polymyxin B sulphate*, *Cycloheximide*), (OXOID Basingstoke, Hampshire, UK).

**Surface inoculation method.** From each water samples, aliquots of 0.2 ml were inoculated without concentration and without acid buffer treatment directly onto GVPC agar. The inoculum was spread with a sterile glass rod. The inoculated culture media with filter membranes and inoculated surfaces were incubated at 37 °C in a humid atmosphere and read at 7–10 days. Observation of colony growth was performed every day. All colonies that grew in the first 2 days of incubation were regarded as associated microflora.

In the next step, confirmatory tests on the BCYE-α medium (*Buffered Charcoal Yeast Extract*) with cysteine (bioMérieux) and BCYE-Cys medium without cysteine (bioMérieux) were performed. The agar media were incubated at 37 °C

for 72 hours. The colonies that grew on BCYE medium with cysteine (BCYE-α) while not grown on the BCYE medium without cysteine (BCYE-Cys) were deemed to be *Legionella*. Identification was made using the latex agglutination test for *Legionella* Latex Test (Oxoid Basingstoke, Hampshire, UK). The test allows the identification of *L. pneumophila* serogroup 1 (SG 1), 2–14 serogroup (SG 2–14) and 7 other species of the genus *Legionella*: *Legionella longbeache* serogroup 1 (SG 1) i serogroup 2 (SG 2), *Legionella bozemanii* serogroup 1 (SG 1) i serogroup 2 (SG 2), *Legionella dumoffii*, *Legionella gormanii*, *Legionella jordanis*, *Legionella micdadei* and *Legionella anisa*.

The results were presented as the number of colony forming units (CFU) of *Legionella* per specific volume of water, or statement of their absence as 'not found' in the test sample volume. Interpretation of the results was made in accordance with Annex No. 1D and No. 7 to the Regulation of the Minister of Health of 29 March 2007 'On the quality of water intended for human consumption'.

**Statistical analysis.** Data were described by mean ± standard deviation and median, and for quantitative variables and by percentages for qualitative variables. The W Shapiro-Wilk test was used to assess normality of distribution of the analyzed quantitative parameters. For comparison, the number of *L. pneumophila* in the examined objects the Kruskal-Wallis test was used. The Spearman's rank correlation coefficient was used to check correlations between water temperature and number of *Legionella*. The percentage of isolates *L. pneumophila* per season: winter (January, February, March), spring (April, May, June), summer (July, August, September), and autumn (October, November, December) were determined. Positivity rates were then compared using the Chi-squared test. Results were considered statistically significant at  $p \leq 0.05$ . The STATISTICA version 9.0 (StatSoft, Warsaw, Poland) was used for statistical analysis.

## RESULTS

*L. pneumophila* was present in 166 samples of hot water, which accounted for 74.77%. In cold water samples, *L. pneumophila* bacteria were not detected. In all tested water samples *L. pneumophila* SG 2–14 strains were detected. The most virulent strain – *L. pneumophila* SG 1 was not detected. Table 3 shows of the results characterizing the contamination of water systems supply by *L. pneumophila* in the examined objects.

For each type of building, the total percentage of samples testing positive were, in hospitals – 78.57% (132/168), in hotels 86.66% (13/15), in the homes of single mothers – 33.33% (2/6), in the industrial plant – 22.22% (4/18), in shopping centres – 100.00% (4/4), and in barracks – 100.00% (11/11).

The mean number of *L. pneumophila* in all positive samples was 294.85 ± 451.87 CFU/100 ml (range 0.02 CFU/100 ml – 3,300 CFU/100 ml). The highest number of *L. pneumophila* were detected in the examined hospitals (mean number 355.10 ± 624.52, median 127.00 CFU/100 ml) and hotels (mean number 279.43 ± 282.48, median 146.00 CFU/100 ml). In other examined objects (i.e. barracks of a military unit, houses for single mothers, shopping centres and the industrial plant) the number of *L. pneumophila* was much lower (mean number 37.51 ± 46.88, median 38.00 CFU/100 ml). The

**Table 3.** Summary of the results characterizing the contamination of water systems supply by *L. pneumophila* in the examined objects

	Number of <i>Legionella pneumophila</i> in 100 ml of water (CFU/100 ml)													
	Positive water samples		Lack/minimal <100				Medium 100-1000				High >1000			
	N	n	Number of objects	Number of samples	%	Range	Number of objects	Number of samples	%	Range	Number of objects	Number of samples	%	Range
71.79%														
HOSPITALS	12	132 (78.57%)	4	87	65.90	0.49-96	7	37	28.03	118-918	1	8	6.07	1035-3300
HOTELS	5	13 (86.66%)	2	3	23.07	28-90	3	10	76.93	123-745	-	-	-	-
SINGLE MOTHER HOUSES	2	2 (33.33%)	2	2	100	0.9-18	-	-	-	-	-	-	-	-
INDUSTRIAL PLANT	1	4 (22.22%)	1	3	75	36-52	-	1	25	138	-	-	-	-
SHOPPING CENTERS	2	4 (100%)	2	4	100	55-80	-	-	-	-	-	-	-	-
BARRACKS	4	11 (100%)	3	10	90.09	0.02-54.9	1	1	9.91	178	-	-	-	-

N – total number of examined objects

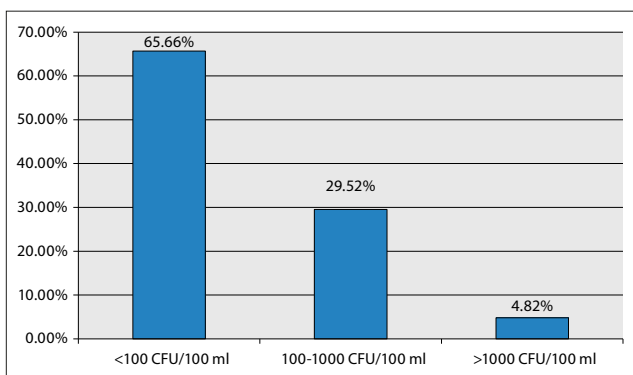
n – number of positive water samples

number of *L. pneumophila* was significantly higher in the examined hospitals in comparison with hotels and other buildings ( $p < 0.0001$ ).

In the accordance with Annex 7 to the Regulation of the Polish Minister of Health of 29 March 2007 'On the quality of water intended for human consumption' (The Journal of Laws of 6 April 2007, No. 61, pos. 417) the number of bacteria of the genus *Legionella* should not exceed 100 CFU in 100 ml of water samples ( $< 10^2$  CFU/100 ml), (ANNEX 1 D). In accordance with the above regulation there are 3 levels of water contamination with bacteria of the genus *Legionella*:

- lack/minimal  $< 100$  CFU/100 ml
- medium 100–1000 CFU/100 ml
- high  $> 1000$  CFU/100 ml
- very high  $> 10\,000$  CFU/100 ml [14].

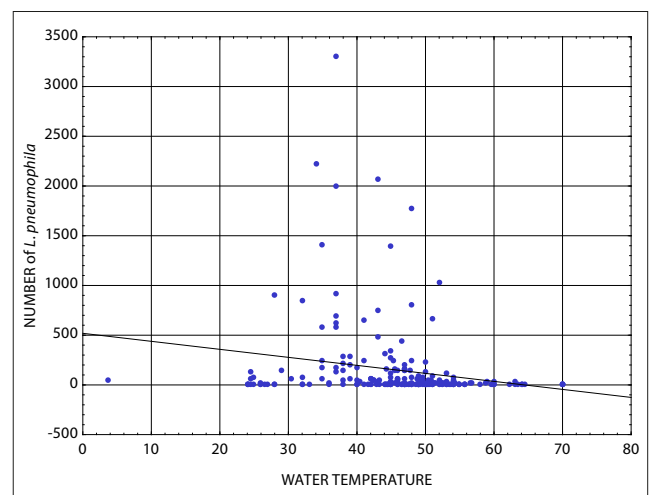
The study showed that in 34.34% ( $n=57$ ) of the water samples tested, the *L. pneumophila* bacteria count exceeded the acceptable level of  $> 100$  CFU/100 ml. In 65.66% ( $n=109$ ) of water samples the levels of *L. pneumophila* were low ( $< 100$  CFU/100 ml). Among the samples that exceeded an acceptable level, 49 samples had an average level of *L. pneumophila* (100–1000 CFU/100 ml), while in 8 samples the levels were high ( $> 1,000$  CFU/100 ml), (Fig. 1).

**Figure 1.** The percentage of water samples tested including the degree of contamination by *L. pneumophila*

In the study it was found that medium level (100–1000 CFU/100 ml) of bacteria occurred more frequently in samples collected from hotels (76.93%) than in samples from the hospitals (28.03%) and other examined objects. High levels of *L. pneumophila* ( $> 1,000$  CFU/100 ml) were

found in examined the hospitals (6.07%), where the highest number of *L. pneumophila* were detected in the Internal Medicine Unit with Intensive Care Unit (3,300 CFU/100 ml), Intensive Care Unit (1,777 CFU/100 ml), and the Cancer Chemotherapy Unit (1,035 CFU/100 ml). In the Transplant Unit of one examined hospital detection of a small number of bacteria – 32 CFU/100 ml, eradication methods were used, because in accordance with current guidelines, in units where patients are treated with immunosuppressive drugs, *L. pneumophila* should not be present in water samples of 1,000 ml volume.

The mean temperature of hot water in the studied objects were as follows:  $47.35 \pm 7.23$  °C (median 48.00 °C) in hospitals,  $49.17 \pm 7.64$  °C (median 50.00 °C) in hotels, and  $38.19 \pm 15.15$  °C (median 35.00 °C) in other objects. Significant differences were found between water temperature in the investigated objects ( $p < 0.0001$ ). Regression analysis between concentrations of *L. pneumophila* in water samples and temperature showed statistically significant inverse correlation ( $R = -0.27$ ;  $p = 0.00003$ ) (Fig. 2).

**Figure 2.** The correlation between the temperature of the hot water and the number of *L. pneumophila*

There was a significant relationship between the number of *L. pneumophila* and water temperature in the examined hospitals ( $R = -0.44$ ;  $p < 0.000001$ ), in hotels ( $R = -0.86$ ;  $p = 0.00004$ ), while in other objects the relationship was not significant ( $R = -0.03$ ;  $p = 0.86$ ).



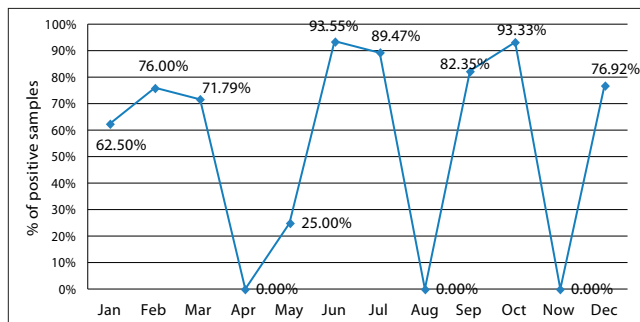


Figure 3. Percentage of positive samples in particular months in 2007-2010

Figure 3 show the percentage of positive samples in particular months in 2007–2010. The seasonal differences in the positive results were not noted (winter – 71.68% positive samples, spring – 71.68%, summer – 33.33%, and autumn – 86.11%) ( $p=0.13$ ). The number of positive samples were different in particular months ( $p < 0.00001$ ). The higher rates of isolation of *L. pneumophila* were observed in June (93.55% positive samples), October (93.33%) and July (89.47%).

## DISCUSSION

*Legionella* spp. are the members of the natural flora of many freshwater environments, such as rivers, streams and impoundments, where they occur in relatively low numbers [18]. Industrialization has created the possibility of colonization by *Legionella* of hot and cold water supplies in residential buildings, hospitals, sanatoria and hotels [1, 2, 3]. There is also an adaptation of bacteria to industrial conditions, such as cooling towers, car washes and air conditioning systems [1, 2, 3].

The factors facilitating the colonization of water distribution systems by *Legionella* are temperature ranging 20 °C – 45 °C, lack of isolation in hot and cold water installations, corrosion, and formation of deposits, the presence of sediments, low concentration of disinfectant, water stagnation, ‘dead legs’ of the installation, and biotic factors (presence of other microorganisms, biofilm) [1, 2, 18, 19, 20]. Frequently, the colonization of a water system by *Legionella* is the result of an incorrect system design, a number of changes introduced during exploitation, the use of inappropriate materials, and improper maintenance of the water system [1, 18, 20].

The presence of *Legionella* in water system creates a real risk of infection for humans, especially for hospitalized patients. The risk of infection increases when the installation is more contaminated by *Legionella*. [21]

To-date, the infective dose has not been precisely defined. Estimated data indicate that in the case of water contamination by *Legionella* at the number of  $10^3$ – $10^5$  CFU/1,000 ml, the disease may occur sporadically, but when the number exceeds  $10^5$  CFU/1,000 ml an outbreak of Legionnaires’ disease can be expected [1, 2, 22]. About 25% – 50% of all cases are associated with a previously taken trip, staying in a hotel, staying indoors or in the outdoor environment where contaminated water and water-air aerosol can spread [2, 22].

The sources of *Legionella* infection can be the hot water system, tanks for storage, dredging cocks, strainers, shower, air conditioning units, humidifiers, and additionally in healthcare facilities – hydrotherapy pools and other facilities used in balneotherapy, breathing air equipment, dental

turbine, dialyzers, cooling water systems in industrial plants [2, 18, 23].

This study allowed determination of the risk of microbial contamination by *L. pneumophila* associated with water systems in different buildings of the Lublin region of eastern Poland. The study showed the presence of *L. pneumophila* in all tested buildings, but the level of contamination varied. *L. pneumophila* bacteria were detected only in hot water samples: of 222 tested hot water samples, in 166 samples *L. pneumophila* bacteria were present which accounted for 74.77%. The frequency of positive samples in the examined objects were: barracks of a military unit and shopping centres – 100.00%, hotels – 86.66%, hospitals – 78.57%, homes for single mothers – 33.33%, and the industrial plant – 22.22%. In 34.33% of water samples the number of *L. pneumophila* exceeded the acceptable level of  $>10^2$  CFU/100 ml and ranged from 108–3300 CFU/100 ml. Of all the tested buildings, very high levels of *L. pneumophila*,  $>1,000$  CFU/100 ml were found not only in the hospitals, with an average level of contamination of 100–1,000 CFU/100 ml *L. pneumophila*, but also occurred significantly often in hotels.

In the presented study, only *L. pneumophila* SG 2–14 were found, whereas the most virulent *L. pneumophila* SG 1 was not isolated. Polish data concerning serogroups of *L. pneumophila* isolated from water systems are varied. According to data from the literature in other European countries the most commonly isolated serogroup of *L. pneumophila* is the SG 1 [2, 22].

In 65.90% of water samples collected from hospitals in this study, there were low levels of *L. pneumophila* ( $<100$  CFU/100 ml), in 28.03% of samples average levels were detected (100–1000 CFU/100 ml), and 6.07% of samples had high levels of *L. pneumophila* ( $>1,000$  CFU/100 ml). Data from the presented research are in accordance with data from the literature, indicating that *L. pneumophila* commonly colonizes hospital water supply systems.

Environmental surveys for *Legionella* in the water systems of hospitals have shown that 12% – 70% were colonized by *Legionella*. [24]

A similar study was conducted by *Matuszewska et al.* in which of 169 water samples collected from a water supply system, air-conditioning and balneotherapy equipment in healthcare facilities (i.e. hospitals, sanatoriums), 132 samples (78.70%) showed the presence of *Legionella* in a number far exceeding the acceptable level of  $>100$  CFU/100 ml, and ranged from  $1.2 \times 10^2$  CFU/100 ml –  $1.3 \times 10^5$  CFU/100 ml. In 19 samples (16.10%), there was also detected the most dangerous of all, *L. pneumophila* SG 1, and in 16 (13.50%) samples, *L. pneumophila* SG 1 were mixed with *L. pneumophila* SG 2–14. *Legionella pneumophila* SG 2–14 were present at the highest percentage in 83.90% of samples [2].

Research carried out between 2001–2008 at the Department of Environmental Hygiene of the National Institute of Public Health/National Institute of Hygiene in Warsaw (Poland) revealed the presence of *Legionella* in hot water of different public buildings (i.e. hospitals, hotels, industrial plants, residential buildings). The frequency of these bacteria in hospitals was 78.60%, in hotels – 68.60%, in residential buildings – 93.00%, and industrial plants – 68.00%. *L. pneumophila* SG 1 was found in about 3% of the examined water samples from all objects. *L. pneumophila* SG 2–14 was detected in 78.80% of cases, while in 18.20% both *L. pneumophila* SG 1 and SG 2–14 were identified [25].

According to the literature, in other countries contamination of hospital facilities by *Legionella* spp. often exceeds 50.00%, and the number of bacteria exceeding 100 CFU/100 ml is a real hazard for health of patients and medical staff (Tab. 4) [2, 26].

**Table 4.** Occurrence of *Legionella* spp. in hospital water systems in selected countries [27, 28, 29, 30]

Country	Number of hospitals	The percentage of hospitals colonized by the <i>Legionella</i>
United Kingdom	40	70%
	69	55%
	17	12%
Spain	20	85%
Poland	8	100%
Canada	84	68%
United States	12	91%
	6	83%

Yu *et al.* examined the water samples from 16 hospitals in Taiwan where *L. pneumophila* was detected in 63.00% (10/16) of water systems, especially SG 1 (80.00% of the isolates) [28]. Stout *et al.* examined 20 hospitals in Barcelona, Spain in which they found the presence of *L. pneumophila* in 85% of the water samples (17/20). The most frequently isolated serogroups were SG 2–14 [29]. The five-year study conducted by Sabrià *et al.* in 20 hospitals of Catalonia in Spain demonstrated the presence of *L. pneumophila* in 17 hospitals (85.00%) [31]. The study by Kool *et al.* consisted of detecting the source of infection, assessment the risk factors for infection and colonization, and the factors contributing to the hot water system colonization by *Legionella* in 12 hospitals in San Antonio, Texas, USA. There were 20 cases of Legionnaires' disease detected in 5 hospitals. *Legionella* was isolated from the water systems of 11 hospitals. In all hospitals, the hot water temperature was too low to inhibit the growth of *Legionella*, and therefore the system had been colonized by the bacteria. According to the authors, to assess the risk of Legionnaires' disease in hospitalized patients, it is better to take into account the percentage of positive samples after examination of the object, than analyze the number of *Legionella* (contamination levels of the water system) in water samples [30].

In the presented study, of 20 water samples collected from 4 hotels, the presence of *L. pneumophila* was detected in 13 (86.66%) samples. In 76.93% of samples, the number of *Legionella* exceeded the acceptable level (>100 CFU/100 ml) with a mean value of 322.42 CFU/100 ml (ranging from 123–745 CFU/100 ml). Similarly high levels of water contamination by *Legionella* in hotels are reported by other investigators. In the water systems of European hotels, *Legionella* colonization ranged from 27% – 75% in several studies [5].

Studies in 40 Italian hotels (119 water samples collected) were conducted by Borella *et al.* In 30 hotels (75.00%) with 72 water samples collected (60.50%) the bacteria of the genus *Legionella* were detected. Among the hotels with positive results, 9 were colonized by *L. pneumophila* SG 1 and 15 by *L. pneumophila* SG 2–14. The *Legionella* level  $\geq 10^3$  CFU/1,000 ml was found in 62.50% of the samples (n=45), and  $\geq 10^4$  CFU/1,000 in 19.40% (n=14) [32]. In a study by Endogan *et al.*, of 52 Turkish hotels, 491 samples were collected (145 swabs and 346 water samples). *Legionella*

were detected in 93 samples: in 29 (20.00%) smear samples and 64 (18.5%) water samples. *L. pneumophila* (SG 1, SG 3, SG 6, SG 7–14) were isolated from 86 (92.50%) of the samples, while species other than *L. pneumophila* were found in 7 (7.50%) samples. The level of *Legionella* <100 CFU/100 ml was detected in 19 (29.70%) samples, 100–1000 CFU/100 ml in 26 (40.60%) samples,  $\geq 1000$  to  $\leq 10,000$  CFU/100 ml in 14 (21.90%) and  $\geq 10,000$  CFU/100 ml in 5 (7.8%) [5].

The exposure to water-air aerosol contaminated by *Legionella* produced by industrial cooling towers provides the risk for sporadic cases and outbreaks of Legionnaires' disease among workers and people living near the sources of infection. Such cases have already been reported, associated with a power station (Morton *et al.*) and a factory producing plastics and ceramics (Muraca *et al.*, Bellido-Blasco *et al.*) [33, 34, 35]. Different studies have demonstrated that approximately 30% – 60% of industrial cooling towers are colonized by *Legionella* [22].

In the presented study, 4 water samples collected from shopping centres were tested. *L. pneumophila* was found in all samples, but at an acceptable level (55–80 CFU/100 ml CFU/100 ml). In 18 hot water samples collected from industrial plants located in the Lublin region, positive results were obtained in 4 (22.22%) cases, with the level of contamination from 3.6 CFU/100 ml – 138 CFU/100 ml.

The most important factor which enables the survival and proliferation of *Legionella* in the water systems of large public buildings is temperature – 20°C – 45°C. The influence of temperature has been seen not only on the survival, but also on the virulence of *Legionella* [36].

The presented shows an association between *L. pneumophila* levels and hot water temperature: by increasing the temperature of the water the number of bacteria was decreased. Similar results were obtained by other researchers. [36, 37, 38].

In cold water samples (temperature range 8°C – 15°C), *L. pneumophila* bacteria were not detected. There is a very low probability of *Legionella* occurrence in a water supply with water temperature lower than 15°C [36].

The current study analyzed seasonal variability in the contamination of water systems by *L. pneumophila*. There were no differences between the season and the positive samples, but in autumn, winter and spring, higher rates of isolation of these bacteria were found. Recent studies suggest that Legionnaires' disease occurs most frequently in the summer but this is not necessarily linked to a higher water contamination by *Legionella* [10].

Research by Legnani *et al.* found higher counts of *Legionella* spp. in all water systems in the period between June – October, with statistically significant differences compared with the remaining seasons of the year. The researchers suggest that the seasonal differences in the levels of *Legionella* in water samples could be due to the variability of hot water temperature during the year. The samples had a lower temperature in summer (mean: 45.64°C) than the remaining seasons (mean: 49.56°C) [38].

*L. pneumophila* bacteria commonly colonize water supply systems in public buildings and healthcare facilities. The level of *Legionella* in the examined objects varied, which also indicates the varied exposure of the occupants of buildings, and patients/medical staff in healthcare facilities. It should be noted, however, that regardless of the level of water contamination by *Legionella*, there is always a risk of illness

because of the presence of a source of infection. In the case of detection of bacteria in water systems, efforts should be made to reduce the risk of *Legionella* infection by eliminating them by using appropriate methods.

## CONCLUSIONS

The examination of hot water samples collected from the water supply system of hospitals and public buildings revealed that acceptable levels of *L. pneumophila* were exceeded, which indicates the risk of infection. The permanent monitoring of water distribution systems is an important element of the control of infections caused by these microorganisms.

The data obtain suggest that high temperature prevents the colonization water systems by *L. pneumophila* (an increase in the water temperature reduces the number of bacteria). This shows that the maintenance of the hot water temperature prevents excessive multiplication of *Legionella*. Therefore, an elevated water temperature is one of the methods used to eradicate *Legionella* from hot water systems [36].

In the case of detection of bacteria in the water systems, reduction of the risk of *Legionella* infection can be achieved by eliminating it by using appropriate methods, which sometimes can be difficult and requires someone to develop a strategy.

## REFERENCES

- Bartram J, Yves Ch, Lee JV, Pond K, Surman-Lee S. *Legionella* and prevention of legionellosis. World Health Organization, 2007.
- Matuszewska R, Krogulska B. Problem występowania pałeczek *Legionella* w instalacjach i urządzeniach wytwarzających aerozol wodno-powietrzny w obiektach służby zdrowia. Nowa Med. 2009; 1: 56–60 (in Polish).
- Breiman RF, Butler JC. Legionnaires' disease: clinical, epidemiological, and public health perspectives. Semin Respir Infect. 1998; 13(2): 84–89.
- Legionella*: human health criteria document. Environmental Protection Agency, United States, 1999.
- Erdogan H, Arslan H. Colonization of *Legionella* species in hotel water systems in Turkey. J Travel Med. 2007; 14(6): 369–373.
- Mekkour M, Driss KB, Cohen N. Prevalence of *Legionella pneumophila* in production networks and distribution of domestic hot water in Morocco. World Environment. 2012; 2(2): 11–15.
- Poupard M, Campèse C, Bernillon P, Che D. Factors associated with mortality Legionnaires' disease, France, 2002–2004. Med Mal Infect. 2007; 37(6): 325–330.
- Kusnetsov J, Neuvonen LK, Korpio T, Uldum SA, Mentula S, Putus T, et al. Two Legionnaires' disease cases associated with industrial waste water treatment plants: a case report. BMC Infect Dis. 2010; 10: 343–350.
- Lock K, Millett C, Heathcock R, Joseph CA, Harrison TG, Lee JV, et al. Public health and economic costs of investigating a suspected outbreak of Legionnaires' disease. 2008; 136(10): 1304–1314.
- Borella P, Montagna MT, Romano-Spica V, Stampi S, Stancanelli G, Triassi M, et al. *Legionella* infection risk from domestic hot water. Emerg Infect Dis. 2004; 10(4): 457–464.
- Erdogan H, Erdogan A, Lakamdayali H, Yilmaz A, Arslan H. Travel-associated Legionnaires' disease: clinical features of 17 cases and a review of the literature. Diagn Microbiol Infect Dis. 2010; 68(3): 297–303.
- www.ecdc.europa.eu (access: 2013.02.09).
- www.pzh.gov.pl (access: 2013.02.09).
- Rozporządzenie Ministra Zdrowia z dnia 29 marca 2007 r. w sprawie jakości wody przeznaczonej do spożycia przez ludzi. Ministerstwo Zdrowia (Dz. U. Nr. 61 poz. 417) (in Polish).
- PN-EN ISO 19458: 2007 „Jakość wody – Pobieranie próbek do analiz mikrobiologicznych”. PKN, 2007 (in Polish).
- PN-ISO 11731: 2002 „Jakość wody. Wykrywanie i oznaczanie bakterii z rodzaju *Legionella*”. PKN, 2002 (in Polish).
- PN-EN ISO 11731–2:2008 „Jakość wody – Wykrywanie i oznaczanie ilościowe bakterii z rodzaju *Legionella* – Część 2: Metodyka filtracji membranowej dla wód o małej liczbie bakterii”. PKN, 2008 (in Polish).
- Guidelines for drinking-water quality. World Health Organization Recommendations – 3<sup>rd</sup> ed. Vol. 1.
- Allen JG, Myatt TA, Macintosh DL, Ludwig JF, Minegishi T, Stewart JH, et al. Assessing risk of health care-acquired Legionnaires' disease from environmental sampling: The limits of using a strict percent positivity approach. Am J Infect Control. 2012; 40(10): 917–921.
- Rakić A, Perić J, Foglar L. Influence of temperature, chlorine residual and heavy metals on the presence of *Legionella pneumophila* in hot water distribution systems. Ann Agric Environ Med. 2012; 19(3): 431–436.
- Tesauro M, Bianchi A, Consonni M, Pregliasco F, Galli MG. Environmental surveillance of *Legionella pneumophila* in two Italian hospitals. Ann Ist Super Sanità 2010, 46(3): 274–278.
- Matuszewska R, Krogulska B. Występowanie bakterii z rodzaju *Legionella* w systemach wody chłodniczej. Roczn PZH. 2008; 59 (4): 445–454 (in Polish).
- Diederer BM. *Legionella* spp. and legionnaires' disease. J Infect. 2008; 56(1): 1–12.
- Lin YS, Stout JE, Yu VL, Vidic RD. Disinfection of water distribution systems for *Legionella*. Semin Respir Infect. 1998, 13(2): 147–59.
- Krogulska B, Matuszewska R. „Bakterie z rodzaju *Legionella* – obowiązujące w Polsce regulacje prawne”, „Bezpieczna woda w szpitalu” warsztaty na temat zakażeń szpitalnych wywołanych przez pałeczki *Legionella* spp. Instytut Gruźlicy i Chorób Płuc w Warszawie, Narodowy Instytut Zdrowia Publicznego; 24 Oct 2008 (in Polish).
- Napoli C, Itta R, Fasano F, Marsico T, Montagna MT. Vairable bacterial load of *Legionella* spp. in hospital water system. Sci Total Environ. 2009; 408(2): 242–244.
- Yu VL. Nosocomial legionellosis. Curr Opin Infect Dis. 2000; 13: 385–388.
- Yu PY, Lin YE, Lin WR, Shih HY, Chuang YC, Ben RJ, et al. The high prevalence of *Legionella pneumophila* contamination in hospital potable water systems in Taiwan: implications for hospital infection control in Asia. Int J Infect Dis. 2008; 12(4): 416–420.
- Stout JE, Yu VL. *Legionella* in the hospital water supply: A plea for decision making based on evidence-based medicine. Infect Control Hosp Epidemiol. 2001; 22(11): 670–672.
- Kool JL, Bergmire-Sweat D, Butler JC, Brown EW, Peabody DJ, Massi DS, et al. Hospital characteristics associated with colonization of water systems by *Legionella* and risk of nosocomial legionnaires' disease: a cohort study of 15 hospitals. Infect Control Hosp Epidemiol. 1999; 20(12): 798–805.
- Sabrià M, Mòdol JM, Garcia-Nuñez M, Reynaga E, Pedro-Botet ML, Sopena N, et al. Environmental cultures and hospital-acquired Legionnaires' disease: a 5-year prospective study in 20 hospitals in Catalonia, Spain. Infect Control Hosp Epidemiol. 2004; 25(12): 1072–1076.
- Borella P, Montagna MT, Stampi S, Stancanelli G, Romano-Spica V, Triassi M, et al. *Legionella* contamination in hot water of Italian hotels. Appl Environ Microbiol. 2005 71(10): 5805–5813.
- Morton S, Barlett CL, Bibby LF, Hutchinson DN, Dyer JV, Dennis PJ. Outbreak of Legionnaires' disease from a cooling water system in a power station. Br J Ind Med. 1986; 43(9): 630–635.
- Bellido-Blasco JB, Pelaz-Antolín C, Delas-González MA, Sarrión-Martínez J, Moreno-Muñoz MR, Herrero-Carot C. Aggregation of cases of *Legionella pneumonia* in workers related to the ceramic industry in Castellon, Spain, 2006. Rev Esp Salud Publica. 2008, 82(1): 111–116.
- Muraca PW, Stout JE, Yu VL, Yee YC. Legionnaires' disease in the work environment: implications for environmental health. Am Ind Hyg Assoc J. 1988; 49(11): 584–590.
- Hrubá L. The colonization of hot water systems by *Legionella*. Ann Agric Environ Med. 2009, 16: 115–119.
- Leoni E, de Luca G, Legnani R, Sacchetti R, Stampi S, Zanetti F. *Legionella* waterline colonization: detection of *Legionella* species in domestic, hotel and hospital hot water systems. J Appl Microbiol. 2005, 98: 373–379.
- Legnani PP, Leoni E, Corradini N. *Legionella* contamination of hospital water supplies: monitoring of private healthcare facilities in Bologna, Italy. J Hosp Infect. 2002, 50: 220–223.