® Postepy Hig Med Dosw (online), 2021; 75: 85-90
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 14.04.2020
Accepted: 30.10.2020
Published: 05.02.2021

Legionella spp. in Polish hospitals in 2009–2013 and 2014–2016: An epidemiological analysis

Występowanie bakterii *Legionella* spp. w szpitalach w Polsce w latach 2009–2013 i 2014–2016: analiza epidemiologiczna

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

Iwona Gładysz[1,A,B,E,F,G], Agnieszka Sikora[2,B,D,E], Małgorzata Wójtowicz-Bobin[2,B,D], Jan Karczewski[1,C,E]

[1]Pope John Paul II State School of Higher Education in Biała Podlaska, Faculty of Health and Social Sciences, Department of Health, Biała Podlaska, Poland
[2]Department and Chair of Medical Microbiology, Medical University of Lublin, Lublin, Poland

Summary

Introduction: Bacteria of the genus *Legionella* cause Legionnaires' disease (atypical pneumonia, often with a severe clinical course) and Pontiac fever (self-limiting infection with flu-like symptoms). *Legionella* spp. are commonly found in natural aquatic environments and artificial water distribution systems.

Material & Methods: An epidemiological analysis of *Legionella* spp. in hospital water supply systems was based on the results obtained from Sanitary-Epidemiological Stations of Poland. The materials for this study were hot water samples collected from 346 hospitals in 2009–2013 and from 221 hospitals in 2014–2016.

Results: Between 2014 and 2016, there was a decrease in the percentage of the number of water samples in Group A (<100 CFU/100 ml). In other groups: B (>100 CFU/100 ml), C (>1000 CFU/100 ml), D (>10 000 CFU/100 ml) was observed an increase in the percentage of the number of water samples, which was a negative phenomenon. The mean number of *L. pneumophila* colonies in analyzed periods 2009–2013 and 2014–2016 were different. In 2009–2013, the most virulent *L. pneumophila* serogroup 1 (SG 1) was detected in 3 hospitals (0.9%), and L. pneumophila SG 2-14 were isolated in 20 (5.5%). Between 2014 and 2016, *L. pneumophila* SG 1 and *L. pneumophila* SG 2-14 were found in 5 hospitals (2.2%) and 18 hospitals (8.1%), respectively.

Disscusion: In this study a comparative epidemiological analysis was performed on the prevalence of *L. pneumophila* in hospital water supply systems in Poland in the following two periods: 2009 –2013 and 2014–2016. The study demonstrated that the water supply systems of Polish hospitals were colonized by *L. pneumophila* at different levels. However, between 2014 and 2016 an upward trend was observed in comparison with 2009–2013.

Keywords: ***Legionella pneumophila*, Legionnaires' disease, hospital water systems, epidemiological analysis**

GICID 01.3001.0014.7249
DOI: 10.5604/01.3001.0014.7249
Word count: 3 446
Tables: 6
Figures: 1
References: 27

Author's address: Iwona Gładysz, Department of Health Pope John Paul II State School of Higher Education in Biała Podlaska, 21-500 Biała Podlaska, Poland; e-mail: iwona37@gmail.com

INTRODUCTION

Bacteria of the genus *Legionella* cause *Legionnaires*' disease (atypical pneumonia, often with a severe clinical course) and Pontiac fever (self-limiting infection with flu-like symptoms). These two diseases are reffered to as legionellosis. Legionnaires' disease and Pontiac fever account for 1 to 5% and 90 to 98% of all infections caused by *Legionella* spp., respectively [12, 20]. Approximately 70% to 90% of all cases of legionellosis are caused by *Legionella pneumophila,* especially *L. pneumophila* belonging to serogroup 1 (SG 1) [14]. Infections with *L. pneumophila* from other serogroups, SG 2-14 are less common (15–20% of all reported cases). The infections caused by *Legionella* species other than *L. pneumophila* (*Legionella*-like) are rare (10–20% of infections) [3, 22, 26].

Legionella spp. are commonly found in natural aquatic environments (e.g. streams, rivers, lakes, ponds, thermal pools) and artificial water distribution systems (human-made water systems). Their ubiquitous spread is associated with high capacity of these bacteria to adapt to various environmental conditions. Optimal growth temperature for *Legionella* spp. is between 45°C and 50°C and the proliferation of the bacteria is inhibited at temperatures higher than 60°C [6].

In water distribution systems, *Legionella* spp. can be found in the biofilm formed on the internal surfaces of pipes and other parts which are in direct contact with water. Bacteria growth is promoted by many factors, among them the occurrence of corrosion or sediment accumulation, retention of water within the system, presence of "dead-legs" of the installation, and the co-existence of protozoa such as *Acanthamoeba* spp., *Naegleria* spp., *Hartmannella* spp. Therefore, water installations constitute a suitable environment for the proliferation of *Legionella* spp., which can survive a broad spectrum of pH and temperatures. Not infrequently, the temperature of hot water in the distribution systems overlaps with the optimum growth temperature for *Legionella* spp. The colonization of water systems by *Legionella* spp. may be a consequence of an incorrect system design, implementation of unplanned changes during exploitation, the use of inappropriate materials and inadequate maintenance [2].

Legionella infection can occur in a community and hospital setting. The infections are classified into hospital-acquired infections/pneumonia, community-acquired infections /pneumonia (at a place of residence), and travel-associated infections/pneumonia (associated with hotel stays) [13, 22].

The most dangerous is the occurrence of *Legionella* spp. in the water distribution systems in hospitals. *Legionella* spp. can colonize medical devices that are in direct contact with water, such as respirators, dialyzers, inhalers, humidifiers, respiratory devices, and nebulizers, as well as nasogastric tubes. This poses a risk of infecting hospitalized patients, especially those with weakened immune systems. In these groups of patients, the course of *Legionella* infection is severe, with a mortality rate up to 30% [12, 20]. The risk of the infection depends on the number of bacteria that colonize the drinking water network, presence of water-air aerosol-generating devices, host's immunity, and the number and virulence of *Legionella* that penetrate to the lungs [14, 22]. Therefore, the water distribution systems in hospitals should be regularly monitored for the presence of *Legionella* spp. Whenever these microorganisms were detected at higher than acceptable levels, appropriate decontamination procedures need to be implemented [14].

The aim of this study was to collect data on the prevalence of *L. pneumophila* in water supply systems in Polish hospitals between 2009 and 2013 and between 2014 and 2016. On this basis, a retrospective comparative assessment of the epidemiological situation of the risk of legionellosis was performed.

MATERIALS AND METHODS

An epidemiological analysis of the prevalence of *Legionella* spp. in water distributions systems of hospitals in Poland was based on the data obtained from Sanitary-Epidemiological Stations, which were asked to provide them. Tests for the presence of *Legionella* spp. in potable water systems were carried out as part of routine monitoring in accordance with current legal regulations in force in Poland [7, 8].

The water samples (1.000 ml in volume) were collected by employees of Sanitary-Epidemiological Stations, in accordance with the standard PN-EN ISO 19458:2007, from hot water intakes in the temperature range of 45oC to 60°C. The water sampling points were: hot water tanks or nearest sites, distal sites from the hot water reservoir, water returning to a boiler (recirculated water), and at selected intermediate points, the number of which depended on the size of the water supply system of the examined hospitals [18].

In the laboratories of Sanitary and Epidemiological Stations, the water samples were tested according to the Polish standards: PN-EN ISO 11731-2:2008 and PN-ISO 11731:2002 [17, 19]. Whenever *L. pneumophila* were detected in the water samples, additional tests were carried out to verify whether the pathogen belonged to serogroup 1 (SG 1) or serogroups 2-14 (SG 2-14). The serological tests were performed using commercial latex agglutination test kits.

The results were analyzed and interpreted on the basis current regulations: Decree of Minister of Health on the quality of water intended for human consumption, Council Directive 98/83/EC on the quality of water intended

Table 1. Levels of water contamination by *Legionella* [9]

Number of *Legionella* spp. in 100 ml of hot water	Assessment of contamination
<100 10^2	lack/minimal (GROUP A)
>100 10^2-10^3	medium (GROUP B)
>1000 10^3-10^4	high (GROUP C)
>10 000 >10^4	very high (GROPUP D)

for human consumption, European Study Group for *Legionella* Infections (ESGLI) guidelines, World Health Organization (WHO) guidelines (Table 1), [4, 7, 8, 10, 15]. Currently, the 2017 Regulation on the quality of water intended for human consumption is in force in Poland [9].

The concentration of *L. pneumophila* in the water samples was determined by membrane filtration [17, 19]. The results were presented as the number of colony forming units (CFU) of *Legionella* per specific volume of water.

The materials for the study were water samples collected in 2009–2013 from 364 hospitals (which accounted for 46.56% of all hospitals in Poland) and 221 hospitals (29.69% of all hospitals in Poland) in 2014–2016 [23, 24].

The water samples were collected in both analyzed periods, from January to December, from the water supply systems in the hospitals. Table 2 shows the number of samples tested in 2009–2013, and Table 3. in 2014–2016). The number of hot water samples per hospital ranged from 3 to 205 in 2009–2013, and from 3 to 104 in 2014–2016. A total of 4.747 samples of hot water were collected in 2009–2013, while 2.765 samples in 2014–2016. The number of monitored hospitals were representative of all public hospitals in Poland.

Statistical analysis of the results was carried out with STATISTICA 7.1 (StatSoft, Poland). The Mann-Whitney U test was used to determine the differences between the analyzed groups. The level of statistical significance was set at $p \leq 0.05$.

RESULTS

Tables 2 and 3 show the number of tested hot water samples in 2009–2013 compared to 2014–2016 depending on the degree of water contamination. Between 2014 and 2016, there was a decrease in the percentage of the number of water samples in Group A (<100 CFU/100 ml). In groups B (>100 CFU/100 ml), C (>1000 CFU/100 ml), and D (>10 000 CFU/100 ml) an increase was observed in the percentage of the number of water samples, which was a negative phenomenon. The mean number of *L. pneumophila* colonies (CFU/100 ml), median, standard deviation (SD) and mean error (SE) in selected years and for the all analyzed periods

Table 2. Number of hot water samples tested in 2009–2013 according to accepted criteria of contamination

Year	Number of water samples	GROUP A number/ percentage of all water samples	GROUP B number/percentage of all water samples	GROUP C number/ percentage of all water samples	GROUP D number/ percentage of all water samples
2009	610	433 (71%)	108 (17.8%)	56(9.1%)	13(2.1%)
2010	985	827 (84%)	110 (11.1%)	43 (4.4%)	5 (0.5%)
2011	1052	920 (87.5%)	82 (7.8%)	47 (4.5%)	3 (0.2%)
2012	1642	1393 (84.9%)	160 (9.7%)	82 (5%)	7(0.4%)
2013	458	394 (86.0%)	55 (12.0%)	9 (2.0%)	0
Total samples in 2009–2013	4747	3967 (83.6%)	515 (10.8%)	237 (5%)	28 (0.6%)

Table 3. Number of hot water samples tested in 2014-2016 according to accepted criteria of contamination

Year	Number of water samples	GROUP A number/ percentage of all water samples	GROUP B number/percentage of all water samples	GROUP C number/ percentage of all water samples	GROUP D number/ percentage of all water samples
2014	832	542 (65.2%)	187 (22.5%)	85 (10.2%)	18 (2.1%)
2015	711	518 (72.9%)	142 (19.9%)	46 (6.5%)	5 (0.7%)
2016	1222	901 (73.7%)	172 (14.2%)	134 (10.9%)	15 (1.2%)
Total samples in 2009–2013	2765	1961 (71.0%)	501 (18.1%)	265 (9.6%)	38 (1.3%)

are presented in tables 4 and 5. Table 6 presents a comparison between the study periods using statistical analysis.

The mean number of *L. pneumophila* colonies in analyzed periods 2009–2013 and 2014–2016 were different. The comparison of the total mean number of *L. pneumophila* colonies in these periods, despite a significant decrease, is not statistically significant.

Between 2009 and 2013, the most virulent *L. pneumophila* serogroup 1 (SG 1) was detected in 3 hospitals (0.9%), and *L. pneumophila* SG 2-14 were isolated in 20 (5.5%). Between 2014 and 2016 *L. pneumophila* SG 1 and *L. pneumophila* SG 2-14 were found in 5 hospitals (2.2%) and 18 hospitals (8.1%), respectively (Fig. 1).

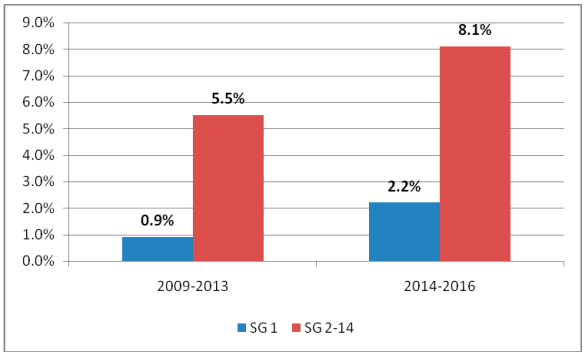

Fig. 1. Prevalence of *L. pneumophila* SG 1 and SG 2-14 in the analyzed hospitals

DISCUSSION

According to the European Legionnaires' Disease Surveillance Network (ELDSNet) report, a total of 8.624 confirmed cases of Legionnaires' disease were recorded in Europe in 2017. Similar to previous years, the majority of the cases reported in 2017 occurred in a community setting (69%); less often, the infections were associated with travel (21%), stay at healthcare facilities (8%) and other facilities (2%) [15]. Based on epidemiological data from the National Institute of Public Health/National Institute of Hygiene in Warsaw (published in the Infectious Disease and Poisoning in Poland bulletin), a total of 39 cases of legionellosis were registered in Poland in 2017; this number included 38 cases of Legionnaires' disease and one case of Pontiac fever. This corresponded to the incidence of 0.102 (per 100.000 population); this figure was nearly 50% higher than the incidence rate for 2016. Four cases were shown to be associated with contaminated drinking water supply systems in healthcare facilities, 33 were autochthonous, and six were related to travel abroad [25]. In Europe and the United States, legionellosis is most often diagnosed in patients hospitalized at intensive care units. Other hospital units associated with high risk of *Legionella* spp. infection include cardiology, hematooncology, hemodialysis, and pulmonology units. The hospital-acquired infections are often associated with bacterial contamination of tap water and medical devices generating a water-air aerosol [14].

An epidemiological analysis was carried out on the presence of *Legionella* in water distribution systems in hospitals in Poland. The conducted epidemiological analysis allowed us to assess the prevalence of *Legionella* spp. in water distribution systems in hospitals in Poland in the years 2009–2013 compared to the years 2014–2016. Studies have shown that the water supply systems in Polish hospitals are colonized with *L. pneumophila* at different levels. However, in 2014–2016 an upward trend was observed compared to the 2009–2013. Unfortunately, we did not have access to the information about the elimination measures undertaken in the hospitals in which *L. pneumophila* counts in hot water samples exceeded the normative value (>100 CFU/100 ml). The recommended corrective actions include maintenance of the temperature regime, removal of water retention from the installation, and thermal or chemical disinfection. Frequent renovations of hospitals and changes in hospital wards, in particular changing the ward profile (e.g. from treatment to conservative) contributes to leaving dead branches, the so-called „dead legs" where *Legionella* find good conditions to [11].

In many European countries the maximum allowable number of *L. pneumophila* in hot water has been defined as <100 CFU per 100 ml (<10^2 CFU/100 ml). According to the currently applicable in Poland Decree of the Minister of Health „on the quality of water intended for human consumption", microbiological requirements to be met by hot water is <100 CFU/100 ml in facilities performing medical activities such as stationary and round-the-clock health services and in collective residence buildings and in public utility buildings, in which water-air aerosol is produced during their use [9]. Furthermore, *L. pneumophila* contamination should not exceed 50 CFU/100 ml in entities performing inpatient and round-the-clock healthcare services for immunocompromised patients, including those undergoing immunosuppressive therapy [9]. The minimum frequency of hot water sampling and the procedures implemented depending on the result of bacteriological testing are specified in the Decree of the Minister of Health "on the quality of water intended for human consumption". In line with current regulations, water contaminated with *Legionella* can be classified as lack/minimal (<100 CFU/100 ml, <50/1000 ml), moderate (≥100 CFU/100 ml, ≥50/1000 ml), high (≥1000 CFU/100 ml, >100/1000 ml) and very high (>10 000 CFU/100ml, ≥1000/1000 ml) [9].

Based on available data, sporadic infections may be observed when the number of *L. pneumophila* in water approximates 103–105 CFU/1000 ml and an outbreak legionellosis is likely to occur if the bacterial count exceeds 105 CFU/1000 ml. The main source of *L. pneumophila* infections in hospitals and other healthcare facilities is the presence of this pathogen in water pipes, taps, showers and medical devices supplied with water [13].

Research by other authors confirm that *Legionella* spp. colonizes water distribution systems in 12–70% of hospitals. The risk of infection is high if the contamination of *L. pneumophila* water is > 100 CFU/100 ml [1, 5].

Table 4. Mean number of *L. pneumophila* colonies isolated from hot water samples in 2009–2013

Year	Number of water samples	Mean number of *L. pneumophila* colonies (CFU/100 ml)	Median	Standard deviation (SD)	Standard error (SE)
2009	610	7134	150	481	525
2010	985	1316	98	346	350
2011	1052	921	60	263	264
2012	1642	3165	79	195	157
2013	458	333	25	120	170
Values for the period 2009–2013	Total: 4747	2574	77	119	102

Table 5. Mean number of *L. pneumophila* colonies isolated from hot water samples in 2014–2016

Year	Number of water samples	Mean number of *L. pneumophila* colonies (CFU/100 ml)	Median	Standard deviation (SD)	Standard error (SE)
2014	832	1611	79	405	359
2015	711	859	110	277	263
2016	1222	2153	210	449	352
Values for the period 2014–2016	Total: 2765	1541	118	381	192

Table 6. Comparison of the mean number of *L. pneumophila* colonies (CFU) isolated from water samples in 2009–2013 and 2014–2016

Year	Total number of water samples	Mean number of *L. pneumophila* CFU	Median	Standard deviation (SD)	Standard error (SE)
2009-2013	4747	2574	77	119	102
2014–2016	2765	1541	118	381	192

1–2: Z = 1,71; p = 0,081; Z– value of the Mann-Whitney U test

Sabrià et al. found *L. pneumophila* (SG 1, SG 2-14 or both) in 73 water samples taken in 17 out of 20 examined hospitals. The level of contamination ranged from 200 CFU/L to 74.250 CFU/L. The most virulent *L. pneumophila* SG 1 were found in 17 out of 20 hospitals (85%). The prevalence of *L. pneumophila* SG 2-14 in hospital water supply and distribution systems was estimated at 70% [21].

According to Yu et al. *L. pneumophila* colonized hot water distribution systems in 10 out to 16 examined hospitals (63%). *L. pneumophila* SG 1 was isolated from 80% of the hospitals. No cases of hospital-acquired legionellosis were reported by the authors [27].

Orsi et al. analyzed the results of a 5-year monitoring program of drinking water systems in Italian hospitals. *Legionella* spp. were found in 10^2 (9.8%) water samples from the systems in which no filters were used (*L. pneumophila* SG 1 – 17.6%, *L. pneumophila* SG 2-14 – 28.4%, non-*L. pneumophila* 53,9%) and in none of 267 samples from the systems equipped with filters. *Legionella* spp. were detected in 23 out of 38 hospitals; in 29 (28.4%) hospitals, *L. pneumophila* counts in samples taken from internal drinking water installation exceeded 103 CFU/L. In 32.3% of the positive samples, the number of bacteria varied between 10^2 CFU/L to 103 CFU/L. Based on the serological analysis, 17.6% of the isolates were *L. pneumophila* SG 1, 28.4% represented *L. pneumophila* SG 2-14 and 53.9% belonged to other *Legionella* species (including one *Legionella* anisa isolate) [16].

The distribution of *L. pneumophila* serogroups in our present study was similar to the one described above, with *L. pneumophila* SG 2-14 isolated more often than *L. pneumophila* SG 1.

CONCLUSION

A retrospective epidemiological analysis showed a greater contamination of the water network in analyzed groups with *Legionella* spp. in 2014–2016 as compared to 2009–2013. Nevertheless, colonization rates documented in this study legitimize constant monitoring of water distribution systems, especially in hospital setting, to minimize the potential risk of nosocomial legionellosis.

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The authors have no potential conflicts of interest to declare.