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e-ISSN 2544-1620

Medical Science Pulse 2019 (13) 4

Original papers

DOI: 10.5604/01.3001.0013.7441

OCCURRENCE OF *LEGIONELLA* SPP. IN POLISH HOTELS BETWEEN 2009-2013 AND 2014–2016: A COMPARATIVE STUDY

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A – study design, **B** – data collection, **C** – statistical analysis, **D** – interpretation of data, **E** – manuscript preparation, **F** – literature review, **G** – sourcing of funding

ABSTRACT

Background: *Legionella* spp. have been found in both natural and manmade water systems, coastal waters, thermal waters, moist soils, clays, and near wastewater discharge sites.

Aim of the study: This study aims to report a comparative, retrospective epidemiological analysis of water testing study results indicating the presence of *Legionella* spp. in Poland between 2009-2013 and 2014-2016. This study is a comparative retrospective epidemiological analysis of individual tests for the presence of *Legionella* spp. in hotels between two different time periods. The two time periods being compared in this study are between 2009-2013 (n=369 hotels) and 2014-2016 (n=174 hotels). Reporting has been performed in accordance with the STROBE checklist.

Material and methods: Hot water samples were collected by employees of the Sanitary and Epidemiological Station in Poland as part of routine water quality monitoring. Samples were taken from the water supply systems of 369 hotels, representing 19.59% of total hotels in Poland, from January 2009 until December 2013. Samples were taken from the water supply systems of 174 hotels, representing 7.51% of total hotels in Poland, from January 2014 until December 2016.

Results: The percentage of facilities classified as Group I remained comparable for the period between 2009 and 2013 compared to the period between 2014 and 2016. The percentage of facilities classified as Group II, however, showed a clear upward trend between 2014 and 2016 compared to the time period between 2009 and 2013. The percentage of facilities classified as Group III showed a clear downward trend between 2014 and 2016 compared to the time period between 2009 and 2013.

Conclusions: The colonization rates observed in this comparative study indicate that the aquatic environment in these facilities requires constant monitoring to lower the risk of legionellosis. The increased colonization of hotel water networks with *Legionella* spp. is a serious health problem that requires constant monitoring.

KEYWORDS: Legionnaires' disease, *Legionella pneumophila*, water

Background

Legionella species are naturally found in aquatic environments and these environments serve as the reservoir from which the bacteria spread. *Legionella* spp. inhabit manmade utility systems that carry both hot and cold water. *Legionella* spp. are found in natural and artificial water systems, groundwater, coastal seawaters, thermal waters, moist soils and clays, and near wastewater discharge sites. The optimal temperature range for bacterial growth is between 32°C and 42°C. This temperature range likely affects the bacteria's ability to multiply within protozoan cells. The presence of

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protozoa in natural aquatic environments promotes *Legionella* growth and persistence by providing a host [1,2]. *Legionella* spp. enter water supplies and distribution systems through water treatment plants. Once inside, bacteria can rapidly multiply inside water supply and distribution systems. Artificial reservoirs for *Legionella* spp. also include water massage equipment like pearl pools, water heating and cooling circuits associated with air conditioning equipment, refrigeration towers, steam condensers, and medical apparatuses like respiratory devices, nebulizers, inhalers, dialysis equipment, and dental turbines. *Legionella* spp. can inhabit moisturizing equipment, fountains, sprinklers, car washes, moisturizing systems and other equipment with the ability to produce aerosols less than 5 μm in diameter [3].

Legionella spp. can also be transmitted through the oil-water mist generated by installation of compressed air. Modern swimming pools that are part of recreational complexes are a great threat to public health if they contain *Legionella* spp. Many recreational complexes create additional implements to attract consumers, and these can harbor *Legionella* spp. Geysers, water whips, underwater massage systems, cascades, artificial rivers, slides, whirlpool baths, and saunas are all potential reservoirs for *Legionella* spp. Currently, most hotels have a recreation area equipped with such facilities [4–6].

The implementing act for the Law on collective water supply and collective wastewater disposal of 7 June 2001 is a Regulation of the Minister of Health regulating the quality of water intended for human consumption. This act implements Directive 98/83 EC of 3 November 1998 (OJ L 330 of 5.12.1998) concerning the quality of water intended for human consumption. The act requires the Member States of the European Union to report every three years on the quality of water intended for human consumption. The Regulation also specified that hot water meet a series of requirements concerning the presence of microbiological organisms. Due to the epidemiological threat of Legionnaires' disease and the increase in cases of the disease, the Regulation requires that hot water be tested for the presence of *Legionella* spp. Aerosol water-air droplets between 2.0 to 5.0 μm in diameter are the main sources of transmitted *Legionella* infection for humans. Droplets containing invasive forms of *Legionella* bacteria are aspirated into the human respiratory system, causing infection [7,8].

Aim of the study

The aim of this study was a comparative retrospective epidemiological analysis of water testing results wherein water samples from hotels were tested for the presence of *Legionella* spp. between two different time periods in Poland. The samples were collected and testing as part of routine hotel water monitoring, and collected and tested between 2009 and 2013 and between 2014 and 2016. The testing was performed in by Poland's State Sanitary Inspectorate. The study findings were reported according to the STROBE checklist.

Material and methods

Study design and sampling

Hot water samples were collected by employees of the local competent Sanitary and Epidemiological Station in Poland as part of routine water quality monitoring. The authors of this paper thank the State Sanitary Inspectorate for making the results of this research available. Arbitrary distribution of the research results was adopted according to the following guidelines. The examined hotels were arbitrarily divided into three groups:

- group I - where a negative result was obtained from each of the hot water samples taken (samples with <100 CFU/100 ml);
- group II - where from the hot water samples taken results were obtained once positive and negative (in which values >100 CFU/100 ml as well as <100 CFU/100 ml were found);
- group III - where a positive result was obtained each time from hot water samples (containing an abnormal number of Legionella spp. in which >100 CFU/100ml was found).

Date collection

Samples were collected from the water supply systems of 369 hotels, representing 19.59% of total hotels in Poland, between January 2009 and December 2013. During the second time period assessed in this study, samples were collected from the water supply systems of 174 hotels, representing 7.51% of hotels in Poland, between January 2014 and December 2016 [9,10]. The number of objects surveyed is a representative sample for objects of this type in Poland.

Standards

All testing was performed by the Sanitary Inspection laboratories. In the Sanitary Inspection laboratories, samples were tested in accordance with the applicable standards and legal acts enforced at the time of the study. Specifically, samples were tested in accordance with: 1) PN-EN ISO 11731-2:2008 Water quality. Detection and enumeration of legionella – Part 2: Part Direct membrane filtration for waters with low bacterial counts 2) PN-ISO11731 December 2002 Water quality. Detection and enumeration of the genus *Legionella* 3) PN-EN ISO19458:2007 Water quality. Sampling for microbiological analyses 4) Regulation of the Minister of Health of 29 March 2007 on the quality of water intended for human consumption (OJ No 61, item 417 as amended) 5) Regulation of the Minister of Health of 13 November 2015 on the quality of water intended for human consumption (OJ 2015, item 1989) 6) Regulation of the Minister of Health of 7 December 2017

on the quality of water intended for human consumption (OJ 2017, item 2294).

Statistical analysis

The results of water testing for the occurrence of *Legionella* spp. was compared between the two different time periods when testing was performed, between 2014 and 2016 and 2009 and 2013. The results were compared and the statistical differences were analyzed. Statistical analysis was carried out using STATISTICA 7.1. The Pearson's Chi square independence test was applied using $p < 0.05$ as the level of significance.

Results

Legionella spp. risk assessment in the studied objects

The sample results were categorized into groups indicated whether they were positive, negative or positive and negative for the presence of *Legionella* spp., as illustrated in Fig. 1. When the tested samples collected between 2014-2016 were compared to the samples collected between 2009-2013, the percentage of samples classified as Group I (negative) were the same, or comparable [11]. The percentage of samples in Group II (negative and positive) showed a clear upward trend. In contrast, the percentage of samples in Group III (positive) showed a clear downward trend. These data show that it is unclear whether the applied methods in place to eradicate *Legionella* spp. from hotel drinking water are sufficient.

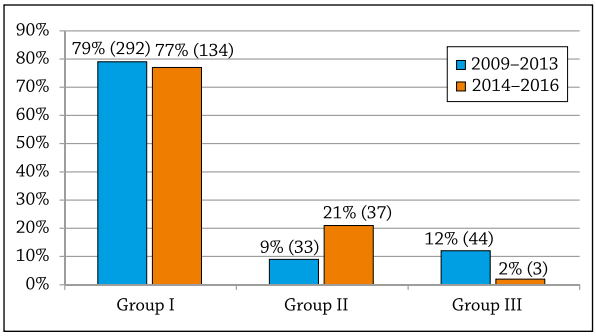

Figure 1. *Legionella* spp. risk assessment in hotels between 2009 and 2013 compared to 2014 and 2016.

The average number of *Legionella* spp. colonies in water samples collected between 2014 and 2016 was significantly higher than water samples collected between 2009 and 2013 (Tab. 1).

Discussion

Factors most likely to affect the occurrence and persistence of *Legionella* spp. in hot water systems include the physiochemical qualities of the water and the maintenance and operation of the colonized object. For example, colonization can be impacted if an artificial water system is used seasonally, if sediment is allowed to accumulate within the system, and if water is stagnant inside the system. Colonization can also be affected by failure of the system to meet temperature requirements that minimize colonization.

Hotels are facilities where travelers stay on a temporary basis. Stress incurred by travelers throughout their journey can result in compromised immune systems in travelers, resulting in increased susceptibility to infections like those caused by *Legionella* spp. [12]. The results of this study indicate that Polish hotels are facilities in which upward trends in *Legionella* occurrence have been observed. This may main that the methods in place to eradicate bacteria from the hotel water system is not functioning properly, or is insufficient. Other studies have indicated that hotel water systems may be reservoirs for *Legionella* spp.

Szczepanek et al. conducted a study testing hot water installation systems in 228 facilities that provide hotel services in Świętokrzyskie voivodeship. A large amount of *Legionella* spp. was found in 19.2% of the total number of samples taken, indicating that bacteria were present in the water sampled from 44 hotels [13]. Hotels outside Poland have also been implicated as reservoirs for *Legionella* spp. Research conducted by Kyritsi et al. in 51 Greek hotels showed the presence of *Legionella* spp. in 38 hotels (75% total samples) [14]. Özen et al. conducted research in 56 hotels located in the tourist region of Antalya, Turkey, and found *Legionella* spp. in 10% of the samples analyzed, indicating the presence of bacteria in the water of 6 hotels [15]. Fragoua et al. investigated 9 hotels in Patras, Greece. The hotels selected for the study had between 50 and 100 rooms. The colonization of the water network with *Legionella* spp. was detected in 5 hotels (55.5% of samples) [16]. Studies by other Greek authors, conducted in 67 hotels with a total of 518 hot water samples taken, showed colonization of the water networks in 43 hotels (64.1% of samples), representing 35.8% (181) of the analyzed hot water samples [17]. De Filippis et al. analyzed 160 water samples from 36 recreational facilities with swimming pools. 10 hotels (57.1% of samples) and 7 sports centers (41.2% of samples) were the most contaminated with *L. pneumophila* [18].

Table 1. Comparing the amount of *Legionella* spp. detected in hotel water samples collected between 2009 and 2013 to samples collected between 2014 and 2016.

Research period	Number of hotels	Average number of colonies	Standard deviation (SD)	Standard error (SE)
2009-2013[1]	369 (19.56% hotels in Poland)	576.39[1]	3899.05	180.04
2014-2016[1]	174 (7.51% hotels in Poland)	932.60[1]	3043.61	207.09

[1] Z=2.12; p=0.0337*

Studies carried out by Napoli et al. in 305 Italian hotels detected bacteria in as many as 66.9% (204) of hotels. A total of 5009 water samples were taken and *Legionella* spp. were detected at 36.5% (1828 samples). Among the positive samples containing *Legionella* spp., bacteria within the range of 100-1000 CFU/L were detected in 30.6% of samples, or 1533 total samples. Bacteria within the ranges of 1000-10,000 CFU/L were detected in 44.6% of samples, or 2234 total samples. Bacteria present at over 10,000 CFU/L were detected in 24.8% of samples, or 1242 total samples [19]. Rakić et al. analyzed 304 hot water samples from 3 hotels in southern Croatia. This study showed the presence of *Legionella* spp. in 20.3% of hotels, representing 62 hotels. The *Legionella* bacteria were present in the range of 500-13,000 CFU/L in these samples [20].

The choice of an appropriate *Legionella* spp. eradication method should be adopted by those maintaining hotel water networks. Future maintenance and the subsequent consequences of wear over time should be anticipating by those maintaining the hotel water networks. These future considerations include increased corrosion and sediment precipitation. There are two main methods of water disinfection. Water disinfection can refer to disinfection during installation and disinfection of the installed equipment. In addition, there is chemical disinfection and physical disinfection. The chemical methods most commonly used in routine disinfection include the use of chlorine and chlorinated compounds. According to *Wanot and Krzypkowska,* chlorination should be used when other methods have failed. The effectiveness of this method depends on pH, temperature, the amount of organic compounds in the water, and the thickness of bacterial biofilms [21]. During the application of this method, halogen compounds may be formed, which pose a threat to human health [22, 23]. One of the procedures based on this method is shock hyperchlorination. Shock hyperchlorination consists of using chlorine compounds in concentrations that result in a concentration of free chlorine that is 10 mg/L. Disinfection time should consist of a two hour period at 30°C. Higher temperatures cause evaporation of chlorine. Finally, the system should be rinsed until free chlorine levels are between 0.1-0.3 mg/L at a pH of between 7.6-8.3.

Limitations of this study

This study is a comparative retrospective epidemiological analysis. This study does not account for physiochemical properties of the water sampled.

Conclusions

The major conclusion of this study were that the observed colonization rates indicate that constant monitoring of the aquatic environment is necessary to prevent the potential risk of legionellosis. The colonization of hotel water networks with *Legionella* spp. is becoming more and more common. This is a serious health problem that requires constant monitoring.

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Word count: 2072 • Tables: 1 • Figures: 1 • References: 23

Sources of funding:
The research was funded by the authors.

Conflicts of interests:
The authors report that there were no conflicts of interest.

Cite this article as:
Gładysz I, Sikora A, Wójtowicz-Bobin M, Karczewska ZM, Karczewski J.
Occurrence of *Legionella* spp. in Polish hotels between 2009-2013 and 2014-2016: a comparative study.
MSP 2019; 13, 4: 27–31.

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Received: 5.09.2019
Reviewed: 18.12.2019
Accepted: 30.12.2019