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THE CONTAMINATION OF HOSPITAL WATER SUPPLY SYSTEMS BY *LEGIONELLA PNEUMOPHILA*

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The risk of severe infectious complications associated with provision of medical care continues to be a pressing issue in modern surgery. *Legionella pneumophila*, characterized by its wide distribution in water supply systems and is highly active in film formation, represents a dangerous/important cause of hospital-acquired pneumonia. Patients requiring immunosuppression, including organ transplant recipients, are in the special risk group. Prevention of hospital-acquired legionellosis in patients at risk is essential due to severe clinical manifestations and high mortality. **Objective:** to summarize the practical experience in detecting contamination of water supply systems by *Legionella pneumophila* strains in multidisciplinary hospitals in Moscow. **Materials and methods.** Isolation of *Legionella pneumophila* strains from water and biofilms of water supply systems in multidisciplinary hospitals in Moscow and serotyping of this pathogen using bacteriological, molecular genetic and enzyme immunoassay methods. **Results.** *Legionella pneumophila* content in water reached high levels. The peculiarities of *Legionella pneumophila* contamination of hot water supply systems included formation of stable biofilms, in which other hospital-acquired pathogens were also identified. The share of *Legionella pneumophila* "SG 1", which causes up to 80% legionellosis cases in the world, was 13% in the water of the hospitals surveyed. The most effective measures for prevention of legionellosis are actions aimed at ensuring water biosecurity. **Conclusion.** There are potential risks of disease in the surgical wards of hospitals providing medical care, including in immunocompromised patients. Due to potential risks, prevention of hospital-acquired legionellosis is a necessary component of ensuring the safety of treatment for immunosuppressed patients.

Keywords: Legionella pneumophila, legionellosis, biofilm, water biosecurity.

Prevention of health care-associated infection (HCAI) is considered an integral component of patient safety during hospital stay. However, if the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacte*r species) pathogens, as the most aggressive causative agents of severe infectious complications, are well known, it is not sufficient for clinicians to just have a knowledge about *Legionella pneumophila* and legionellosis caused by this pathogen as a hospital-acquired infection.

Currently, the incidence of legionellosis is 1.1 cases per 100,000 of the population in the European Union and 1.62 cases per 100,000 of the population in the United States [1]. The risk group includes patients over 25 years old in hospitals where immunosuppressive therapy is actively used (departments of transplantology, oncology, intensive care, burns, surgical, etc.); patients with diabetes, cardiovascular diseases, respiratory failure; patients whose treatment is accompanied by intubation and lung ventilation; mortality in this group of patients may reach 40–60% [2, 3].

Legionella's high adaptive capacity allows it to colonize artificial water systems; water supply systems and medical equipment serve as reservoirs for the accumulation of the pathogen in cases of hospital-acquired legionella. Unlike planktonic forms, microorganisms that make up biofilms are more resistant to antibiotics and disinfectants [4].

Legionella infection is characterized by the development of multi-organ pathology in patients, high mortality, and absence of specific clinical symptoms, which makes it possible to be differentiated from severe pneumonia caused by other etiological agents [5].

The likelihood of legionellosis cases depends on the level of water contamination with the pathogen, efficiency of aerosols containing Legionella, the rate of spread of the pathogen, as well as the virulence of strains and patient's individual susceptibility. At the same time, the infectious dose of the bacteria required to infect a person cannot be named, since it also depends on the ratio and

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interaction of the listed factors and can vary significantly under specific conditions [6].

All water supply systems are characterized by biofouling processes, or biofilm formation on the inner surfaces of pipes, which leads to secondary microbial contamination of water. In closed water systems, biofilms form on the inner surface of pipes, in shower heads, faucets, and various water filters. The most intensive legionella colonization is shown for rubber and plastic surfaces. However, metal corrosion, which is very common in hot water supply systems with metal pipes, also promotes reproduction of microorganisms to concentrations unusual for natural ecological niches and dangerous for humans [7].

Objective: to summarize the practical experience of identifying contamination of water supply systems by *Legionella pneumophila* strains in multidisciplinary hospitals in Moscow.

MATERIALS AND METHODS

The study was based on the results of bacteriological examination of hot water supply systems in 7 major multidisciplinary hospitals in Moscow; samples were taken in operating rooms, organ transplantation wards, hematology, burns and intensive care units (hereinafter referred to as risk wards).

The applied algorithm for epidemiological assessment of the state of water supply systems consisted of 3 stages:

1) Conducting a preliminary assessment of the epidemiological hazard of the facility. (water screening test using real-time polymerase chain reaction).
2) Assessment of the presence of contamination using a bacteriological method, which determines the exact concentration of the pathogen.
3) Epidemiological hazard assessment based on serotyping of isolated Legionella pneumophila strains.

During sampling, preliminary disinfection of the tap and water drainage were not carried out, which does not contradict the requirements by the National Standard of the Russian Federation "Drinking water. Sampling at water treatment plants and pipeline distribution systems." GOST R 56237-2014.

Samples of water, biofilms, washings from hot water supply systems were investigated in accordance with methodological guidelines MUK 4.2.2217-07 "Detection of *Legionella pneumophila* bacteria in environmental objects" by bacteriological method on BCYE medium using latex agglutination kits SLIDEX (Biomerieux, France), as well as RT-PCR using AMPLI-LEG-RV (CJSC Syntol), a test system for quantitative detection of *Legionella pneumophila*.

Serotype characteristics of the isolated strains were studied using an international panel of monoclonal antibodies by enzyme immunoassay. Monoclonal antibody panel was provided by Dr. J. Helbig and Dr. K. Luck (German Reference Center for Legionellosis, Institute of Medical Microbiology and Hygiene, Technical University, Dresden, Germany) [8].

The effectiveness of the final filtration method and the dynamics of biofilm formation involving *Legionella pneumophila* were evaluated using antimicrobial water filters (PallMedical, UK).

RESULTS AND DISCUSSION

The study examined the peculiarities of *Legionella pneumophila* contamination of hot water systems and identified the factors contributing to it.

Legionella pneumophila culture was isolated in the water distribution systems of all surveyed hospitals, in 14 out 18 surveyed buildings. The percentage of samples in which Legionella was isolated was 41%. The level of Legionella pneumophila contamination in the water supply system varied from 1.2×10^2 to 6.4×10^5 CFU/L. *Legionella pneumophila* concentrations exceeded the risk levels in half of the wards surveyed. Systemic colonization of water distribution networks by Legionella was detected in 4 hospitals (isolated in two or more sections of the water supply system).

In addition, in 9% of the water samples studied in association with *Legionella pneumophila*, microorganisms that are also pathogens of infections associated with health care were isolated: *Acinetobacter spp., Pseudomonas aeruginosa, Brevibacterium vesicularis, Micrococcus luteus*. Species diversity of the isolated microflora indicates that preventive measures taken to ensure water safety are not effective enough.

To determine the epidemiological hazard of potentially dangerous water distribution systems, the serotypic characteristics of the isolated strains of *Legionella pneumophila* were studied. The study confirmed the serotypic diversity of Legionella circulating in hot water systems. The isolated strains belonged to 12 out of 15 *Legionella pneumophila* serotypes. It was found that in 87% of cases, contamination with SG 6 (44%, $p < 0.01$) and SG 5 (26%) serotype strains was detected. The proportion of strains of the first serotype is significantly lower – 13% ($p < 0.01$).

Due to significant contamination of water distribution networks, the epidemiological risk for patients remains significant, because the disease can be caused not only by the Legionella pneumophila serotype SG 1, but also by others. Legionella, which is part of the biofilm, can cause nosocomial *Legionella pneumonia* due to water aspiration by the patient, because the dose of the pathogen capable of causing disease in people with reduced immunity is much lower than for healthy people.

To evaluate the final filtration method, sections of water supply system were selected. A sufficiently high level of contamination with *Legionella pneumophila* serotype SG 1 (more than 10^3 CFU/L of water) was detected.

In the present study, the following pathogen content values in water were taken as reference:
- For non-risk hospital wards, *Legionella pneumophila* level in water was 10^3 CFU\L,
- For risk wards, no *Legionella pneumophila* in water.

For hot water supply systems, a moderate degree of risk occurs when the quantitative level of Legionella in water is 10^3 CFU/L, a high degree of risk occurs at 10^3 CFU/L levels and above. A high degree of risk occurs even in the presence of the pathogen of not only SG 1 *Legionella pneumophila* in water, but also other serotypes and species of Legionella.

The study showed the possibility of completely eliminating *Legionella pneumophila* by means of additional water treatment with 9.9×10^3 CFU/L pathogen levels. There was no additional water protection at the intake points, and the original level of contamination remained. After changing the filter, biofilm samples were examined. Separate microcolonies formed on the outer surface of the filter already on the second day. After a week, biofilm formed on the filter surface. In the biofilm structure, various aquatic microorganisms were isolated, including HCAI pathogens: *Legionella pneumophila, Pseudomonas aeruginosa, Pseudomonas spp., Acinetobacter spp.* and etc.

Analysis of the incidence of legionellosis indicates that the proportion of healthcare-associated legionellosis in different countries ranges from 5 to 20% among all cases of legionella infection. In the United States, health care-associated legionellosis accounts for 23% of all reported legionella infections, with deaths ranging from 9% to 100%. In Italy, pneumonia resulting from legionella is 7.1% of the total number of registered hospital-acquired pneumonia (33.3% mortality in 2008). In the Netherlands, over a ten-year observation period, it was found that 6% of legionellosis cases were associated with transmission in medical institutions [9, 10].

Measures to ensure water safety and prevent legionellosis have become an obligatory component of the prevention of hospital-acquired infections in the United States, Europe, Japan, etc. Prevention is regulated by relevant documents at the national and regional levels aimed at constant monitoring of Legionella levels in water. The effectiveness of these measures is evidenced by the absence of major outbreaks associated with this pathogen. However, foci are periodically recorded in 10–30 cases [6].

Despite species diversity of the *Legionellaceae* family, numbering 50 species, over 90% of cases are caused by the *Legionella pneumophila* species. SG 1 strains cause up to 80% of infections in immunocompromised individuals [11].

External risk factors for the colonization of water systems are: water quality that does not meet the established requirements for both microbiological and chemical indicators; problems in the water distribution system (stagnation and slow flow); piping and tank materials that promote bacterial growth and biofilm formation; insufficient or ineffective water disinfection; water temperature within 25–50 °C; presence of biofilm; aerosol formation; inadequate training of personnel in the maintenance of water distribution systems and prevention of legionellosis [12].

Contamination of medical equipment and instruments associated with intubation and ventilation procedures, surgical intervention, and parenteral nutrition of the patient poses a danger [13]. Inhalation of Legionella-contaminated aqueous aerosol by a patient can occur if breathing devices, ventilation tubing, and nebulizer compartments are rinsed or filled with tap water. Cases of patients being infected during dental procedures and by aspiration during feeding through a nasogastric tube in which enteral feeding mixtures, diluted with water infected with Legionella (SG 6 serotype), were introduced, have been described [14].

In immunocompromised patients, delaying initiation of therapy significantly worsens the prognosis. On average, it takes 8 days from the start of treatment to the response to legionellosis therapy in cancer patients [15]. So, a delay in antibiotic therapy even for a day reduces the effectiveness of treatment; timely diagnosis of legionellosis provides adequate therapy and reduces mortality rates.

Methods of laboratory diagnosis of the infection are standardized on the basis of 3 main methodological approaches: isolation of the pathogen from the detachable lower part of the respiratory tract; identification of a 4-fold or more increase in antibody titers in the serum of patients; determination of soluble legionella antigen in the urine of patients in the acute stage of the disease. Introduction of diagnostic standards in many countries has significantly improved the quality of laboratory diagnosis of infection, which has contributed to increased number of detected epidemic outbreaks and sporadic cases of legionellosis [16].

According to G.M. Galstyan et al., if urine tests are negative for legionellosis in patients with severe pneumonia, then bronchoalveolar lavage examination is necessary to obtain a sufficient number of alveolar macrophages in which legionella reproduce [17, 18].

Disinfection of potentially contaminated water systems and reducing Legionella in them to safe levels is the only real way to prevent legionellosis. The choice of disinfection method is based on analysis of the sensitivity of legionella to various chemical and physical factors. Sodium hypochlorite, monochloramine, chlorine compounds, etc. are usually used for water disinfection [19].

In addition, there is special focus on disinfection and control of medical equipment and instruments in the risk wards, where intensive immunosuppressive therapy is actively used. Operation of medical devices such as a nebulizer is monitored. If the legionellosis pathogen is isolated from water, additional methods of disinfection of water supply system are used or antibacterial filters are installed at the end points of the water intake in the ward. Restrictions are imposed on patients visiting the shower against the background of severe immunosuppressive therapy; patients who have undergone stem cell or solid organ transplantation are encouraged to use sterile water for brushing teeth, drinking and for rinsing nasogastric tubes; tap water is prohibited in the wards of patients at risk to avoid legionella-contaminated aerosol [20].

Taking into account the need to ensure the safety of water, the Russian Government approved Decree No. 10 dated January 6, 2015 "On the procedure for implementation of production control of the quality and safety of drinking water, hot water". Based on this law, organizations operating water supply systems are required to carry out departmental control of indicators approved by regulatory documents.

Depending on the epidemic situation at the facility, there are three periods of operation of artificial water systems:

– Safe period of operation;
– Risk period when test results indicating epidemically significant levels of Legionella in water are reported;
– Dangerous period when cases of legionellosis associated with colonization of water systems are reported.

In the safe period (in the absence of laboratory-confirmed legionellosis cases), it is necessary to:

– conduct staff training: instructing physicians to be more vigilant about probable cases of healthcare-associated legionellosis, as well as to use reliable diagnostic methods; instructing nursing staff, technical (engineering) staff on measures to prevent hospital-acquired legionellosis.
– control: cases of suspected healthcare-associated legionellosis; laboratory examination (analysis for the detection of antigen in urine, bacteriological analysis of bronchoalveolar lavage) of patients at high risk of infection with suspected legionellosis;
– control over the equipment used for clinical diagnostic laboratory with the necessary diagnostic systems;
– control of operation and disinfection of various medical devices; after cleaning and disinfection of nasal inhalers or other devices, including mechanical ventilation systems, use sterile water to rinse the device; in the absence of sterile water, use filtered water; Use only sterile water to fill the inhaler tank.

In the risk period, it is necessary to take measures provided for by the algorithm of actions during the safe period: to install special water filters in risk wards; to test water for legionella in risk wards within a year (monthly), or within 3 months in other wards.

In a danger period, it is necessary: to register cases of legionellosis or suspicions of legionellosis according to established procedure; exclude the use of potentially colonized water systems by patients from risk wards and risk groups; discuss the results of the investigation of the hotbed of the disease at the commission for the prevention of nosocomial infections; conduct a retrospective epidemiological investigation by analyzing the microbiological, serological and autopsy results to identify possible previous cases.

Thus, general hygienic and disinfection measures can significantly reduce the colonization of water supply systems, while the assessment of the initial level of contamination contributes to determining the most effective strategy for preventive measures. The most effective measures from the standpoint of preventing legionella infection are those aimed at ensuring the biological safety of water.

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REFERENCES

1. *Torres A, Blasi F, Peetermans WE, Viegi G.* The aetiology and antibiotic management of community-acquired pneumonia in adults in Europe: a literature review. *Eur J Clin Microbiol Infect Dis.* 2014; 33: 1065–1079.
2. *Campese C, Decludt B.* Notified cases of Legionnaires disease in France. *Eurosurveillance*. 2004; 7: 121–128.
3. *Luderowski E, Harris CM, Khaliq W, Kotwal S.* Severe Atypical Pneumonia Causing Acute Respiratory Failure. *Am J Med.* 2020; 133 (6): e230–e232. doi: 10.1016/j.amjmed.2019.10.034.
4. *Olson M, Ceri H, Morck DW et al.* Biofilm bacteria: Formation and Comparative Susceptibility to antibiotics. *Can J Vet Res*. 2002; 66: 86–92.
5. *McCormick D, Thorn S, Milne D et al.* Public Health response to an outbreak of Legionnaires Disease in Edinburgh. United Kingdom. *Eurosurveillance*. 2012; 17 (28): 200–216.
6. Legionella and prevention of Legionellosis / Ed. by J. Bartram. *WHO*. 2007.
7. *Colbourne JS, Ashworth J.* Rubbers, waters and Legionella. *Lancet*. 1986; 2 (8506): 583.
8. *Dronina YuE, Tartakovskij IS, Lyuk K, Hel'big Yu, Sadretdinova OV, Karpova TI i dr.* Monoklonal'nye antitela k liposaharidu legionell i ih znachenie dlya tipirovaniya vozbuditelya. *ZHizn' bez opasnostej. Zdorov'e. Profilaktika. Dolgoletie*. 2010; 4: 122–125.
9. Legionellosis-USA (New Jersey) Nosocomial, fatal. *ProMED-mail, Newsday*. 2008, Oct. 3.
10. *Den Boern JW, Euser SM, Brandsema P et al.* Results from the National Legionella Outbreak Detection Program, the Netherlands, 2002–2012. *Emerging Infectious Diseases*. 2015; 7: 1167–1173.
11. *Chuchalin AG, Sinopal'nikov AI, Tartakovskij IS i dr.* Prakticheskie rekomendacii po diagnostike i lecheniyu legionelleznoj infekcii, vyzyvaemoj *Legionella pneu-*

mophila serogruppy 1. Posobie dlya vrachej. *Rossijskoe respiratornoe obshchestvo*. M.: MAKMAH, 2009.
12. *Borellia P, Montagna M, Romano-Spica V et al.* Legionella infection risk from domestic hot water. *Emerg Infect Dis*. 2004; 10: 457–464.
13. *Ryder MA*. Catheter-related infections: it's all about biofilms. *Topics in advanced practice nursinge Journal.* 2005; 5 (3): 1–6.
14. *Venezia RA, Agresta MD, Hanley EM et al.* Nosocomial legionellosis associated with aspiration of nasogastric feedings diluted in tap water. *Infect Control Hosp Epidemiol*. 1994; 15: 529–533.
15. *Jacobson KL, Miceli MH, Tarrand JJ, Kontoyiannis DP. Legionella pneumonia* in cancer patients. *Medicine (Baltimore).* 2008; 87 (3): 152–159.
16. *Tartakovskij IS, Gincburg AL, Lazikova GF i dr.* Standarty laboratornoj diagnostiki legionelleza i ih primenenie vo vremya epidemicheskoj vspyshki pnevmonij v g. Verhnyaya Pyshma. *ZHurnal mikrobiologii, epidemiologii i immunobiologii*. 2008; 2: 16–20.
17. *Galstyan GM, Kostina IE, Katrysh SA i dr.* Klinicheskie proyavleniya legionelleznoj pnevmonii u gematologicheskih bol'nyh. *Terapevticheskij arhiv.* 2014; 86 (3): 45–52.
18. *Tartakovskij IS, Gruzdeva OA, Galstyan GM, Karpova TI.* Profilaktika, diagnostika i lechenie legionelleza. M.: Studiya MDV, 2013. 344 p.
19. *Thomas V, Bouchez T, Nicolas V et al.* Amoebae in domestic water systems: resistance to disinfection treatments and implication in Legionella persistence. *J Appl Microbiol*. 2004; 97 (5): 950–963.
20. Guidelines for preventing health-care associated pneumonia 2003: recommendation of CDC and the Health care Infection Control Practices Advisory Committee. *MMWR Recomm Rep.* 2004; 26, 53 (RR-3): 1–36.

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