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 form 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 pneumonias (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, Cohn’s disease, in patients post organ transplantation and patients treated with corticosteroids or anti-TNF α 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].
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), it 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. 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 1 (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]

<table>
<thead>
<tr>
<th>NUMBER of Legionella spp. in 100 ml of water</th>
<th>ASSESSMENT OF CONTAMINATION</th>
<th>PROCEDURE</th>
<th>TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10²</td>
<td>lack / minimal</td>
<td>if the majority of the samples is positive, the water system is considered as colonized by bacteria of the genus Legionella, 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</td>
<td>after 1 year and after 3 years²</td>
</tr>
<tr>
<td>10⁻²⁻¹⁰⁴</td>
<td>medium</td>
<td>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</td>
<td>after 1 week of cleaning and disinfecting then every 3 months³</td>
</tr>
<tr>
<td>10⁻¹⁻¹⁰⁴</td>
<td>high</td>
<td>equipment and hot water systems immediately excluded from the operation; carry out procedures for cleaning and disinfection</td>
<td>after 1 week of cleaning and disinfecting then every 3 months³</td>
</tr>
<tr>
<td>&gt;1⁰⁴</td>
<td>very high</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Explanation:

1. 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
2. If in further studies there were <100 CFU/100 ml annually
3. 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:

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


The membrane filtration method was used for all tested water samples. For samples with an expectedly large number of bacteria of the genus Legionella (>10^6 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 longbeachae serogroup 1 (SG 1) i serogroup 2 (SG 2), Legionella bozemanni 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 ≤ 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 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).
number of \textit{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 \textit{Legionella} should not exceed 100 CFU in 100 ml of water samples (<100 CFU/100 ml), (ANNEX I D). In accordance with the above regulation there are 3 levels of water contamination with bacteria of the genus \textit{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 \textit{L. pneumophila} bacteria count exceeded the acceptable level of >100 CFU/100 ml. In 65.66% (n=109) of water samples the levels of \textit{L. pneumophila} were low (<100 CFU/100 ml). Among the samples that exceeded an acceptable level, 49 samples had an average level of \textit{L. pneumophila} (100–1000 CFU/100 ml), while in 8 samples the levels were high (>1,000 CFU/100 ml), (Fig. 1).

![Figure 1](image)

**Figure 1.** The percentage of water samples tested including the degree of contamination by \textit{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 \textit{L. pneumophila} (>1,000 CFU/100 ml) were found in examined the hospitals (6.07%), where the highest number of \textit{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 \textit{L. pneumophila} – 32 CFU/100 ml, eradication methods were used, because in accordance with current guidelines, in units where patients are treated with immunosuppressive drugs, \textit{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 ± 7.23°C (median 48.00°C) in hospitals, 49.17 ± 7.64°C (median 50.00°C) in hotels, and 38.19 ± 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 \textit{L. pneumophila} in water samples and temperature showed statistically significant inverse correlation (R = -0.27; p=0.00003) (Fig. 2).

![Figure 2](image)

**Figure 2.** The correlation between the temperature of the hot water and the number of \textit{L. pneumophila}
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 (< 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 10³–10⁵ CFU/1,000 ml, the disease may occur sporadically, but when the number exceeds 10⁶ 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 >10⁵ 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, whereby 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 × 10⁶ CFU/100 ml – 1.3 × 10⁹ 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 are 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 industrial buildings – 93.00%, and industrial plants – 68.60%. *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]

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of hospitals</th>
<th>The percentage of hospitals colonized by the Legionella</th>
</tr>
</thead>
<tbody>
<tr>
<td>United Kingdom</td>
<td>40</td>
<td>70%</td>
</tr>
<tr>
<td>Spain</td>
<td>17</td>
<td>12%</td>
</tr>
<tr>
<td>Poland</td>
<td>8</td>
<td>100%</td>
</tr>
<tr>
<td>Canada</td>
<td>84</td>
<td>68%</td>
</tr>
<tr>
<td>United States</td>
<td>12</td>
<td>91%</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>83%</td>
</tr>
</tbody>
</table>

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 ≥10⁶ CFU/100 ml was found in 62.50% of the samples (n=45), and ≥10⁷ CFU/100 ml 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, ≥1000 to ≤10,000 CFU/100 ml in 14 (21.90%) and ≥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 obtained 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
