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Original scientific paper

INFLUENCE OF AIR CONDITIONING DEVICES AGE, DISTRIBUTION INSTALLATION MATERIALS, MAINTENANCE AND DYNAMICS OF USE ON THE APPEARANCE OF *LEGIONELLA*: A STUDY CONDUCTED IN THE TERRITORY OF THE FEDERATION OF BOSNIA AND HERZEGOVINA

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Abstract: Because man strives to ensure the comfort in living and working spaces, there is a lot of air conditioning devices to achieve the desired temperature. Bacteria from the genus *Legionella* is a pathogenic bacterium that can cause the disease to occur in very severe form. It most often inhabits water systems created by human activity, among which are air conditioning devices, which is why there is a need to test factors that favors bacteria colonization. The study included 79 facilities from the territory of the Federation of Bosnia and Herzegovina, of which 42 or 53.2% facilities with centralized, and 37 or 46.8% facilities with individual air conditioning devices. Data were collected using a survey and laboratory analysis of samples. The analysis was performed individually, taking into account the different requirements and complexity of the two types of air conditioning devices. The objectives were related to the correlation between the age, distribution installation material, project, the maintenance dynamics and the need to use the air conditioner with *Legionella* spp. colonization. The results indicate a positive influence of the air conditioner age and steel distribution installation on the *Legionella* spp. colonization. Significant correlation was not observed between the collected survey responses (project, maintenance and the air conditioner use frequency) and the *Legionella* spp. colonization. The identification of factors that positively correlate with the presence of *Legionella* spp. in air conditioning devices has significant public health and technical contribution, especially in the context of legionellosis prevention, the implementation of adequate monitoring and maintenance.

Keywords: Legionellosis, monitoring, public health contribution, technical contribution, Water systems.

INTRODUCTION

In recent decades, global warming and climate change have contributed that people for achieving the necessary temperature parameters inside closed spaces use air conditioning, and create comfort with an artificial climate. Based on previous reports (Hernandez-Martin et al., 2025, Liu et al., 2024, Grassie et al., 2025), there is a justified indication for installing air conditioning devices. However, attention should also be paid to the risks posed by conditioned air, since the colonization of bacteria from the genus *Legionella* (*L. spp.*) has been established in air conditioning devices (Elsanousi, R. M. A. & Elsanousi, S. M. 2017, Gea-Izquierdo, et al., 2023, Yao et al., 2024). It is a well-known fact that *L. spp.* is the cause of a disease called legionellosis (Obradović, 2013). In general, the trend shows an increase in the number of people suffering from legionellosis in many European countries and the mortality rate is high (Barskey, et al. 2022, European centre for disease prevention and control, 2024). People with weakened immunity and existing comorbidities are most often affected, and a lethal outcome often occur. The disease can occur in all age groups, including children, but it most often affects middle-aged and elderly people (Kalenić, et al., 2013).

It has been proven that the disease occurs three times more often in men than in women (Dautović-Krkić & Čengić, 2010). In facilities with increased mobility of people and the air is contaminated, the disease spreads very quickly through aerosols (Seppänen & Fisk, 2004). From the aspect of unfavorable influence of conditioned air, in addition to *legionella*, other Gram-negative bacteria and viruses can threaten human health. Contamination of ventilation openings and air conditioning devices with various microorganisms often occurs (Apte et al., 2004). Air conditioning devices based on refrigerant (most often freon) so-called individual air conditioning devices are used where strict control of temperature and humidity in the air-conditioned space is not required, when the investor is limited by financial means, the deadline for installing the device, and when there is space limitation (Selection Of Air Conditioning Systems, 2024). Centralized air conditioning devices represent an air conditioning plant, made up of several interconnected components, which are used to condition the air. The demanding technical performance makes them much more complex compared to individual air conditioning devices (Nyers, 2016). Centralized air conditioning devices are most often installed in larger facilities, such as hotels, hospitals and larger office buildings. They are used for economic reasons and to achieve optimal quality of conditioned air (2002 report of the refrigeration, air conditioning and heat pumps technical options committee (RTOC), 2023), and facilities that operate seasonally are at greater risk of *L. spp.* colonization (Obradović, 2013). These air conditioning devices most often use water for their operation (Selection of Air Conditioning Systems, 2024), and it is known that water is the most suitable medium for the occurrence and proliferation of *L. spp.* (Ontario Agency for Health Protection and Promotion, 2019). The materials of distribution ducts are often made of steel or galvanized steel (Todorović, 2005), while recently, plastic pipes have also been installed more frequently (Nyers, 2016). The materials must meet many criteria, including durability, flexibility, easy cleaning and smooth internal surfaces (Todorović, 2005). An integral part of a good project are the parts of the installed equipment. It is also necessary to take into account the size of the facility and its purpose for which it is necessary to ensure certain microclimatic conditions, while not neglecting environmental awareness (Kaladzija, 2024). It is the responsibility of the designer to ensure that the volume of dead space is reduced within the design of all water supply and air-conditioning devices due to the accumulation and stagnation of sediment, as such conditions, especially in biofilm, are conducive to the development of *L. spp.* (Gheraout et al., 2022). Cooling towers are often used to change the water temperature and remove excess heat (Kaladzija, 2024). Stagnation and circulation of water, as well as occasional use of the device, certainly have a beneficial effect on the colonization of bacteria in the cooling tower (*Legionella* – treating the critical risks in cooling towers: Section 8, 2024). Defective and old parts of the tower, as well as damaged ones, are conducive to the formation of biofilm, and servicing is a preventive measure when it comes to contamination by various microorganisms (Legionnaires' Disease Outbreaks, are Cooling Towers High Risk?, 2024). In the Federation of Bosnia and Herzegovina, there is no single regulation dedicated exclusively to *L. spp.* in relation to air-conditioning. Prevention of *L. spp.* colonization in air-conditioning devices is regulated through a combination of health, technical and sanitary regulations (Law on the Protection of the Population from Infectious Diseases, 2005, Rulebook on technical properties of ventilation and air conditioning systems, 2009, Rulebook on technical properties of heating and cooling systems, 2009). The existing regulatory framework indicates the need for additional research into the real risks and the level of compliance with the prescribed hygiene and technical standards in practice.

THE OBJECTIVES OF THE RESEARCH ARE:

To examine the relationship between the age of the air conditioning devices and the *L. spp.* colonization,

To examine the relation between the materials of the distribution installation of the air conditioning devices and the *L. spp.* colonization,

To determine the impact of the project and the dynamics of servicing the air conditioning devices with the *L. spp.* colonization,

To establish the connection between the dynamics of the use of the air conditioning device and the *L. spp.* colonization.

MATERIAL AND METHODS

The research was conducted at the territory of the Federation of Bosnia and Herzegovina, Bosnia and Herzegovina (2023 – 2024). The survey covered 79 buildings. The condition for inclusion in this study was that they were public and larger facilities. Smaller facilities and facilities that people rarely visit are not included in the research. Out of total, 42, or 53.2%, of buildings with centralized air-conditioning devices and 37, or 46.8% of buildings with individual air-conditioning devices are covered. In all investigated facilities, sampling (N=173) was carried out from previously defined critical places of the air conditioning device, based on professional and scientific literature (water cooling tower and water tank, condensed water, filters, diffusers and turbines). The number of samples per facility ranged from 1 to 8, and the air conditioning device was considered contaminated if *L. spp.* was isolated in only one sample. Sampling was performed using the wet swab method using a sterile stick (BAS EN ISO 18593:2019) and by pouring water from the outlet that circulates through the air conditioning system (BAS EN ISO 19458:2008). The samples were taken within the stipulated time transported to the agreed laboratory in Sarajevo, which is in the process of accreditation according to the requirements of the BAS EN ISO 17025:2018 standard. All the collected samples were cultured, due to the low number of bacteria, and membrane filtered 11731-2:2018. Furthermore, a structured interview was conducted in each of the investigated facilities using a paper-based questionnaire in charge of air conditioning maintenance, managers or deputies of the investigated facilities. The questionnaire used is exclusively the author's work, and was created on the basis of 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 seven questions. The questions were of a closed type, three of which offered a YES/NO answer.

STATISTICAL METHODS

The results are presented in tables and/or charts by absolute number and percentage. The chi-square test was used to test the differences of discrete variables. The results of the mentioned test are considered statistically significant at the confidence level of 95%, or 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

In Table 1. are presented the collected responses to the survey question regarding the age of the air conditioner device. The analysis was performed based on the classification according to the type of air conditioner device, the findings on *L. spp.* and the survey responses. According to the presented data, an increased frequency of *L. spp.* colonization was observed in older air conditioning devices. The result of the statistical analysis using the chi-square test shows that there is a statistically significant difference ($p < 0.05$) in favor of a higher prevalence of *L. spp.* colonization in air conditioning devices which are more than ten years old.

Table 1. Relationship between the age of the air conditioning device and the colonization with *L. spp.* (N = 79)

Survey question and answers collected in the facilities		Type of air conditioning device				Total	
		Centralized		Individual			
		<i>L. spp.</i>		<i>L. spp.</i>			
		Not detected	Detected	Not detected	Detected		
Age of the air conditioning device	Up to 5 years	N	7	0	18	0	25
		%	100.0	0.0	100.0	0.0	31,6
	From 6 to 10 years	N	13	3	10	0	26
		%	81.3	18.8	100.0	0.0	32,9
	More than 10 years	N	11	8	7	2	28
		%	57.9	42.1	77.8	22.2	35,4
Total	N	31	11	35	2	79	
	%	73,8	26.2	94.6	5.4	100.0	

$\chi^2=15.899$, $p=0.005$, *L. spp.* - bacteria from the genus Legionella;

The collected answers to the survey question related to the material of the distribution installation of the air conditioning device are shown. The analysis was done on the basis of the classification according to the type of air conditioning device, the obtained findings on *L. spp.*, and survey responses. It is evident that regardless of the materials of the distribution installation, the possibility of colonization with *L. spp.* is not excluded, but the result of statistical analysis using the chi-square test shows that there is a statistically significant difference ($p<0.05$) in favor of a positive correlation of steel distribution installation with the appearance of *L. spp.* in air conditioning systems (Table 2).

Table 2. Correlation of the distribution installation material of the air conditioning devices with the settlement of *L. spp.* (N = 79)

Survey question and answers collected in the facilities		<i>L. spp.</i>		Total	
		Not detected	Detected		
Distribution installation of the air conditioning device (material type)	Galvanized	N	23	1	24
		%	95.8	4.2	
	Steel	N	8	10	18
		%	44.4	55.6	
	Plastic	N	35	2	37
		%	94.6	5.4	
Total	N	66	13	79	
	%	83,5	16.5	100.0	

$\chi^2=25.939$, $p=0.0001$, *L. spp.* - bacteria from the genus Legionella

Table 3 shows the collected survey responses related to the design and servicing of centralized air conditioning devices. The analysis was performed based on the obtained findings on *L. spp.* and survey responses. Although the air conditioning devices were installed according to the design, signed by an authorized person, and have the appropriate documentation and a contract with an authorized service, it was observed that the colonization of *L. spp.* is not excluded. The obtained data show that *L. spp.* less frequently colonized air conditioning devices when servicing is performed several times a year. However, the results of the statistical analysis using the chi-square test show that there is no statistically significant difference ($p>0.05$).

Table 3. Relationship between the design and maintenance of air conditioning devices and the colonization of *L. spp.* – centralized air conditioning devices (N = 42)

Survey question and answers collected in the facilities		<i>L. spp.</i>		Total	
		Not detected	Detected		
The project was signed by an authorized person	Yes	N	31	42	
		%	73.8	26.2	100,0
Having the necessary documentation	Yes	N	31	42	
		%	73.8	26.2	100,0
Service frequency $\chi^2=2.439$, $p=0.295$	Once a year	N	18	8	26
		%	69.2	30.8	61,9
	Twice a year	N	10	1	11
		%	90.9	9.1	26,2
	As needed	N	3	2	5
		%	60.0	40.0	11,9
Having a contract with an authorized service $\chi^2=0.417$, $p=0.687$	No	N	1	0	1
		%	100.0	0.0	2,4
	Yes	N	30	11	41
		%	73.2	26.8	97,6
Total	N	31	11	42	
	%	73,8	26.2	100.0	

L. spp. - bacteria from the genus *Legionella*.

Survey responses related to the design and servicing of individual air conditioning devices were collected. The analysis was conducted based on the findings obtained on *L. spp.* and survey responses. *L. spp.* is more often colonized in air conditioning devices that are installed without a project signed by an authorized person, and if the facility does not have the necessary documentation regarding the air conditioning device. Colonization with *L. spp.* was not observed in air conditioning devices that are serviced at least once a year. However, the results of statistical analysis using the chi-square test show that there is no statistically significant difference ($p>0.05$) (Table 4).

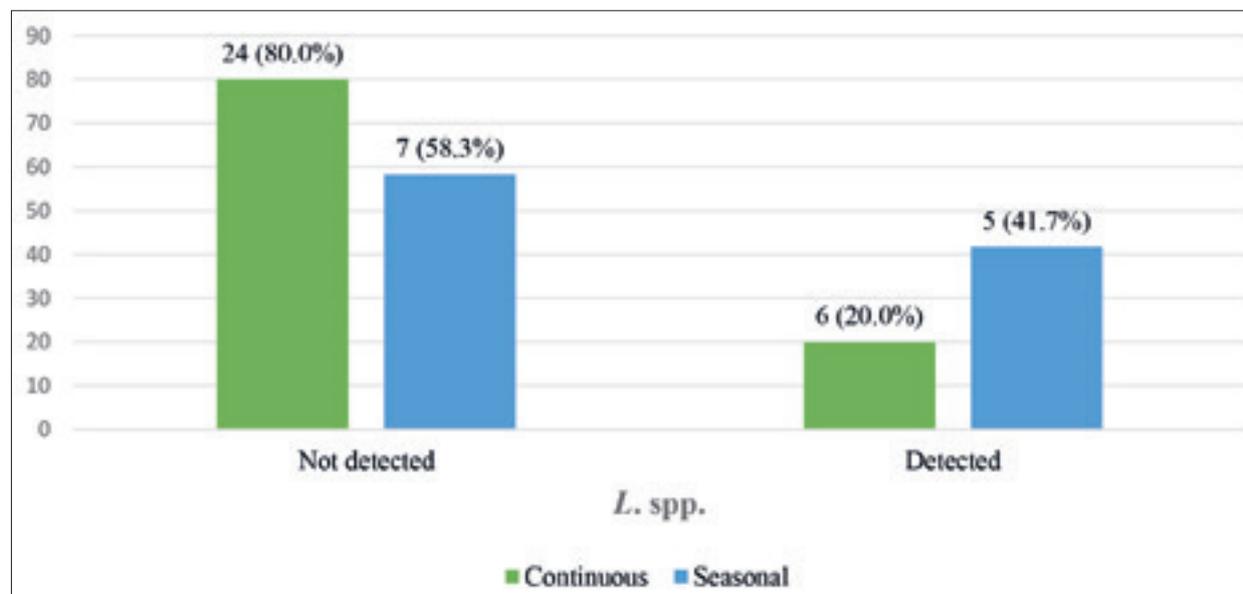
Table 4. Association of air conditioning device design and maintenance with colonization of *L. spp.* – individual air conditioning devices (N = 37)

Survey question and answers collected in the facilities		<i>L. spp.</i>		Total	
		Not detected	Detected		
The project was signed by an authorized person $\chi^2 = 0,680$; $p = 0,410$	No	N	26	2	28
		%	92.9	7.1	75,7
	Yes	N	9	0	9
		%	100.0	0.0	24,3
Having the necessary documentation $\chi^2 = 0,680$; $p = 0,410$	No	N	26	2	28
		%	92.9	7.1	75,7
	Yes	N	9	0	9
		%	100.0	0.0	24,3
Service frequency $\chi^2 = 2,232$; $p = 0,328$	Once a year	N	6	0	6
		%	100.0	0.0	16,1
	Twice a year	N	13	0	13
		%	100.0	0.0	35,1
	As needed	N	16	2	18
		%	88.9	11.1	48,6

Having a contract with an authorized service $\chi^2 = 0,566$; $p = 0,452$	No	N	9	1	10
		%	90.0	10.0	27,0
	Yes	N	26	1	27
		%	96.3	3.7	73,0
Total		N	35	2	37
		%	94,6	5,4	

L. spp. - bacteria from the genus *Legionella*.

Chart 1. Distribution of facilities according to the dynamics of air conditioning use and the connection with the colonization of *L. spp.* – centralized air conditioning devices (N = 42)



$\chi^2=2.082$, $p=0.243$, *L. spp.* - bacteria from the genus *Legionella*

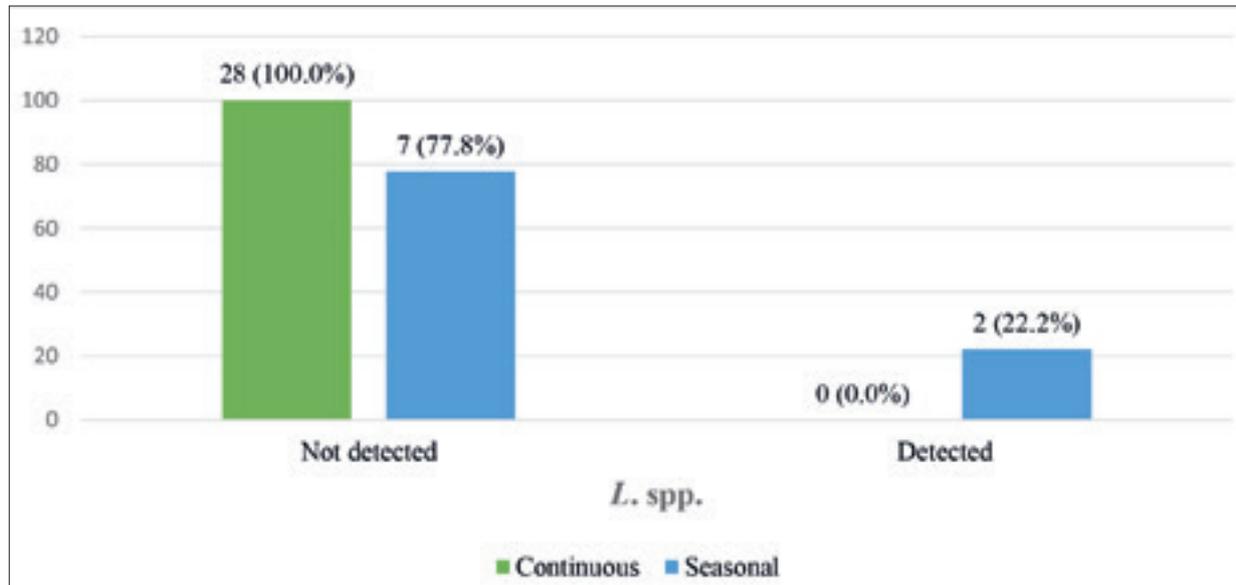
Chart 1. shows the collected survey responses related to the need to use centralized air conditioning devices. The analysis was conducted based on the obtained findings on *L. spp.* and survey responses. The obtained data indicate the possibility of colonization with *L. spp.* in both categories of investigated facilities, but the result of statistical analysis using the chi-square test shows that there is no statistically significant difference ($p>0.05$).

The collected survey responses related to the need to use individual air conditioning devices are presented. The analysis was performed based on the obtained findings on *L. spp.* and survey responses. The colonization with *L. spp.* was not present in air conditioning devices that are used continuously, with the result of the statistical analysis using the chi-square test showing that there is no statistically significant difference ($p>0.05$) (Chart 2).

DISCUSSION

The focus of this research, in accordance with the set goals, was to expand knowledge about the factors that influence the *L. spp.* colonization in air conditioning devices. Previously the relationship between the physical and chemical parameters of water in air-conditioning devices and the *L. spp.* colonization was examined, where the results showed that there is a connection between higher temperature and lower concentration of residual chlorine, and higher concentrations of iron and copper, with the *L. spp.* colonization in air-conditioning

Chart 2. Distribution of buildings according to the dynamics of air conditioning use and the connection with the colonization of *L. spp.* – individual air conditioning devices (N = 37)



$\chi^2 = 6,578$; $p = 0,054$; *L. spp.* - bacteria from the genus *Legionella*

devices (Vukotić et al., 2025). Our research included two categories of air conditioning devices, which were analyzed separately due to different requirements and complexity. Results showed a correlation between the age of the air conditioning devices and *L. spp.* colonization in both groups of investigated air conditioning devices ($p < 0.05$). The results of a study conducted in the Split-Dalmatia County, where hot water from the water supply system was tested for the presence of *L. spp.*, also indicate a correlation between the age of the system and bacteria colonization (Rakić, 2014). It is characteristic of an older plumbing installation that if the bacterium survives the water treatment in very small numbers, growth and reproduction will continue as soon as it acquires favorable conditions (Barna, et al., 2016). In contrast, the first case of Legionnaires' disease in Bosnia and Herzegovina occurred towards the end of the 1980s, and the source of the infection was an air conditioning device in a newly built hotel, which indicates the possibility of colonization by *L. spp.* regardless of the device age (*Legionella* is nothing new and there is no threat of an epidemic, 2024). Regarding the materials of the distribution installation, our results showed the correlation between the steel distribution installation with the *L. spp.* colonization of the air conditioning unit ($p < 0.05$). Based on the results of research in the area of Split-Dalmatia County, a higher concentration of *L. spp.* was found in water samples from galvanized distribution installations compared to plastic ones. The explanation given is that on the smooth inner surfaces of plastic pipes there is less possibility of biofilm attachment and greater possibility of mechanical tearing due to increased water flow (Rakić, 2014). Different interactions with the environment affect the deterioration of material properties, which is especially true for most metals. Metals have the ability to return to their original oxidized state and this process is inevitable (Shaw & Kelly, 2006). Increased concentrations of oxidants and appropriate water flow reduce the concentration of iron released from corroded pipes (Sarin et al., 2004). Water supply systems use water in a temperature range that is favorable for the proliferation of *L. spp.* During operation, aerosols are produced, and in addition, water often stand still and corrosive deposits are present, phenomena that favorably affect the growth and reproduction of *L. spp.* (Martin et al., 2020). Lack of water flow allows the deposition of sludge, from which bacteria draw nutrients. Sludge also contributes to the occurrence of corrosion. Furthermore, all this leads to inhibition of biocides and they cannot reach all parts of the water system in the appropriate concentration (*Legionella* risks – identification and analysis: Section 6., 2024). By analyzing several available sources, it was found that the importance of the design and project of the distribution installation, professional installation, maintenance and regular servic-

ing of air conditioning devices is indicated in order to avoid the risks of the appearance of *L. spp.* and other contaminants, as well as other consequences caused by neglecting the above (Ghernaout et al., 2022, Principle of operation of air conditioners, 2024, López-Gómez et al., 2012, 10 most common malfunctions of air conditioners, 2024). The results of our research on these issues and the connection with the settlement of *L. spp.* did not show statistically significant differences ($p > 0.05$), which refers to both categories of investigated air conditioning devices. It is evident that the colonization of bacteria is not excluded, and that the *L. spp.* colonization is more frequent in facilities where air conditioning devices are serviced less often. Preventing the formation of biofilm in the distribution network of water systems is an important measure when it comes to the proliferation of *L. spp.* The connection of biofilm with installation projects has been established, due to redundant pipelines and blind corners. Also, a bad design leads to still water and poor flow, which disrupts the necessary water temperature regime (Ontario Agency for Health Protection and Promotion, 2019). It is considered that individual air conditioning devices do not pose a risk in terms of colonization with *L. spp.* (We've busted the *Legionella* myth: No, the air conditioning in your home won't transmit the dangerous bacteria to you, but something else might, 2025). Although our results showed a low prevalence of facilities in which *L. spp.* colonize an individual air conditioning device, the possibility is not excluded. It is evident in older devices and devices that are less frequently serviced. Irregular maintenance and servicing of certain components of the device can cause the formation of a watery environment in the indoor unit (Air Conditioning. The complete guide for home owners, 2024). It is common for ice to form on the evaporator of the indoor unit. Possible reasons for this are lack of gas, dirty filters or fan turbine (10 most common malfunctions of air conditioners, 2024). A relative humidity of 60% has a positive effect on the survival of this bacterium (Legionnaires' disease outbreak investigation toolbox, 2024). During servicing, replacement or cleaning of air conditioning device filters is a mandatory part of the process. Filter materials of air conditioning devices as well as low humidity reduce the risk of microbial contamination (Ventilation system hygiene. A review of published information on the occurrence and effects of contamination, 2024). By analyzing samples from car air filters for *L. spp.*, the findings showed that *L. ph.* was isolated in a third of the samples (Alexandropoulou et al., 2013). When it comes to centralized air conditioning systems, HEPA filters have been used in practice for many years. This type of filter is easily damaged, so it is necessary to pay attention when installing it and to check and change it often. It is important that they are firmly placed in the housing to avoid the risk of leaking unfiltered air into the room (Anil, 2008). In addition, it is necessary to continuously invest in the education of all persons who are in any way connected with the air conditioning processes, primarily in connection with the issue of implementing the correct practice of device maintenance and an increased level of knowledge about the consequences of the occurrence of *L. spp.* (Acquaye, et al., 2020). Regarding the need to use an air conditioning device, our results did not show a statistically significant difference between the two groups of facilities and the type of air conditioning device ($p > 0.05$), which was also confirmed by the results of the research in the Split-Dalmatia County. Based on the conclusions of the mentioned research, the concentration of bacteria in samples from facilities that work seasonally is much higher than in the other group of samples (Rakić, 2014). During the coronavirus (COVID-19) pandemic, tourist facilities in Federation of Bosnia and Herzegovina were closed for a long period of time. Before reopening, and in relation with the issue of *L. spp.*, the Ministry of Health of the Federation of Bosnia and Herzegovina and the Institute of Public Health of the Federation of Bosnia and Herzegovina issued guidelines to all responsible persons in order to minimize the risks of legionella infection in such facilities. Some of the operational tasks should have been performed before the actual opening of the facility, accompanied by an educated person from the area. Mechanical cleaning, hyperthermia and hyperchlorination of all risky places and places that are subject to the formation of scale, sediment or biofilm are recommended before opening the facility (Basic guidelines for managers of hotels and other accommodation units on actions to be taken before reopening facilities in order to reduce the risk of infection with bacteria of the

genus *L. spp.* during the COVID-19 pandemic, 2021). By analyzing the regulations of some European countries regarding air conditioning devices and the prevention of *L. spp.* colonization, it seems that in Germany the most clearly defined regulations, guidelines and recommendations are based on which air conditioning devices and the *L. spp.* colonization. Recommendations of the Federal Office for Environmental Protection (Verein Deutscher Ingenieure, 2018). When it comes to the limitation of this research, it can be taken into consideration the reliability of the data collected using the survey questionnaire, which depended on the honesty of the respondents in the facility and the correct understanding of the questions asked. In addition, there was no verification that hygienic measures were applied in practice, nor were the detection of biofilms and dead pipes was included as a project task. Based on the evidence so far, factors for the survival of *L. spp.* which are very important. In accordance with the set goals of the research, the lack of investigation of these factors can represent a significant limitation and affect the reliability of the obtained results.

CONCLUSION

Based on the analysis of the collected survey data and laboratory findings, as well as the summarized results of this research, it is possible to interpret the data in accordance with statistically significant differences. Regarding the age and material of the distribution installation of the air conditioning device, positive correlations were established with the *L. spp.* colonization. Statistically significant differences exist in the group of air conditioning devices older than 10 years, and steel distribution installations, which indicates that the age of the device and the material of the air conditioning installation positively correlates with the colonization with *L. spp.* Regardless of the distribution of the examined air conditioning devices, in terms of design, maintenance and dynamics of use, the results of this study did not show statistically significant differences. It is evident that bacteria colonize the air conditioning device, regardless of the design or maintenance dynamics, although it is less likely if the device is serviced several times during the year. Furthermore, regarding the dynamics of the use of the air conditioning device, the bacteria colonization was not established in the group of buildings where the device is used continuously, and only when it comes to individual air conditioning devices. From a public health perspective, the results of the research enable the identification of risk factors for *L. spp.* colonization in air conditioning devices, which contributes to the prevention of legionellosis and the protection of the health of all facility users. Furthermore, the presented results from the technical aspect can be useful because they indicate the importance of regular and adequate maintenance of the air conditioning devices, and based on which it is possible to improve the maintenance practice and contribute to greater safety and efficiency of the system. Recommendations refer to the modernization of older devices or damaged components, continuous monitoring program of air conditioning devices, regular and documented servicing and staff training. In addition, it would be desirable to continue the research on a larger sample of objects with additional objectives, for the sake of a more complete understanding of the factors that positively influence the *L. spp.* colonization in air conditioning devices.

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Conflict of Interest

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

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