

# HYGIENE MEASURES AND MICROBIOLOGICAL INDICATORS AS FACTORS RELATED TO THE COLONIZATION WITH *LEGIONELLA* SPP. IN AIR CONDITIONING DEVICES

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## ABSTRACT

**Introduction:** *Legionella* spp. represents a significant public health concern due to its association with water and air-conditioning systems.

**Goal:** The objective of the study was to determine the presence of *Legionella* spp. on the surfaces of air-conditioning devices in public buildings using the wet swab method. Furthermore, the study aimed to examine the relationship between the implementation of hygiene measures and the presence of *Legionella* spp., as well as the association between aerobic mesophilic bacteria, enterobacteria, and the presence of *Legionella* spp. Additionally, one of the objectives was to determine the concentrations of the investigated

microorganisms and to assess their association with the presence of *Legionella* spp. in air-conditioning devices.

**Methods:** The research was conducted in the territory of the Federation of Bosnia and Herzegovina. Air-conditioners devices in 62 public buildings were included in the study. The collection of samples was carried out from 2023 to 2024. In total, 119 samples were collected and analyzed in the laboratory. The samples were microbiologically analyzed for *Legionella*, aerobic mesophilic bacteria and enterobacteria using appropriate laboratory methods. A survey was also conducted in the investigated facilities (N=62).

**Results:** *Legionella* spp. was detected in 12 (19%) facilities and isolated in 19 (16%) samples. It was found that the likelihood of the presence of *Legionella* spp. was lower when disinfection was performed using chlorine ( $p < 0.05$ ). *Legionella* spp. was isolated in one (2.4%) sample, which was negative for aerobic mesophilic bacteria ( $p < 0.05$ ), while no enterobacteria were isolated in four (9.3%) samples, and the findings were positive for *Legionella* spp. ( $p < 0.05$ ). In the group of samples in which *Legionella* spp. was isolated, the concentrations of both analyzed pathogens were higher compared to the other group ( $p < 0.05$ ).

**Conclusion:** The results of this study indicate that the hygienic condition of air-conditioning is associated with the presence of *Legionella* spp. Furthermore, the findings highlight the need for the establishment of a clearly defined regulatory framework for the hygienic maintenance, control, and monitoring of air-conditioning devices in public buildings, with the aim of strengthening preventive measures.

**Keywords:** Aerobic mesophilic bacteria, enterobacteria, Federation of Bosnia and Herzegovina, public facilities, wet swab method

## INTRODUCTION

*Legionella* spp. (*L. spp.*) belong to a group of microorganisms that are found everywhere and are widely distributed, especially in aquatic environments (1). The natural habitat of *L. spp.* is the waters of rivers, lakes, ponds, hot springs and moist soil (2). In anthropogenic systems, appropriate factors play a key role in order for bacteria to multiply (3). Factors favoring the reproduction of *L. spp.* are biofilm formation, temperature, weak disinfectant, water stagnation, and change in water pressure (4). Air conditioners can be a significant source of *L. spp.* contamination (1, 2, 5). *L. spp.* is important cause of pneumonia in humans (6) and nosocomial infections (1) that often occur in epidemic form (7, 8). Hospital infections of legionellosis have a very severe clinical picture (2) and mortality is from 15% to 20%, while in cases of hospital epidemics it can be up to 60% (5). The disease caused by *L. spp.* is transmitted by aerosol (2). It has not been established that the disease is transmitted from human to human, nor has the presence of the bacterium been proven in animals (9). In European countries, the number of cases of Legionnaires' disease is increasing year by year, from about 9,000 cases in 2017 to 10,723 cases in 2021, with an estimated

mortality of 6.6% in 2021, with the largest share of cases in France, Germany, Italy and Spain (10, 11). During 2025, significant legionellosis outbreaks were reported in the US, Canada and Europe, including New York, London, Albertville and Vorarlberg, which were linked to contaminated cooling towers and large air conditioning or water systems. In all cases, the aerosol route of transmission was confirmed, with serious public health consequences (12, 13, 14, 15). The presence of elevated values of aerobic mesophilic bacteria (AMB) and enterobacteria (EB) is an indicator of inadequate implementation of hygiene measures (16, 17). Recent evidence indicates that enterobacteria, including strains resistant to  $\beta$ -lactam antibiotics, may be present in the air of public spaces, indicating a potential health risk for exposed individuals (18). Studies have also shown that high concentrations of AMB in closed spaces, including opportunistic pathogens such as *Staphylococcus* and *Klebsiella*, may be associated with an allergic reaction, irritation of the respiratory tract or may worsen existing respiratory conditions, and that their level increases with the density of users and poor ventilation of the space. Staying in an area where the air is contaminated with AMB is a particular risk for the elderly, children or immunocompromised persons (19). By adhering to the surface, aquatic microorganisms achieve a mechanism against unfavorable conditions, most often the lack of nutrients and unfavorable temperatures. On different surfaces, both in natural and artificial sources, the possibility of the existence of biofilm has been established (20). *L. spp.* integrated in the biofilm of water systems can contaminate the entire system even after chemical treatment, especially when the level of biocide in the water decreases (21). A higher resistance of *L. spp.* grown in biofilm was found compared to the same species that exists in the water system (20). The occurrence of legionellosis is often attributed to the formation of biofilms. *L. spp.* is most often included in the microbial community in which it is extremely resistant to chemical and physical treatments. Appropriate conditions lead to persistence, spread, and increased virulence (22). *L. spp.* are sensitive to chlorine (2), while based on other available sources, they are resistant to lower concentrations (5), and that due to their resistance to the action of chlorine, they survive in water systems (1). Corrosion, contaminants in inaccessible places, heat exchanger tube design, roughness and unsuitable clearances are elements that can adversely affect cleaning and disinfection procedures. The importance of the design of cooling devices in terms of cleaning and disinfection efficiency is emphasized, and innovative devices that have the ability to perform the aforementioned processes automatically are preferred (23). The implementation of prophylactic interventions, as well as the use of predictive tools based on statistical processing, are key to effective control of *L. spp.* colonization (24). The need to implement a mandatory prevention program is particularly important for high-risk facilities such as healthcare institutions, nursing homes, gyms, and seasonal hotels (25). Although in the Federation of Bosnia and Herzegovina (FBiH) there is currently no special regulation that explicitly and in detail prescribes the monitoring of *L. spp.* in air conditioning devices, as is the case in some European countries (26), health institutions provide guidelines and recommendations for prevention (27, 28). In accordance with the current

legal regulation (29), competent public health institutes are authorized to carry out epidemiological measures and issue recommendations to prevent the spread of disease. The recommendations of the Institute for Public Health of the Federation of Bosnia and Herzegovina serve as a practical guide for implementing preventive measures and monitoring facilities of public importance with a focus on hospitals, hotels and homes for the elderly (27). In FBiH, the owner or responsible person in the facility is legally responsible for hygienic conditions and preventive measures against infectious diseases (29).

The objectives of the research were:

- To determine the presence of *L. spp.* on the surfaces of air-conditioning devices in buildings of public importance using the wet wipe method;
- Examine the relation between the implementation of hygiene measures and the presence of *L. spp.* in air-conditioning devices;
- To isolate AMB and EB in the collected samples and then analyze their association with the presence of *L. spp.* in air-conditioning devices;
- To determine the concentrations of AMB and EB in the analyzed samples and to examine their association with the presence of *L. spp.* in air-conditioning devices.

## **METHODS**

The research was conducted in FBiH, Bosnia and Herzegovina. The research included air-conditioning devices, and 119 samples were collected from 62 public facilities and analyzed in the laboratory. The collection of samples was carried out from 2023 to 2024. The number of collected samples per object ranged from 1 to 8, which depended on the size of the object and the complexity of the air-conditioning device. The facilities included in the research are classified into four categories, namely tourist and hospitality facilities (hotels, restaurants, cafes), office buildings (in which profit and non-profit business, management of local, cantonal and state administrations, and business related to public services are carried out), service activities (shopping centers, food stores, public transport services, services for aesthetic and hygienic arrangement and sports and recreation, care for children of preschool age and the third age) and health institutions (homes of health, pharmacies, dental surgeries, public health institutes, medical schools). Ten objects were included from the first category, 13 objects from the second category, 32 objects from the third category, while seven objects from the fourth category were included in the research. The research included facilities of public importance with installed air-conditioning devices, a large number of employees and frequent presence of citizens, while smaller and rarely visited facilities were not included in this research. The samples were collected using the wet swab method 18593:2019 (30). On the basis of professional and scientific literature, the sampling locations were previously defined (air conditioning chamber filter, internal filter, diffuser and fan). The collected samples were delivered to the laboratory within the recommended

time frame, with controlled transport conditions and appropriate temperature. The laboratory is in the process of accreditation according to the requirements of standard 17025:2018 (31). Isolation and counting of *L. spp.* in the analyzed samples were performed by cultivation. Sowing was carried out on media with buffer agar of yeast extract and activated carbon (BCYE) (Liofilchem, Italy). Incubation on the substrate at a temperature of 36 °C in a humid environment with 2.5% CO<sub>2</sub> lasted for ten days. Examination with a dissecting microscope was performed on the third and fifth day (11731-2:2018) (32). Furthermore, the collected samples were analyzed in the laboratory for AMB and EB with detection and quantification. The analysis of the samples was carried out in accordance with the regulation of defined parameters according to the methods for AMB - 4833:2014 (33) and EB - 21528: 2018 (34). The results were read for both AMB and EB in accordance with the norms of microbiological cleanliness for objects, surfaces and hands that come into contact with food (35). In the researched facilities (N = 62), a short structured interview was conducted using a paper standardized survey questionnaire. The survey questions were most often answered by persons in charge of air conditioning maintenance. The questionnaire used is exclusively the author's work, and was created based on a review of numerous professional and scientific literature, recommendations from authorized air conditioning service providers and on the basis of previous competence and expected evidence in practice. The questionnaire consisted of three questions. The questions were of a closed type, two of which offered a YES/NO answer.

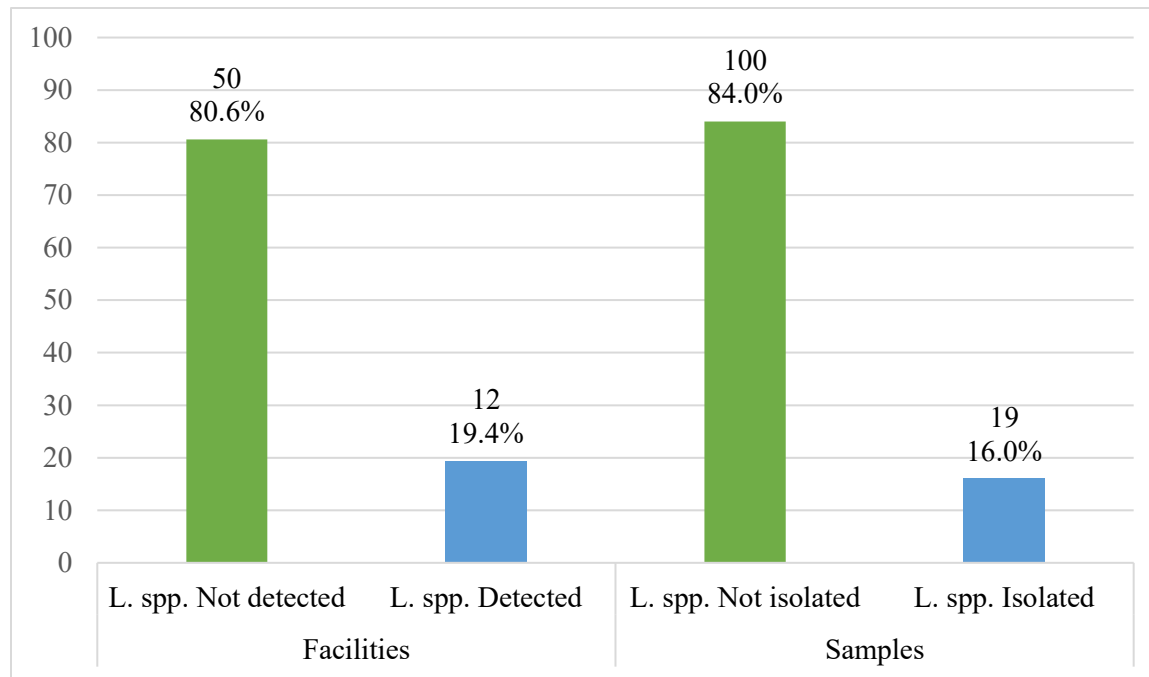
For the purpose of consistent interpretation of the results, the following terms were used in this study: “detection” refers to the determination of the presence of *L. spp.* in the examined sites based on laboratory analysis; “isolation” refers to the recovery and culturing of *L. spp.* from collected samples; while the term “presence” is used as a general expression for the confirmed finding of *L. spp.* on the surfaces of air-conditioning systems. These terms were applied in accordance with their microbiological meaning and used consistently throughout the study.

### **Statistical methods**

The results of the research are presented tabularly and/or graphically by absolute number, percentage, median and interquartile range, depending on the type of data. The chi-square test was used to test the differences of discrete variables, and the Mann-Whitney test was used for continuous variables. The results of all the mentioned tests are considered statistically significant at the confidence level of 95%, i.e. with a value of  $p < 0.05$ . The analysis was performed using the statistical package IBM Statistics SPSS v 25.0 (Chicago, Illinois, USA).

## RESULTS

The study included 62 facilities and 119 samples were collected and analyzed in the laboratory. The variable of the presence of *L. spp.* was used to form two groups of samples, after which a statistical analysis of the association with other variables was performed, including survey responses and microbiological analysis results (AMB and EB), in accordance with the research objectives.



Legend: *L. spp.* - bacteria from the genus *Legionella*;

Figure 1. Presentation of the tested facilities (N=62) and the results of the analyzed samples (N=119) regarding the presence of *L. spp.*

Out of the total number of tested facilities (N=62), *L. spp.* was detected in 12 (19%) facilities, while out of the total number of analyzed samples (N=119), there were 19 (16%) samples in which *L. spp.* was isolated (Figure 1).

Table 1 shows the collected survey responses on the implementation of hygienic measures of air-conditioning devices in the included facilities (N = 62). Statistical analysis was performed based on the presence of *L. spp.* in air-conditioning devices and survey responses to three questions. It was observed that the presence of *L. spp.* is not excluded in air-conditioning devices regardless of the survey answer. The results of the statistical analysis using the chi-square test showed only one statistically significant difference, indicating a lower likelihood of the presence of bacteria in air-conditioning devices in facilities that reported using chlorine as a disinfectant ( $p < 0.05$ ). Although *L. spp.* was detected only in one facility

(5.3%) where hygienic measures in the air-conditioning device are carried out twice a year, no statistically significant difference was observed compared to the other facilities.

Table 1. Presentation of survey responses on the implementation of hygiene measures and the connection with the presence of *L. spp.* (N = 62)

Survey questions and answers collected in facilities		<i>L. spp.</i>		Total	
		Not detected	Detected		
Having a contract with authorized services on the implementation of hygiene measures $\chi^2 = 0.678; p = 0.410$	No	N	11	4	15
		%	73.3	26.7	24.2
	Yes	N	39	8	47
		%	83.0	17.0	75.8
Frequency of washing, cleaning and disinfection $\chi^2 = 3.485; p = 0.085$	Once a year	N	32	11	43
		%	74.4	25.6	69.4
	2 times per year	N	18	1	19
		%	94.7	5.3	30.6
Using chlorine as a disinfectant $\chi^2 = 5.367; p = 0.034$	No	N	38	5	43
		%	88.4	11.6	69.4
	Yes	N	12	7	19
		%	63.2	36.8	30.6
Total		N	50	12	62
		%	80.6	19.4	100.0

Legend: *L. spp.* - bacteria from the genus *Legionella*;

From the total number of samples (N= 19) in which *L. spp.* was isolated, 18 (23.1%) samples were also positive for AMB, and only one (2.4%) sample isolated *L. spp.*, which was negative for AMB. The result of the statistical analysis using the chi-square test shows that there is a statistically significant difference and a positive correlation of AMB with the presence of *L. spp.* ( $p < 0.05$ ) (Table 2).

Table 2. Presentation of the results of the samples analysis for AMB and *L. spp.* and mutual correlation (N = 119)

		<i>L. spp.</i>			Total
			Not isolated	Isolated	
AMB – finding cfu/g	Negative	N	40	1	41
		%	97.6	2.4	34,5
	Positive	N	60	18	78
		%	76.9	23.1	65,5
Total	N	100	19	119	
	%	84,0	16.0	100.0	

Legend:  $\chi^2 = 8,531$ ;  $p = 0,003$ ; *L. spp.* - bacteria from the genus *Legionella*; AMB - aerobic mesophilic bacteria.

Statistical analysis using the Mann-Whitney test showed that there is a relation between the concentration of AMB and the samples in which *L. spp.* was isolated, the difference is statistically significant ( $p < 0.05$ ). The measured median value and the interquartile range were significantly lower in the group of samples in which *L. spp.* was not isolated, compared to the group of samples in which the bacterium was isolated (Table 3).

Table 3. Presentation of AMB concentration (N = 78) and correlation with the presence of *L. spp.* (N = 119)

		<i>L. spp.</i>		p
		Not isolated	Isolated	
AMB	Median	50.00	140.00	Z=-3.015 p=0.003
	Q1	20.00	96.00	
	Q3	125.00	200.00	

Legend: *L. spp.* - bacteria from the genus *Legionella*; AMB - aerobic mesophilic bacteria;

From the total number of samples (N=19) in which *L. spp.* was isolated, 15 (19.7%) samples were also positive for EB, while in four (9.3%) samples EB was not isolated, and the findings were positive for *L. spp.* The result of statistical analysis using the chi-square test shows that there is a statistically significant difference and a positive correlation of EB with the presence of *L. spp.* ( $p < 0.05$ ) (Table 4).

Table 4. Presentation of the results of the samples analysis for EB and *L. spp.* and mutual correlation (N = 119)

		<i>L. spp.</i>			Total
			Not isolated	Isolated	
EB – finding	Negative	N	39	4	43
		%	90.7	9.3	36,1
cfu/g	Positive	N	61	15	76
		%	80.3	19.7	63,9
Total		N	100	19	119
		%	84,0	16.0	100.0

Legend:  $\chi^2 = 8.531$ ;  $p = 0.003$ ; *L. spp.* - bacteria from the genus *Legionella*; E – enterobacteria.

Statistical analysis using the Mann-Whitney test showed that there is a relation between the concentration of EB and the samples in which *L. spp.* was isolated, the difference is statistically significant ( $p < 0.05$ ). The measured median value and the interquartile range were significantly lower in the group of samples in which *L. spp.* was not isolated, compared to the group of samples in which the bacterium was isolated (Table 5).

Table 5. Presentation of EB concentration (N = 76) and correlation with the presence of *L. spp.* (N = 119)

		<i>L. spp.</i>		p
		Not isolated	Isolated	
EB	Median	10.00	30.00	Z=-2.804 p=0.005
	Q1	8.00	12.00	
	Q3	22.00	53.00	

Legend: *L. spp.* - bacteria from the genus; E - enterobacteria;

## DISCUSSION

The results of this study contribute to the existing body of knowledge regarding factors that may be positively correlated with the presence of *L. spp.* in air-conditioning devices. The analysis of samples collected by the wet swab method from the surfaces of air-conditioning devices in public facilities in the

Federation of Bosnia and Herzegovina revealed the presence of *L. spp.* in several of the analyzed samples. Other objectives of this research were related to the connection of the presence of bacteria with the dynamics of implementation of hygiene measures and the connection of settlement with AMB and EB as well as their concentrations. The objectives of another study also carried out in the FBiH area were to examine the relation between the physicochemical parameters of water in air-conditioning devices and the presence of *L. spp.* The results of that study showed a positive correlation between higher temperatures, lower residual chlorine concentrations, higher iron and copper concentrations, and the presence of *L. spp.* in air-conditioning devices (36). In this study, regarding the frequency of hygiene measures implemented in air-conditioning devices, comparison of the collected questionnaire responses with the findings for *L. spp.* showed no statistically significant difference between the two groups of facilities (facilities performing hygiene measures once a year and those performing them twice a year) ( $p > 0.05$ ). A total of 47 (75.8%) facilities reported having a contract with an authorized service company. However, analysis of the two groups of facilities (those with and those without such a contract) and comparison with the findings for *L. spp.* also revealed no statistically significant difference ( $p > 0.05$ ). Furthermore, another study conducted in Bosnia and Herzegovina aimed to investigate the association between hygienic conditions and the presence of *L. spp.* in water supply systems, heating and cooling systems, and fountains in public and tourist facilities. The results were summarized based on microbiological analysis of collected water samples and a questionnaire used to assess the hygienic status of the examined water systems. The findings indicated an association between hygienic conditions and the presence of *L. spp.* in water systems in public and tourist facilities (37). Considering that the presence of AMB and EB indicates poor hygienic conditions (16, 17), and that one of the objectives of this study was to determine their association with the presence of *L. spp.*, based on the obtained AMB and EB findings and the comparison of two groups of *L. spp.* results (unsatisfactory/satisfactory), it may be suggested that there is an association between the hygienic condition of air-conditioning devices and the presence of *L. spp.* In the group of samples in which *L. spp.* there is a significant representation of samples in which AMB and EB are isolated compared to the other group of samples, the difference is statistically significant ( $p < 0.05$ ). The measured concentrations of AMB and EB may additionally support the association between the hygienic conditions of air-conditioning devices and the presence of *L. spp.* In the group of samples in which *L. spp.* was isolated, the concentrations of the examined pathogens were significantly higher than in the other group of samples, the difference is also statistically significant ( $p < 0.05$ ). In Nigeria, air conditioner filters and conditioned air have been researched. Out of the total number of tested samples ( $N = 100$ ) of filters, all 100 samples were contaminated with bacteria, while fungi were present in 73 samples. Additionally, the air of the air-conditioned space was tested and the presence of bacteria or fungi was established in 41 samples out of the total number ( $N = 50$ ) of analyzed samples. The most common contaminants isolated were *Bacillus* species

and *Aspergillus niger*, followed by *Aspergillus flavus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* (38). In Thailand, water from nursing homes and thermal pools was tested for the presence of *L. spp.*, and the results showed the presence of *L. spp.* in five (16.67%) samples from nursing homes and 13 (43.33%) from thermal pools, out of the total number of analyzed samples (N = 30). The presence of other Gram-negative bacteria, microorganisms dangerous to human health, especially in elderly people and people with a weakened immune system was also found in the samples (39). Based on the results of research conducted in the Republic of Croatia, the presence of *L. spp.* in hot water systems can only be controlled by consistent application of technical and hygienic measures. Maintaining the appropriate water temperature, ensuring circulation and regular cleaning and disinfection of the system are key to preventing colonization with this bacteria. Occasional and insufficiently implemented measures increase the risk of *L. spp.* colonization (40). The analysis of the survey responses regarding the disinfection of the air-conditioning device indicate a statistically significant difference between the two groups of facilities (using/not using chlorine) ( $p < 0.05$ ), which coincides with the previous research where residual chlorine concentrations were measured in water samples and a positive correlation was established between the group of samples in which *L. spp.* was isolated, with lower values of free chlorine (36). A study was conducted that included and analyzed 824 water samples from the distribution system, in which the concentration of residual chlorine was between 0.09 and 0.12 mg/l. Of the nine samples tested for *L. ph.*, four were positive. When increasing and maintaining the level of residual chlorine in water between 0.27 and 0.51 mg/l, and by analyzing a three-year period in water samples, positivity for *L. spp.* was not proven (41). After it was reported that a foreign citizen had contracted Legionnaires' disease in a hotel in the FBiH, the necessary hygiene measures and hyperchlorination were implemented in the water systems of the hotel. However, the presence of *L. spp.* was detected again after three years (42). Long-term research has shown that shock disinfection and hyperchlorination reduce the level of contamination immediately after the procedure, but that this is not a long-term solution when it comes to the reoccurrence of pathogens. The new approach of pre-filtering, filtering, use of pipe protection products and remote control of chlorine dioxide ( $\text{ClO}_2$ ), all integrated into the process, shows satisfactory results without damage within the system (43). Much earlier, it was established that broad-spectrum organophosphorus microbiocides show high efficiency even at low concentrations. They are effective when completely disinfecting and maintaining hygiene. They prevent corrosion and are used to deodorize air conditioning devices. They also do not have a harmful effect on the environment and are not toxic to humans and animals (44). According to the conclusions of the research conducted in Turkey, *L. spp.* represents a major public health threat. The researchers believe that, especially in high-risk facilities, it is a priority to establish appropriate policies that include frequent control and disinfection of water systems, as these are essential tools as part of preventive measures. They also believe that local communities should develop and

implement a program that would cover, control and monitor the water supply systems of all large buildings. It is believed that faster ways to detect *L. spp.* in water systems and clinical samples would greatly improve prevention measures (45). In the Netherlands, the prevention and control of *L. spp.* is regulated through a legally binding risk management system. The regulations require a mandatory risk assessment, development of a management plan for *L. spp.*, regular sampling and laboratory testing of water in systems that can generate aerosols, including cooling towers and complex air conditioning systems. Thresholds for the concentration of *L. spp.* have been defined, upon exceeding which corrective measures must be implemented, and in the case of higher values, the competent authorities must be notified. A register of cooling towers and obligations to keep documentation on maintenance and monitoring was also established (26). According to the guidelines of the Association of German Engineers, hygiene measures in ventilation and air conditioning systems include regular hygiene inspections, cleaning and maintenance at defined time intervals, whereby it is recommended to carry out inspections and hygiene procedures at least once or twice a year, depending on the type of system and the purpose of the object (46). According to available data, 6 cases of Legionnaires' disease have been recorded in the FBiH in the past six years (47, 48), while 65 cases and four fatalities were reported to neighboring Croatia in 2023 alone (49). The aforementioned data may indicate differences in surveillance, diagnostic, and disease reporting systems. The observed low number of reported cases of Legionnaires' disease in the FBiH, compared to the higher number of reports in the Republic of Croatia, further emphasizes the importance of this research in order to better understand potential sources of exposure and improve the surveillance and prevention system. A limitation of this research may relate to the reliability of data collected using a questionnaire, where the honesty of the respondents could have significantly affected the results obtained. In addition, the relatively limited sample size may represent a methodological limitation and affect the reliability and general applicability of the results obtained. Although this research did not directly examine the correlation between microclimatic conditions in buildings and the microbiological presence in air conditioning devices, there are indications that these factors may be interconnected. The lack of research into microclimatic conditions in buildings can be considered a limitation of this research, which indicates the need for cautious interpretation and application of the results obtained.

## **CONCLUSION**

Based on the results of this research, *L. spp.* was isolated from the samples collected from the surfaces of air-conditioning devices in public facilities in the FBiH using the wet wipe method. The results showed a lower likelihood of bacterial presence in air-conditioning devices when disinfection procedures were performed using chlorine ( $p < 0.05$ ). The analysis of two groups of facilities, regarding the frequency of

hygiene measures and the possession of a contract with an authorized company, as well as their association with the presence of *L. spp.* in air-conditioning devices, revealed no statistically significant difference ( $p > 0.05$ ). In contrast, a positive correlation was observed between the group of samples in which *L. spp.* was isolated and the groups of samples in which AMB and EB were detected, and the difference was statistically significant ( $p < 0.05$ ). Also, the concentrations of AMB and EB are brought into relation with the presence of *L. spp.* in air-conditioning devices. The concentrations of tested pathogens were significantly higher in the group of samples in which *L. spp.* was isolated compared to the group of samples in which the bacterium was not isolated, the difference being statistically significant ( $p < 0.05$ ). Based on the microbiological results of the analyzed samples, it can be said that the hygienic condition of air-conditioning devices is associated with the presence of *L. spp.* The results of this study are important for a better understanding of the factors that can be associated with the presence of *L. spp.* in air-conditioning devices, as well as the presence of other pathogenic bacteria. The obtained results can be used for the purpose of establishing precise and clearly defined regulatory measures regarding hygienic maintenance, control and supervision of air conditioning devices of all public facilities on the territory of the FBiH, which is what the results of this research really indicate. The importance of new research on factors associated with the presence of *L. spp.* in air-conditioning devices, as well as on the health consequences caused by pathogenic contaminants, will further contribute to strengthening this important public health issue and the establishment of preventive measures.

## **FINANCING**

Funding: No funding was received for this study.

## **STATEMENT OF INTEREST**

„The authors declare that they have no conflict of interest“

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