Temperature as a determining factor in the occurrence of Legionella sp. bacteria in hot water systems in hospitals and social welfare homes

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▌Abstract

Introduction and Objective. Water in installations in hospitals and social welfare homes (SWHs) should meet the requirements of the Directive of the European Parliament and of the Council on the quality of water intended for human consumption and national regulations issued on its basis. At present, 60 species of bacteria of the genus Legionella sp. are known (of which 30 are considered as pathogenic for humans), and more than 80 serologic groups. The aim of the study was determination of the effect of temperature on contamination of hot water systems with Legionella sp. bacteria in buildings of hospitals and SWHs in the Kielce Province, Poland, based on measurement of the temperature of domestic hot water during the period 2014–2018.
Materials and Method. 631 results of temperature measurements and examinations of the quality of hot water were analyzed for the presence of the occurrence of Legionella sp. bacteria, conducted in 30 buildings of hospitals and 32 buildings of social welfare homes.
Results. From among 581 samples, only 8.95% (n=52) had a temperature of ≥55 °C. The mean temperature of water in the installations of buildings, calculated based on measurements carried out while collecting samples for the study, was 46 °C in 2014, and up to 47 °C in 2018. The allowable limit of bacteria was exceeded in 13.04% (n=69) of the samples at the temperature < 55 °C, and in 3.85% (n=2) of the samples in which the temperature was higher or equal to 55 °C.
Conclusions. Water temperature ≥55 °C significantly reduced the occurrence of excessive contamination of samples with Legionella sp. bacteria. Proper temperature of domestic water in water systems in hospitals and SWHs is indispensable for the provision of health safety of the patients.

▌Key words

temperature, hospitals, Legionella pneumophila, social welfare homes

INTRODUCTION

Water in installations of hospitals and social welfare homes (SWHs) should meet the requirements issued in the Directive of the European Parliament and of the Council on the quality of water intended for human consumption, and national regulations based on the directive. In the Polish legal system, health issues concerning water are governed by the regulation of the Minister of Health of 7 December 2017 on the quality of water intended for human consumption. According to this regulation, water is safe for use if it is free from pathogenic microorganisms and parasites in numbers posing a potential threat to human health. In addition, domestic hot water should contain less than 100 cfu of *Legionella* sp. bacteria in samples of the volume of 100 ml collected from installations in public buildings, such as hospitals and SWHs. More stringent standards apply to samples collected in hospital buildings where immunocompromised patients stay, including those under immunosuppressive treatment, where the number of bacteria must be lower than 50 cfu/1000 ml [1].

Legionella bacteria are Gram-negative, aerobic, flagellated, non-spore-forming bacilli of the size 0.3–0.9 x 2.0–4.0 μm. At present, 60 species of bacteria of the genus *Legionella* sp. are known (of which 30 are considered as pathogenic for humans), and more than 80 serologic groups [2]. *Legionella* bacteria are commonly found in natural and artificial water reservoirs. In conducive conditions they colonize swimming pools, fountains, air conditioners, cooling towers, hydromassage bathtubs, dental units, inhalers, and hot water installations in buildings [3]. Legionella was recovered from samples of well water, tap water from rural dwellings, tap water from urban dwellings, and water from medical appliances – with the isolation frequency of 27.8–50.0%, and the low concentrations ranging from 0.7–13.3 × 101

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cfu/l. Strains of the Legionella pneumophila types 2–14 predominated, forming 89.9% of total Legionella isolates, while other species of Legionella formed 10.1%. Study of tap water and showerhead samples from hospitals isolated Legionella from 67% of the water samples. The most common strains were Legionella pneumophila types 2–14, accounting for 74.6% of all Legionella isolates. Legionella pneumophila type 1 strains accounted for 13.5% of the total, while other Legionella species (referred to as Legionella spp.) accounted for 11.9% of the total. Legionella concentrations in positive water samples ranged from 3–350 cfu/100 ml, which the authors assessed as posing a moderate health risk [4, 5].

Infection of the human body occurs through aspiration into the lungs of water aerosol contaminated with bacteria. Droplets with a diameter of 2–5 μm are considered particularly dangerous. The pathogen causes 3 types of legionellosis which differ by strength and intensity of symptoms: pulmonary (legionnaires' disease), non-pulmonary (Pontiac fever), and a severe non-pulmonary form. In legionnaires' disease, mortality is 8–10% and depends on age, state of health, and life style [6].

According to the latest report by the European Centre for Disease Prevention and Control (ECDC), in 2021 in the European Union and in Iceland, Norway and Liechtenstein, 10,723 cases of legionellosis were registered (2.4 per 100,000 population). In Poland, the morbidity rate was only 0.1 per 100,000 population (47 cases). According to preliminary data in 2023, in Poland, as many as 438 cases of legionellosis were registered (morbidity rate 1.2), of which 165 cases were noted in Rzeszów at the turn of August and September during an epidemic of unknown source [7–9].

Legionella sp. bacteria have high adaptability to changing environmental conditions. Factors exerting an effect on the presence, survival and titre of bacteria are: pH, dissolved oxygen content, cations (Fe, Zn, K, Mn, Cu, Ag, Na), and the presence of protozoa and biofilm. Temperature is considered as the main factor conditioning the presence of the pathogen in the water environment. The bacilli were isolated from water at temperatures between 5.7–63 °C. At a temperature of 70 °C, they can only survive for a few seconds, proliferate within a wide range of temperatures from 25–45 °C, with the optimum temperature for growth being 35 ± 2 °C. The temperature at which *Legionella sp.* grow affects their capability to cause the disease. Most pathogenic are bacterial cells growing at temperatures from 37–42 °C. *Legionella pneumophila serogroup 1* bacteria isolated from domestic water systems are able to survive up to 6 months at the temperature of 30 °C, whereas at the temperature of 4 °C, the period of survival is 17 days [10–13].

Elevation of water temperature in installation up to at least 70 °C, leads to the killing of bacteria by denaturing proteins and ribosomes, and damage to DNA structure, which is used in thermal disinfection of hot water systems [14]. The disinfection process takes place in 2 stages. The first step is to raise the temperature of the water above 70 °C, closing all draw-off points and turning on water circulation, and lasts until stabilization of temperature on the return to the heating place (furnace, heat exchanger). In the case of high contamination, the process should last even up to 3 days. At the second step, the draw-off points are opened and rinsed one by one for the period from 5–30 minutes [15–17]. When rinsing the system, it is necessary to check whether water temperature at points furthest from the heater is not less than 60–65 °C. Using too low temperatures and short rinsing times may contribute to the selection of thermophilic strains of Legionella sp. bacteria, and the emergence of adaptive mechanisms, which will make it difficult to use the thermal method again in the future [18, 19].

OBJECTIVE

The aim of the study was determination of the effect of temperature on contamination of hot water system with *Legionella* sp. bacteria in building of hospitals and SWHs in the Kielce province of Poland, based on measurements of the temperature of domestic hot water during 2014–2018.

MATERIALS AND METHOD

A total of 631 results of measurements of temperature and examinations of the quality of hot water for the presence of *Legionella* sp. bacteria were analyzed, carried out by employees of the National Sanitary Inspectorate in 30 buildings in hospitals and 32 buildings in the SWHs in the Kielce Province. In addition, in 2018, the results of measurements and studies conducted were analyzed in 16 hospital buildings where immunocompromised patients were staying, including those under immunosuppressive treatment. The temperature was measured while collecting water samples using calibrated electronic thermometers. Based on the instruction '*Collection of water samples for detection and determination of Legionella sp. bacilli*' the temperature was measured in the water stream approximately 2–3 minutes after opening the tap. In order to determine contamination with *Legionella sp.* bacteria, hot water samples were collected into sterile glass bottles of the volume of 1,000 ml., and subsequently, based on methodology specified in the standard PN-EN ISO 11731–2:2008 or PN-EN ISO 11731, examined by the membrane filtration method. Samples of the volume of 1 ml, 10 ml, 100 ml and 500 ml were inoculated through cellulose filters with the pore size of 0.45 μm. The accompanying microflora was removed by flooding the filters for 5–10 minutes with buffer with pH 2.2, and rinsing with Ringer's solution (dilution 1:40). Filters were placed on buffered charcoal yeast extract (BCYE) agar plates with the addition of glycine, vancomycin, polymyxin B and cycloheximide (GVPC). The cultivation was carried out at 36±2 °C in a humid atmosphere for 7–10 days, observing the growth of the colony every day (colonies that grew in the first 2 days were considered as the accompanying microflora). Confirmatory testing was performed using BCYE with cysteine (growth of *Legionella* bacteria), and BCYE without cysteine (lack of growth of *Legionella* bacteria).

The temperature of 55 °C was adopted as the limit value between proper and improper temperature considering the risk of an excessive occurrence of *Legionella* sp. bacteria, and in accordance with applicable law. Based on the Regulation by the Minister of Health of 7 December 2017 on the quality of water intended for human consumption, samples of the volume of 100 ml containing ≥100 cfu, and samples of the volume of 1000 ml containing ≥50 cfu were considered as contaminated with *Legionella* bacteria.

Using a questionnaire constructed for the purpose of the study and using the method of a diagnostic survey, the temperature of hot water in the installations of the buildings,

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according to managers of the facilities, was examined. Independence of the qualitative variables was investigated using the non-parametric χ² test. Relationships between temperature of the sample and the determined number of *Legionella sp.* bacteria was investigated by means of the Pearson correlation coefficient. The differences in water temperature between individual facilities (hospitals and SWHs) were evaluated using parametric Student's t-test, or non-parametric Mann-Whitney U test.

The results of measurements and examinations were collected in a database prepared in the software EXCEL included in the Microsoft Office suite 2021. Calculations were performed using statistical package STATISTICA (v.13). The p values p<0.05 were considered statistically significant.

RESULTS

Measurements of water temperature were performed routinely while collecting samples. From among 581 samples, only 8.95% (n=52) had a temperature of ≥55 °C. Proper water temperature was observed significantly more often (p=0.014) in installations of hospital buildings, compared to SWHs (Tab.1).

Detailed data for individual buildings in hospitals and SWHs illustrate the problem of improper water temperature in installations (Fig. 1). In the majority of water sampling sites, the water temperature was lower than 55 °C (this problem also concerns averaged water temperature in installations). The minimum temperature of water allowable in accordance with legal regulations (55 °C) was marked on charts with a
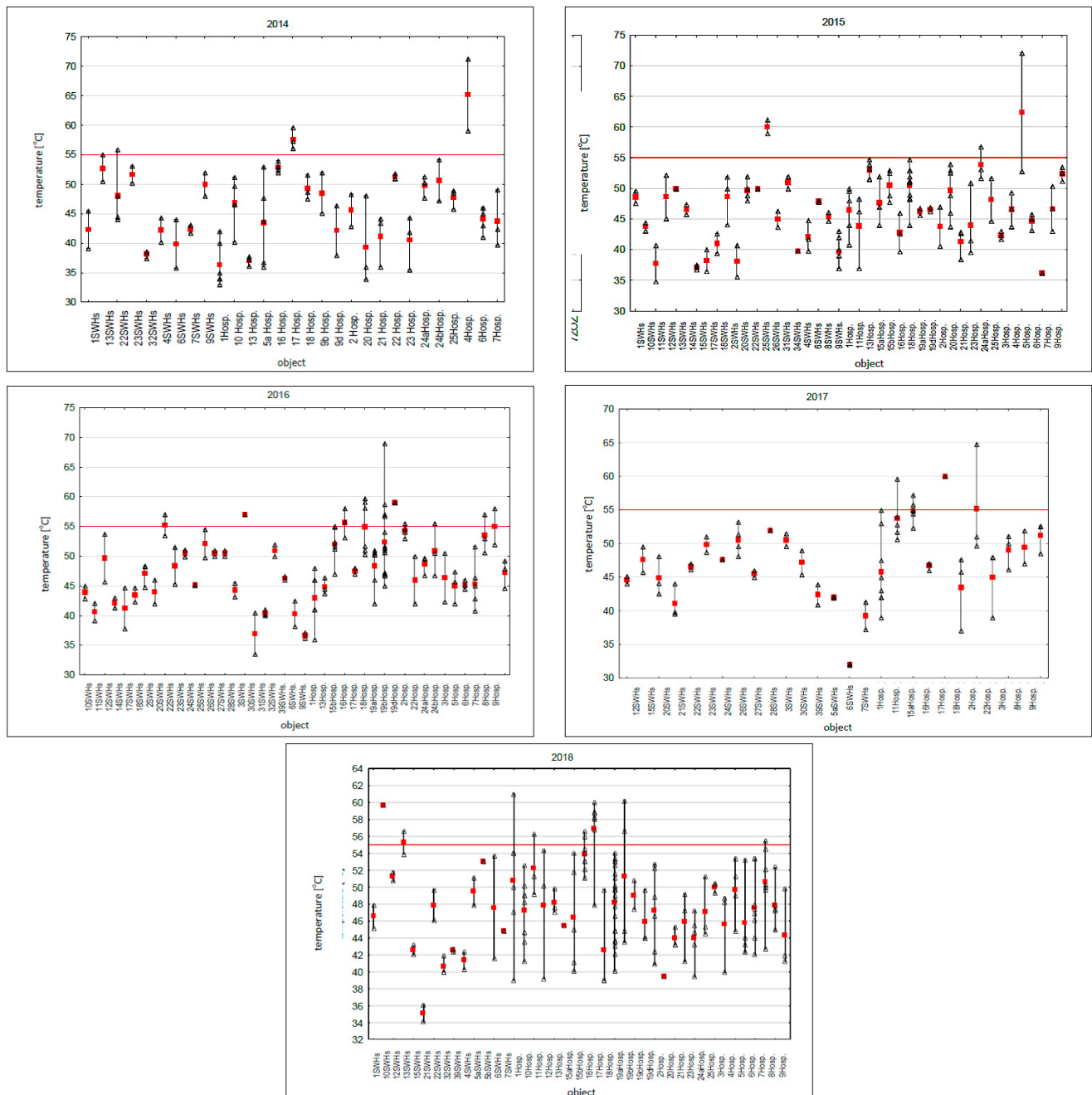

Figure 1. Temperature of water measured while collecting samples in hospital buildings s and SWHs during 2014–2018

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Table 1. Temperature of water samples collected from hot water systems in hospitals and SWHs

Sample temperature	Hospitals		SWHs		Total	
	n	%	n	%	n	%
< 55 °C	361	89.14	168	95.45	529	91.05
≥ 55 °C	44	10.26	8	4.55	52	8.95
Total	405	100.00	176	100.00	581	100.00
	test: chi-square; p=0.014*					

n – sub-group size; SWH – social welfare home; χ² – chi-square test; % – percentage; p – level of significance; *p<α, °C – degree Celsius

horizontal red line. In some facilities, the measured water temperature was below 40 °C which exerted a negative effect on the health safety and comfort of persons using taps and showers.

The mean water temperature in installations of the buildings (Tab. 2), calculated based on the results of measurements performed while collecting samples for the study, was from 46 °C in 2014 (M:46.24; SD±6.71; Me:45.60) up to 47 °C in 2018 (M:47.36; SD±4.73; Me:47.41). For individual buildings, the mean water temperature remained within the range 32.00 °C – 65.20 °C. In all analyzed years, the mean water temperature was higher in hospital buildings, compared to SWHs. In 2016 and 2017, these differences were statistically significant (p=0.019; p=0.016). In 2014–2018, in both groups of facilities, the mean temperature of domestic hot water increased (Fig. 2 and 3); however, it still differed from the minimum required by law (red horizontal line).

The percentage of facilities in which the temperature of water in installations in individual years was compliant with applicable standards was from 4.65% (n=2) in 2015, up to 13.95% (n=6) in 2016. In the group of buildings belonging to SWHs in 2 years (2014 and 2017) the correct temperature
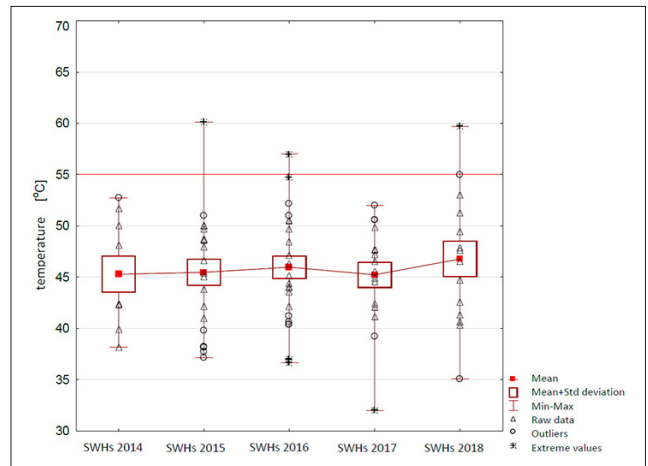

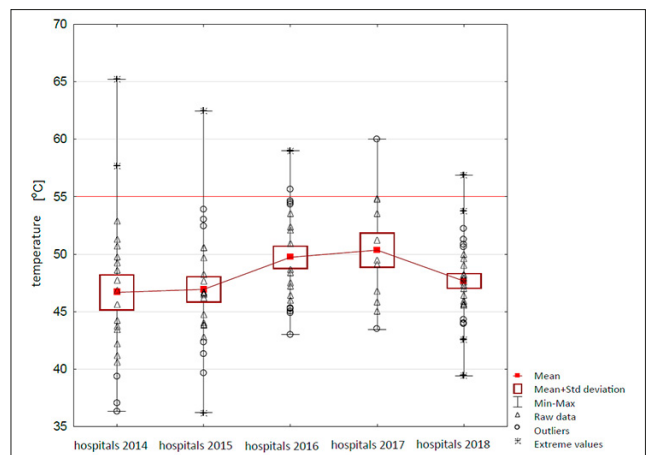**Figure 2.** Mean temperature of water in SWHs buildings during 2014–2018

Figure 3. Mean temperature of water in hospital buildings during 2014–2018

Table 2. Descriptive statistics of water temperature in installations of buildings in hospitals and SWHs during 2014–2018

Year	Parameter	Hospitals	SWHs	Total	test	p
2014	n	20	9	29	t	0.600
	M (SD)	46.67 (6.98)	45.28 (5.39)	46.24 (6.47)		
	Me (IQR)	46.22 (41.70 – 50.23)	42.40 (42.25 – 50.00)	45.60 (42.20–50.00)		
	Range	36.33 – 65.20	38.17 – 52.75	36.33 – 65.20		
2015	n	23	20	43	t	0.403
	M (SD)	46.93 (5.49)	45.47 (5.81)	46.25 (5.62)		
	Me (IQR)	46.55 (43.80 – 50.52)	46.00 (40.40 – 49.18)	46.55 (42.33 – 49.70)		
	Range	36.20 – 62.45	37.13 – 60.10	36.20 – 62.45)		
2016	n	20	23	43	t	**0.019***
	M (SD)	49.72 (4.51)	45.97 (5.46)	47.72 (5.33)		
	Me (IQR)	48.50 (45.67 – 53.92)	45.15 (41.25 – 50.50)	47.23 (44.00 – 52.03)		
	Range	43.00 – 59.00	36.65 – 57.00	36.65 – 59.00		
2017	n	11	16	27	t	**0.016***
	M (SD)	50.35 (5.06)	45.23 (5.08)	47.5 (5.07)		
	Me (IQR)	49.45 (45.81 – 54.76)	46.03 (42.20 – 48.75)	47.4 (44.9 – 50.57)		
	Range	43.46 – 60.00	32.00 – 52.00	32.00 – 60.00		
2018	n	28	14	42	MWU	0.647
	M (SD)	47.66 (3.55)	46.78 (6.62)	47.36 (4.73)		
	Me (IQR)	47.41 (45.64 – 49.79)	47.05 (41.3 – 51.25)	47.41 (44.70 – 49.97)		
	Range	39.40 – 56.87	35.10 – 59.70	35.1 – 59.70		

n – size of subgroup; SWH – social welfare home;; p – level of significance;*p<α; M- mean; SD – standard deviation; Me – median; IQR – interquartile range; t – Student's t-test; UMW – Mann–Whitney U test

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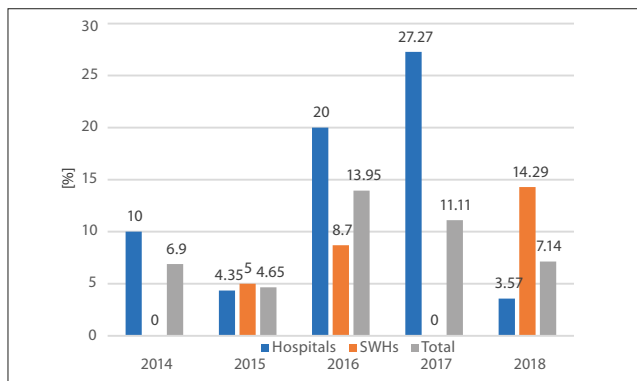
Figure 4. Percentage of facilities in which the mean hot water temperature was compliant with applicable standards (< 55 °C)

inhibiting the multiplication of *Legionella sp.* bacteria was not recorded in any of the buildings (Fig. 4).

According to the managers' statements, 20.00% of hospitals (n=6) and 24.24% of SWHs (n=8) were equipped in installation providing proper water temperature. In 13.33% of hospitals (n=4) and 6.06% of SWHs (n=2) the temperature of water in installation remained within the range 30–40 °C, whereas in 3.33% of hospitals (n=1) and in 3.06% of SWHs (n=1) the temperature was only 20–30 °C.

Analysis of temperature measured while collecting samples in hospitals and SWHs and which were declared in the questionnaire, showed statistically significant differences (p=0.004). According to the questionnaire, the proper temperature was maintained in 22.22% of buildings (n=14); the measurements of temperature (the last measurement was taken into account) showed the correct value in only 4.76% of buildings (n=3) (Tab. 3).

Table 3. Comparison of declared water temperature and temperature measured in hospital buildings s and SWHs

Water temperature	Measured		Declared	
	n	%	n	%
< 55°C	60	95.24	49	77.78
≥ 55°C	3	4.76	14	22.22
Total	63	100.00	63	100.00
test: chi-square; p=**0.004***				

n – size of subgroup; % – percentage; p – level of significance; *p<α; °C – degree Celsius

In hospitals where immunocomprised patients stayed, including those under immunosuppressive treatment, the measurements of temperature were performed only in 2018 while collecting samples of the volume of 1,000 ml. The temperature of measured water samples remained within the range 44.66–52.25 °C. The mean temperature of domestic hot water in installations of hospital buildings was 48 °C (*M*:48.46; SD±2.35; Me:48.59; IQR: 46.52–50.37). In all hospital buildings (n=16), the mean hot water temperature measured was lower than 55 °C. This value differed from the value declared by managers of the facilities. According to the data obtained based on the questionnaire, in 18.75% of buildings (n=3) the water temperature was above 55 °C.

The percentage of samples in which the observed number of *Legionella sp.* bacilli exceeded the standard significantly differed according to water temperature measured while collecting samples (p=0.028). The allowable limit of

Table 4. Relationship between temperature of water samples collected during 2014 – 2018 in hospital buildings and SWHs, and contamination with *Legionella sp.* bacteria

Contamination	< 55°C		≥ 55°C		Total	
	n	%	n	%	n	%
No	460	86.96	50	96.15	510	87.78
Yes	69	13.04	2	3.85	71	12.22
Total	529	100.00	52	100.00	581	100.00
test: chi-square; p=**0.028***						

n – size of subgroup; % – percentage; p – level of significance; * p<α; χ²

bacteria was exceeded in 13.04% of samples (n=69) with a temperature <55 °C, and in 3.5% of samples (n=2) the temperature was higher or equal 55 °C (Tab. 4). In 2018, excessive contamination was observed in 13.8% of hospital buildings and 9.7% of SWHs buildings, and in as many as 37.5% of hospitals providing inpatient and 24-hour health services, where immunocomprised patients stayed, including those under immunosuppressive treatment.

Analysis of correlations showed a negative relationship between the sample temperature and the determined number of *Legionella sp.* bacteria. A significant negative correlation was observed in facilities where the examination of the quality of hot water was performed from a sample with a volume 100 ml (r=-0.108; p=0.011), as well as 1,000 ml (r=-0.348, p=0.013).

DISCUSSION

Temperature is one of the main factors determining the presence and growth of *Legionella sp.* bacteria, not only in the natural environment, but also in hot water systems in buildings. The occurrence of contamination of domestic water supply systems in building of hospitals and SWHs is considered as particularly hazardous. Mortality among patients and residents of SWHs is considerably higher than among general population, and may reach from 30–50% [12].

The presented study demonstrates that the provision of a temperature higher than 55 °C in hot water systems in building of hospitals and SWHs decreases the frequency of occurrence of excessive contamination with *Legionella sp.* bacilli, which is consistent with other studies.

The effect of the degree of colonization of hot water installations by *Legionella sp.* bacilli was considered as one of the main determinants in the report by Rhoads et al. [19]. Rakić et al. collected 127 samples for *Legionella sp.* from installations of hotels and homes for the disables and old age people in the region of Split, Croatia, where the temperature of 48.82% of water samples was above 55 °C. All samples at this temperature were free from contamination [10].

Based on a comprehensive analysis including 718 buildings (among others, hospitals, long-term care facilities) in Germany, Krause et al. confirmed a strong relationship between the water temperature and degree of colonization. The mean temperature of water samples collected from hospital installations was 54.9 °C, while in long-term care facilities – 60.4 °C. In 233 out of 718 buildings (32.7%), *Legionella sp.* bacteria were identified. Contamination on a high or mediocre level concerned 148 (20.6%) buildings. Compared to other types of buildings, contamination on

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mediocre and high levels was more frequently found in hospitals. The highest levels of contamination concerned samples of the temperature of 30–44.9 °C [20].

Deiane et al. confirmed a statistical relationship (p<0.05) between temperature and the number of positive samples. The collected samples were divided into 3 ranges according to temperature (< 32 °C, 32.1–47.2 °C, > 47.3 °C). In the first tertile, 63.9% of samples contained bacteria, whereas in the second and third tertiles – 47.1% and 5.3%, respectively. Mean sample temperature – 39.5 °C [21].

In the current study, a statistically significant negative correlation was observed between sample temperature and the determined number of bacteria. The correlation was found in facilities where the quality of hot water was examined based on samples of the volume of 100ml (r= -0.1076; p=0.011) and 1,000 ml (r=-0.3481; p=0.013).

A study carried out by Zaborowska-Dobosz et al. in hospitals in the Bydgoszcz district in north-east Poland, also showed a strong relationship between water temperature and number of bacteria. A high level of colonization was accompanied by a low water temperature [22]. Analysis of the relationships between temperature and the number of bacteria in the samples collected from hospital water system performed by Sikora et al. also showed a negative correlation (r = −0.44; p<0.000001) [23].

Opposite results were presented by Fragou et al., according to whom within the temperature range 20–50 °C there was a lack of correlation between water temperature and degree of colonization (p>0.05). A considerable decrease in the number of *Legionella sp.* bacilli was observed only among the samples with the temperature above 60 °C [24]. The lack of correlations within the mean range of temperatures and the inhibitory effect of temperature above 60 °C on *Legionella sp.* bacteria was also observed by Wojtyła-Buciora et al. [25].

A study by Gavalda et al. confirmed that the minimum temperature in hot water installations should be 55 °C. The percentage of samples in which bacteria were not found was 83.2% when the water temperature was ≥55 °C, while within the temperature range 50.1–4.0 °C, this percentage decreased to 64.9%, and to 51.6% in the samples with a temperature of ≤50 °C [26]. Godić Torkar et al. observed a bactericidal effect at the temperature of 55 °C on *Legionella pneumophila* bacterial cultures grown in laboratory conditions [27].

Despite the fact that water temperature is considered as the main factor exerting an effect on reproduction and growth of *Legionella sp.* bacteria, only in a few facilities in the study could its mean value be assessed as being compliant with applicable law (≥55 °C). It is particularly dangerous that in all installations of hospital buildings where immunocomprised patients stayed, including those under immunosuppressive treatment, the measured water temperature was lower than 55 °C. Difficulty maintaining the proper hot water temperature may result from the complexity of installation, materials used, renovations, and frequent reorganization of the facilities, which is conducive to the formation of 'blind ends', i.e. the occurrence of places where water remains stagnant [25, 26, 28, 29]. Insufficient temperature may be due to too low power of the heat source and lack of proper thermal insulation of the installation [22]. The subsequent factor exerting an effect on water temperature is the ageing of water supply systems. Facilities constructed after 16 December 2002 were designed and built in accordance with

the Regulation of the Minister of Infrastructure on technical conditions to which buildings and their location should conform, according to which the water temperature should be between 55 °C – 60 °C, with the possibility of periodic overheating up to 70 °C. In older buildings constructed before 16 December 2002, where installations were not modernized, lower hot water temperature is allowable, from 45 °C – 55 °C.

In the case of occurrence of an excessive number of *Legionella sp.* bacteria and too low water temperature, corrective actions should begin with an inspection of the technical condition and the project assumption of the installation; simply raising the temperature of the furnace or exchanger may not bring the expected results. In improperly insulated extensive water installations, due to heat loss, in the most distant taps and showers the temperature will still be too low [12, 20]. In this type of installations, maintaining the correct water temperature at the distal points requires the achievement of temperatures exceeding 60 °C at the output from heating devices, which generates considerable financial outlays. Also, water temperature higher than 60 °C creates the risk of burns to the users of buildings of hospitals and SWHs, especially children, persons at old age, and those intellectually disabled [26]. The solution to the problem is the installation of local flow heaters at the final, critical points of the system. This type of devices contribute not only to the maintenance of proper water temperature, but also decrease financial costs and the risk of burns [12].

Implementation of a number of organizational and technical solutions, monitoring of water temperature, maintenance of its correct value (>55 °C), cleaning and disinfection of hot water installation elements, continuing education of staff, and preparation of documentation regarding water quality supervision, should be considered as necessary actions aimed at reducing the risk of infection with *Legionella sp.* bacteria among patients in hospitals and residents of SWHs.

The World Health Organization (WHO) recommends the development and implementation of the Water Safety Plan (WPS), a document which includes all issues related with the supervision of in-building installations, risk assessment, and staff training [15].

The above-mentioned solutions are compliant with Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption, especially with Article 10: 'Risk assessment of domestic distribution systems'. Provisions of the Directive (changing the approach to supervision over domestic water systems and the quality of hot water) should have been implemented into Polish legal system by 12 January 2023, which, however, has not happened to date, i.e. 1 October 2024.

CONCLUSIONS

1. In the majority of hospitals and SWHs in the Kielce Province of Poland, the temperature of hot water was too low, which is inconsistent with the provisions of law.
2. Water temperature measured while collecting the samples was lower than the value provided in the survey form by facility managers.
3. Excessive contamination with *Legionella sp.* bacteria occurred statistically more rarely in water samples at the temperature ≥55 °C.

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4. It is necessary not only to systematically monitor temperature and examine the quality of water, but also to implement procedures, schemes of management and training programmes dealing with problems concerning the occurrence of *Legionella sp.* in facilities.

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