® Postepy Hig Med Dosw (online), 2019; 73: 47-52
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 04.04.2017
Accepted: 04.10.2018
Published: 07.02.2019

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Prevalence of *Legionella* spp. in hot water samples from Polish hospitals in 2009-2013

Występowanie bakterii z rodzaju Legionella spp. w próbkach wody ciepłej w szpitalach w Polsce, w latach 2009-2013

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Background:

Material/Methods:

Summary

Microbiological threat from *Legionella* spp. is associated with the current widespread use of air-condition systems and frequent colonization of hot water distribution systems in public facilities (hospitals, hotels, nursing homes). This poses a particularly high risk in hospitalized patients, as many of them are immunocompromised. More than 50 species and 72 serogroups of *Legionella* spp. have been described thus far, including more than 20 species being pathogenic to humans.

Results:

Microbiological threat posed by *Legionella* spp. was assessed based on a retrospective analysis of microbiological quality of water from hot water distribution systems in Polish hospitals, determined in 2009-2013 at certified laboratories of the Sanitary-Epidemiological Surveillance. The results were kindly provided by the hospitals' administration upon request, for which the authors would like to express their deepest gratitude. The study material included samples of hot water from internal distribution systems in 379 Polish hospitals, collected by the State Sanitary Inspectorate.

Discussion:

Based on the results of microbiological analyses, we were able to estimate *Legionella* spp. colonization rates in hospital water distribution systems and to assess the activities undertaken by the State Sanitary Inspectorate to reduce excessive bacterial counts in this reservoir. Microbiological analyses conducted in 2009-2013, i.e. after implementing a statutory obligation to monitor *Legionella* spp. in hospital hot water distribution systems, showed elevated bacterial counts in 3.92% to 12.7% of the samples. These findings justify further microbiological monitoring of hospital water distribution systems.

The prevalence of the most pathogenic serotype SG1 in hot water distribution systems of Polish hospitals is relatively low compared to other European countries. To maintain this favorable status, hospital water systems should be monitored not only for the presence of *Legionella pneumophila*, but also for the prevalence of serogroup 1 *L. pneumophila*.

Keywords: ***Legionella spp.*** • ***Legionella pneumophila*** • **hospitals**

GICID 01.3001.0013.0172
DOI: 10.5604/01.3001.0013.0172
Word count: 3190
Tables: 1
Figures: 1
References: 23

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INTRODUCTION

The source of infection for humans is water-air aerosol with 2.0 to 5.0-µm droplet diameter, containing invasive forms of *Legionella* spp., and its aspiration to the respiratory tract [6]. Microbiological threat from *Legionella* spp. results from the widespread use of air-condition systems and hydrotherapy. A total of 50 *Legionella* species and 72 serogroups (SGs) have been described thus far, among them more than 20 species that are considered pathogenic to humans. The main *Legionella* spp. isolated from the outbreaks were *L. pneumophila* SG 1 and SG 2-14. *L. pneumophila* contributes to the largest proportion of hospital-acquired legionnaires disease cases (80-90%) of all representatives of the *Legionellaceae* family. In fact, 60-90% of these cases are caused by *L. pneumophila* SG 1, and *L. pneumophila* from serogroups other than SG 1 (usually SG 4, SG 6, SG 2-14) contribute to ca. 20-30% of the infections [8]. In spite of this, the Minister of Health Regulation on the quality of water intended for human consumption that was effective at the time of this study, did not specify which measures need to be undertaken if the most pathogenic *L. pneumophila* SG 1 and SG 2-14 were detected in water samples [15].

The risk of infection with *Legionella* spp. is particularly high in hospitalized immunocompromised patients (after organ transplantations and/or immunosuppressive treatment, with diabetes mellitus, cancers or chronic respiratory diseases), as well as in individuals staying in rooms with high air humidity or those working in air-conditioned rooms [16]. Hospitalization is associated with a high risk of *Legionella* spp. infection. Hospital-acquired pneumonia (HAP) may be caused by *Legionella* spp. colonizing air-condition systems, hot water distribution systems (showers) and medical devices [1, 14]. HAP caused by *Legionella* spp. is associated with substantially higher mortality.

To reduce the risk of hospital-acquired infection, the regulation on the quality of water intended for human consumption has imposed a statutory obligation to monitor the microbiological status of water in hospital distribution systems by the State Sanitary Inspectorate [15]. Monitoring includes determination of *Legionella* spp. counts in hot water samples, as well as the elimination of identified risks and repeated control. The aim of this study was to assess the microbiological threat posed by *Legionella* spp. based on a retrospective analysis of microbiological quality of water from hot water distribution systems in Polish hospitals, determined in 2009-2013 at certified laboratories of the State Sanitary Inspectorate. Moreover, we assessed the activities undertaken by the State Sanitary Inspectorate to reduce excessive bacterial counts in this reservoir.

MATERIAL AND METHOD

The study material included samples of hot water from internal distribution systems in 379 (46.6%) Polish hospitals, collected by the State Sanitary Inspectorate inspectors [9]. Overall, between January 2009 and December 2013, inspectors from 34 State Sanitary Inspectorate branches, which have jurisdiction over a given district, have collected a total of 4,747 hot water samples within a framework of routine water quality monitoring. The examination was conducted as part of routine control procedures, and was not related to the presence of legionnaire disease among the hospitalized patients. The samples were examined in line with the Minister of Health Regulation of 29 March 2007 on the quality of water intended for human consumption (Journal of Laws of the Republic of Poland, 2007, no. 61, pos. 417, with further amendments). In line with this act, the maximum count of *Legionella pneumophila* in hot water should be lower than 10^2 colony forming units (CFU) per 100 ml. The minimum frequency of hot water sampling and measures that need to be implemented depending on the result of the microbiological testing are specified in the Minister of Health Regulation on the quality of water intended for human consumption [15].

The samples were examined according to the following standards:

- PN-EN ISO 11731-2:2008 "Water quality – Detection and enumeration of *Legionella* – Part 2 Direct membrane filtration for waters with low bacterial counts".
- PN-ISO 11731:2002 "Water quality – Detection and enumeration of *Legionella*".
- PN-EN ISO 19458:2007 "Water quality – Sampling for microbiological analysis".

To determine the dynamics in *Legionella* spp. counts in hot water distribution systems, water samples from 127 hospitals have been collected repeatedly in at least two consecutive years.

Controlled hospitals were arbitrarily divided into three groups: with only positive, both negative and positive, and only negative results of microbiological testing for *Legionella* spp. in hot water from internal distribution systems. The aim of the statistical analysis was to verify the significance of intergroup differences and year-to-year intragroup differences.

STATISTICAL ANALYSIS

A statistical analysis of the results was conducted with the STATISTICA 7.1 package. The significance of intergroup differences was verified with Pearson chi-squared test and Student t-test for normally distributed data. The differences were considered significant at $p \leq 0.05$.

RESULTS

The microbiological analysis of hospital water distribution systems, conducted in 2009-2013, demonstrated that the proportion of facilities with exceeded maximum allowable count of *Legionella* spp. (>100 CFU) was the highest in 2009 (12.77%), and then decreased to 3.92% in 2010, 4.93% in 2011, 10.45% in 2012, and 4.29% in 2013. The proportion of facilities in which both negative and positive results of microbiological testing were recorded during the study period, ranged between 42.16% in 2010 and 28.57% in 2013, and did not show a decreasing tendency. Irrespective of the analyzed year, a proportion of examined samples tested negatively for *Legionella* spp. (<100 CFU). The largest proportion of facilities with the negative result of microbiological testing was recorded in 2013 (67.14%). This does not allow for an ultimate conclusion, whether preventive measures undertaken by hospital administration during the analyzed period, typically rinsing hot water distribution systems with water with higher temperature or less often, water disinfection, really produced a satisfactory result. This observation seems to be supported by the results presented in Table 1 containing mean numbers of bacterial colonies for consecutive years, along with information about statistical significance of year-to-year differences.

As shown in Table 1, the results of microbiological testing for consecutive years differed significantly.

Statistically significant year-to-year differences in the number of bacterial colonies in hot water samples are designated with superscript numbers.

The results of microbiological testing differed significantly between 2009 and 2010, between 2009, 2010 and 2012, as well as between 2009-2013, 2010-2013 and 2012-2013. The most favorable situation was observed for 2013, which might reflect the effectiveness of surveillance and hospital administration in eradication of bacteria from hot water distribution systems.

To assess microbiological threat posed by *Legionella pneumophila* SG 1 and *Legionella pneumophila* SG 2-14, additional tests for the presence of these serotypes were conducted in hospitals where *Legionella* spp. were isolated from hot water samples. Overall, the additional tests were conducted in 55 Polish hospitals examined in 2012-2013.

L. pneumophila from the most virulent serogroup, SG 1, were detected in 3 hospitals (5.45%), and *L. pneumophila* SG 2-14 in 20 (36.36%). A total of 282 hot water samples were collected in 2012-2013, including 5 (1.77%) that tested positively for *L. pneumophila* SG 1, and 192 (68.08%) testing positively for *L. pneumophila* SG 2-14.

DISCUSSION

Legionella spp. counts in water samples from hospitals controlled in 2009–2013 showed considerable variability, as shown in Table 1 and Figure 1. Samples from 26.77% of hospitals tested positively both in the first and in subsequent years, which might reflect problems with the eradication of *Legionella* spp. or the use of an inappropriate method to reduce bacterial counts. Conversion of negative results to positive ones was documented in 11.81% of hospitals; this might result from new colonization of hospital water distribution systems with *Legionella* spp. or from inefficient prevention of repeated colonization. Conversion of positive results to negative ones was documented in 31.50% of hospitals, and probably reflected the effectiveness of measures undertaken by the State Sanitary Inspectorate to reduce excessive

Table 1. Results of microbiological testing of water samples collected in Polish hospitals in 2009-2013

No.	Year of sampling	Mean $\bar{x}$	Standard deviation $^{+}_{-}$SD	Standard error SE
1	2009	7134.2^{5}	48125.7	5250.9
2	2010	1316.1^{5}	3467.9	350.3
3	2011	921.4	2634.4	264.8
4	2012	3165.2^{5}	19591.8	1578.8
5	2013	$333.6^{1,2,4}$	1207.1	170.7

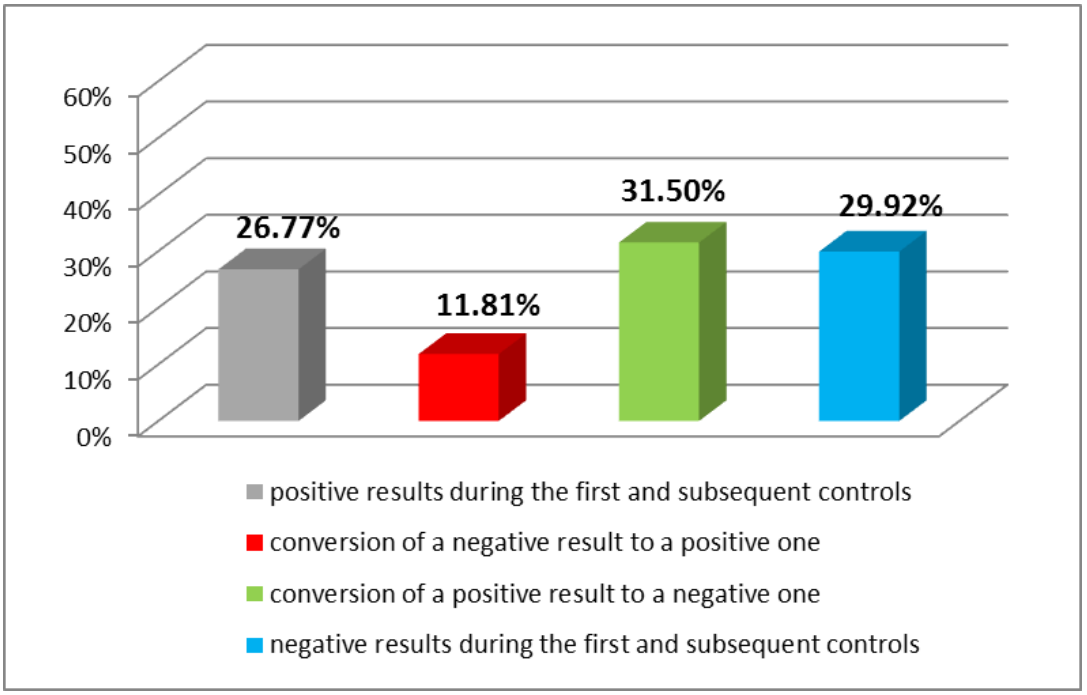

Fig. 1. Conversion of the results of microbiological testing conducted in 2009-2013

bacterial counts. In 29.92% of hospitals, *Legionella* spp. were detected during both the first and subsequent controls. *Legionella* spp. colonizing hospital water distribution systems are etiological factor of legionnaires disease and therefore, constitute a threat to human health and life.

Based on the results of microbiological analyses, we were able to estimate *Legionella* spp. colonization rates in hospital water distribution systems and to assess the activities undertaken by the State Sanitary Inspectorate to reduce excessive bacterial counts in this reservoir. Hospitalization and exposure to excessive counts of *Legionella* spp. constitute critical risk factors of legionnaires disease.

Excessive growth of *Legionella* sp. in hot water systems may be a consequence of frequent renovation and reorganization of hospital facilities, in particular changes in hospital departments' profiles; such activities often result in the formation of the so-called "dead ends" within the water pipe systems, providing a favorable growth environment for *Legionella* spp.

Previous studies conducted in Poland documented the presence of *Legionella* spp. in 8.19% of samples from hospital water distribution systems. In up to 72.8% of positive samples, bacterial counts exceeded the maximum allowable limit [11]. Hospitals constituted the largest proportion (63%) among public facilities whose water distribution systems turned out to be colonized with *Legionella* spp. [10].

In one previous study conducted by other authors, *Legionella* spp. count exceeded the maximum allowable limit in 250 out of 508 (49%) hot water samples [22]. In another study of hot water distribution systems in hospitals from Podlaskie Province, the maximum allowable limit for *Legionella* spp. count was exceeded in 62.07% of the samples. The same study demonstrated a relationship between the temperature of water and *Legionella* spp. colonization rate: the higher the temperature of water at the point of sampling, the lower the *Legionella* spp. count in the sample [17]. Excessive *Legionella* spp. counts were also found in 63.6% of the water samples collected in hospitals from Kuyavian-Pomeranian Province [23].

The results of studies conducted in other European countries vary considerably. *L. pneumophila* were detected in 33% of 91 samples from water systems in five Greek hospitals [4]. An Italian study conducted in a hospital in Apulia demonstrated the presence of *Legionella* spp. in 384 out of 1,056 (36.4%) water samples examined between January 2008 and December 2011. However, the proportion of positive samples varied from year to year, from 27.8% in December 2008, to 14.1% in 2009, 16.4% in 2010, and up to 41.7% in 2011. Also the number of CFU in positive samples underwent substantial changes, from 100 to 180,000 per liter. The proportion of samples with <1000 CFU was 47.0%, whereas those with 1 000-10 000 CFU and >10 000 CFU represented 37.5% and 15.5%, respectively [5]. In another Italian study, which including a total of 129 hospitals, *Legionella* spp. were found in 93% and 34% of samples from 73 state-owned and 56 privately-owned facilities, respectively. The proportion

of positive samples containing more than 100 CFU per liter was 35.4% for state-owned hospitals and 22.4% for privately-owned units [12]. In 18.8% of positive samples (with >100 CFU per liter) from two Italian hospitals contained *L. pneumophila* SG 1. However, the most commonly isolated *L. pneumophila*, found in 68.3% of the positive samples, belonged to SG 2-14 [20]. In another Italian study, *L. pneumophila* SG 1 and *L. pneumophila* SG 2-14 were detected in 15.79% and 42.11% of samples, respectively [3]. British authors analyzed water samples from a hospital in England. A total of 300 samples have been collected; *L. pneumophila* SG 1 (50 CFU per liter) was detected in a birthing pool at the obstetrical department [19]. *Legionella* spp. were found in 27.3% of hot water samples from 41 Greek hospitals examined in 2004-2007; 66.9% of positive samples contained *L. pneumophila* SG 1, and *L. pneumophila* SG 2-14 were detected in 34.3% of the samples [21]. *Legionella* spp. were also found in 6.6% of water samples from 15 hospitals in Morocco; in 4% of the examined hospitals, positive samples contained *L. pneumophila* SG 1 [17].

In our present study, the most pathogenic *L. pneumophila* SG 1 were found in 3 out of 55 examined hospitals, and *L. pneumophila* SG 2-14 in 20. The regulation on the quality of water intended for human consumption, which was effective at the time of the study, did not specify which measures need to be undertaken if *L. pneumophila* SG 1, contributing to 50-75% of legionnaires disease cases, were detected in water samples [7]. *L. pneumophila* SG 1 is the most common cause of legionnaires disease, isolated from up to 70% of patients with confirmed diagnosis of this condition. *L. pneumophila* SG 1 and *L. pneumophila* SG 2-14, as well as other species: *L. anisa, L. feeleii* and *L. micdadei*, were the main pathogens isolated from legionnaires disease outbreaks [13].

A study conducted in Polish hospitals and sanatoria documented presence of *L. pneumophila* SG 1 and *L. pneumophila* SG 2-14 in 16.1% and 83.9% of positive samples, respectively [11]. Also water samples from an outbreak of legionnaires disease in Jastrzębie Zdrój, where 3 persons died, contained *L. pneumophila* SG 1 and *L. pneumophila* SG 2-14 [2]. Similarly, in the abovementioned studies conducted abroad, hot water distribution systems were shown to be primarily colonized with *L. pneumophila* SG 1 and *L. pneumophila* SG 2-14.

The minimum frequency of hot water sampling and measures that need to be implemented whenever *Legionella* spp. count exceeds the maximum allowable limit are specified in the Minister of Health Regulation on the quality of water intended for human consumption. In our present study, we analyzed changes in the prevalence of *Legionella* spp. in hospital hot water distribution systems during the first and subsequent years after the regulation was implemented [15].

After analyzing the colonization rates of hospital water distribution systems with *Legionella* spp. in 2009-2013, we found a steady decreasing tendency beginning in 2010. It implies that both the activities undertaken by the State Sanitary Inspectorate and the measures implemented to improve technical condition of water distribution systems and to reduce excessive counts of *Legionella* spp. were effective.

Microbiological analyses conducted in 2009-2013, i.e. after implementing a statutory obligation to monitor *Legionella* spp. in hospital hot water distribution systems, showed elevated bacterial counts in 3.92% to 12.7% of the samples. These findings justify further microbiological monitoring of hospital water distribution systems. Importantly, no improvement in water quality was documented in 26.7% of controlled hospitals, and secondary colonization with *Legionella* spp. was observed in another 11.8%, which implies that both interventions and preventive measures implemented in these facilities were not fully effective.

In this study, the prevalence of the most pathogenic serotype SG1 in hot water distribution systems of Polish hospitals turned out to be relatively low compared to other European countries. To maintain this favorable status, hospital water systems should be monitored not only for the presence of *Legionella pneumophila*, but also for the prevalence of serogroup 1 *L. pneumophila*.

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The authors have no potential conflicts of interest to declare.