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**RISK OF HOSPITAL-ACQUIRED LEGIONELLOSIS  
FROM MICROBIAL CONTAMINATION OF POTABLE WATER  
AT A BONE MARROW TRANSPLANT UNIT IN A CZECH  
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*The investigated of the potable water treatment room and the sanitary facilities of patient boxes was held. The potable water treatment room had three collection points (ball valves), while in the sanitary facilities potable water was collected from the tap, shower, and the flush tank. A swab was taken from the inside wall of the toilet tank. The samples and swabs of the flush tank water were Legionella pneumophila sg 1 and sg 6A positive. Disinfection of flush tanks with chlorine agents was recommended.*

**Keywords:** hematopoietic stem cell transplantation, *Legionella* spp., potable water, terminal filters.

**Introduction**

*Legionella* spp. is Gram-negative coccobacilli that are ubiquitous in aquatic and moist environments (soil), in association with amoeba, other protozoa, and in biofilm [1]. They can be isolated from water with temperatures ranging from 6 to 60 °C. Growth occurs optimally in the temperature range of 25 to 42 °C, especially when the water is stagnant. The *Legionellaceae* family consists of a single genus *Legionella*, which contains 52 species, 20 of which are considered to be human pathogens. Subspecies belong to over 70 serogroups [2]. In humans *Legionella* spp. can cause Pontiac fever (self-limited flu-like illness) and Legionnaire's disease (severe pneumonia with multisystem dysfunction). Legionnaire's disease (LD) occurs as sporadic cases or as outbreaks and is either community or hospital-acquired [3]. Hematologic malignancies and immunodeficiency are typical risk factors for legionellosis. In most instances, *Legionella* spp. is transmitted to humans by inhalation of aerosol containing

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the bacteria. *Legionella pneumophila* is associated with 90 % of human disease, and within the 15 serogroups, *L. pneumophila* sg 1 causes more than 84 % of LD worldwide [4]. The majority of LD cases in Europe are caused by *L. pneumophila* sg 1, 3 and 6 [5]. In 2008, a total of 5,789 cases of LD were reported across 28 EU and EEA/EFTA countries. The main age group affected is > 65 [6]. In 2010, the Czech Republic reported a total of 43 cases of legionellosis. From 2001 to 2010 a total of 163 cases of legionellosis were reported [7]. Hospital water supplies often contain *Legionella* spp., representing thus a potential source of hospital-acquired infection (HAI) – nosocomial legionellosis. All water systems to which the patient is exposed during the incubation time of 2 to 10 days might be the source of the infection. Primarily, patients after autologous or allogeneic hematopoietic stem cell transplantation (HSCT) need to stay in "reverse isolation wards" (bone marrow transplant units) during the first few weeks after the transplantation in order to successfully survive. These patients are particularly susceptible to legionella infections due to the long intervals of neutropenia and abnormalities in cell-mediated immunity. The minimum number of microbes required to cause legionellosis is unknown. Concentration below 1 CFU/mL could lead to infection in transplant patients [8]. For these patients, "sterile water" free of bacterial contamination is prepared from potable water from the water system using special technology and a terminal tap water filter. In the bone marrow transplant unit, potable water from the hospital water system was used only to flush toilets.

### Experimental

Technology and equipment for the production of specially treated water – "sterile water" at the Bone Marrow Transplant Unit, Department of Hemato-Oncology, Olomouc University Hospital.

*Formerly used technology.* The basic water treatment technology can be divided into two parts: mechanical technology and chemical technology [9].

Mechanical technology consists of microfiltration, ultrafiltration and sterilizing filtration in cartridge filters made by "Millipore" (USA). Sterilizing filtration is conducted through a sterilizing filter with the micronage of 0.22 µm and represents the pipe of cold water. The water runs through the second sterilizing filter into two accumulating water heaters (made of stainless steel). Here, the sterilized water is warmed to 70 °C and distributed as the pipe of warm water. An end-point IONPURE filtration unit is mounted on the outlet spots (showers and water taps). The bacteria retention is secured

by a hydrophilic polysulfone microporous membrane with the pore sizes of 0,22  $\mu\text{m}$ .

The chemical technology secures the perfect disinfection of inlet water (potable water from the water system). Trichloroisocyanuric acid (TIKA) is used for disinfection and is automatically dosed in 0,2 % solution.

*Currently used technology.* Aqua Osmotic 100 kompakt (Aqua Osmotic Systems, CZE).

The device was installed in 2008. Purified through a mechanical impurity filter, the water from the water system flows into a water softener, which removes calcium and magnesium. This device is fully automatic. Tablet salt (NaCl) is added. The regeneration of ion exchange resin starts at 2:00 AM and lasts 3 h. The pre-treated water runs through a fine mechanical filter that needs to be replaced depending on the contamination level. It is recommended to check the filter once every 14 days. Impurities must not enter the inside of the filter. The carbon filter is replaced every 2 months. Free of mechanical impurities, the water flows through a solenoid valve to the pump. The pump increases the water pressure to a value required for reverse osmosis to begin. Subsequently the water enters reverse osmosis modules for desalination. The permeate clusters in the pipe in the middle of the module and the trapped salt and mineral molecules are drained away as a concentrate into the waste. The water then goes into the ion exchange column, which completes the water purification to less than 0,2 mcsm. The treated water flows through the UV lamp into a tank via a 0,22  $\mu\text{m}$  microbial filter. From the tank the water flows out under maximum pressure of 3.5 bar to the collection points. Behind the pump there are another two 0.22  $\mu\text{m}$  microbial filters: one on the branch leading to the boilers and the other on the cold water branch. The treated water complies with the United States Pharmacopeial Standards (USP), Purified Water [10].

*Environmental Sampling – Water Sampling.* As part of legionella surveillance, 8 samples of specially treated water by device Aqua Osmotic 100 kompakt (Aqua Osmotic Systems, CZE) – (4 showers, 4 faucets) were taken from the sanitary facilities of the individual patient rooms of the Bone Marrow Transplant Unit in March 2011. The showers and the faucets are equipped with point-of-use high purity water capsule filters (terminal filters) with 0,22  $\mu\text{m}$  pore size (Siemens, GER). In the sanitary facilities of rooms 1 and 3, samples of water from the toilet tank (water designated for flushing; cold potable water from the hospital water system) were taken. Temperatures of the samples taken in the sanitary facilities were within range 11,2 to 13,6 °C. In June 2011 a similar sampling (4 showers, 4 faucets) was conducted, with water being collected from the

flushing containers in all 4 sanitary facilities. In addition, 4 swabs were taken using a cotton swab from the inside wall of the flushing container. A separate sampling of water was carried out in the shower of the changeroom, which uses hot water treated with chlorine dioxide from the hospital water system. The sample was taken through the shower head. The sample was taken from the cold water systems. Temperature of the sample taken in the changeroom was 14,4 °C. All samples were taken into 500 ml sterile containers. The sampling of specially treated water by device Aqua Osmotic 100 kompakt (Aqua Osmotic Systems, CZE) and the collection of water from the shower of the changeroom were carried out in line with standards SN EN ISO 5667-1 [11], SN EN ISO 5667-3 [12]. However, the Czech Republic has no standard concerning flushing container water sampling.

*Microbiological Examination and Sequence-Based Typing.* The samples collected were kept at ambient temperature and protected from direct light when transported to the National Reference Laboratory for Legionella, to be examined in accordance with standards CSN ISO 11731 [13] and CSN ISO 11731-2 [14]. The samples were cultured on buffered charcoal yeast extract agar (BCYE) [13]. Results were expressed in colony-forming units (CFU) /100 mL.

The isolates were identified using serologic typing (serum panel *L. pneumophila* sg 1-16). In a *L. pneumophila* isolate from March 2011 the molecular typing was conducted using DNA sequencing – Sequence-Based Typing (SBT). Developed by the European Working Group for Legionella Infections (EWGLI: [www.ewgli.org](http://www.ewgli.org)) [15], SBT is applied to the epidemiological typing of clinical and environmental isolates of *L. pneumophila*. SBT is a simple, rapid, discriminatory and portable method for typing *L. pneumophila* strains.

Genomic DNA is extracted and then amplified using primers targeting seven specific gene loci (i.e. *flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA*, *neuA*). Following purification, amplicons are sequenced directly with forward and reverse primers and the resulting consensus sequences trimmed and compared to previously assigned allele numbers using the online database. Using a predetermined order (i.e. *flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA*, *neuA*), the combination of alleles is defined as a 7-digit allelic profile (e.g. 1,4,3,1,1,1,1) and a sequence type represented by a number (e.g., ST1).

## Results and discussion

In March 2011, 8 samples of specially treated water by device Aqua Osmotic 100 kompakt (Aqua Osmotic Systems, CZE) were taken, proving to be culture

negative, i.e. *Legionella* spp. free. At the same time, 2 samples of cold potable water were collected from the flushing container. The sample from isolation room no. 3 was culture-positive. *L. pneumophila* sg. 1 was established in an amount of 2 CFU/100 mL. This strain was subjected to typing using the SBT method with the following result: allelic profile 1,4,3,1,1,1,1; Sequence Type Match = ST1. In June 2011 8 samples of specially treated water by device Aqua Osmotic 100 kompakt (Aqua Osmotic Systems, CZE) were taken; all the samples were culture-negative, i.e. *Legionella* spp. free. At the same time, 4 samples of cold potable water were collected from the flushing containers. Samples from isolation rooms no. 1 (4 CFU/100 mL) and 4 (14 CFU/100 mL) were culture-positive, where *L. pneumophila* sg 6A was identified. In addition, 4 swabs from the inside wall of the flushing containers were taken. In isolation room no. 1, *L. pneumophila* sg 6A was cultured and identified (Table 1, 2).

Table 1. *Legionellae* isolated from the flushing toilet tank water (water not chemically treated)

Water from the flushing toilet tank				
Date	Isolation room nr.	Cultivation	Strain – serogroup type	CFU / 100 mL
16.03.2011	1	Negativ		
	3	Positive	<i>L. pneumophila</i> sg 1	2
01.06.2011	1	Positive	<i>L. pneumophila</i> sg 6A	4
	2	Negative		
	3	Negative		
	4	Positive	<i>L. pneumophila</i> sg 6A	14

Table 2. *Legionellae* isolated from the flushing toilet tank swabs

Swabs from the flushing toilet tank wall			
Date	Isolation room nr.	Cultivation	Strain - serogroup type
01.06.2011	1	Positive	<i>L. pneumophila</i> sg 6A
	2	Negativ	
	3	Negative	
	4	Negative	

A separate sampling of water from the changeroom (water from the hospital water system, treated with chlorine dioxide) proved to be culture-negative, i.e. *Legionella* spp. free. Temperatures of the samples taken in the sanitary facilities were within range 11,2 to 13,6 °C. Temperature of the sample taken in the changeroom was 14,4 °C. As confirmed by other studies, point-of-use high purity water capsule filters (terminal filters) with 0,22 µm pore size placed in the shower and the water faucet prevent *Legionella* spp. from entering the outlet water [16]. In agreement with the paper by Camps et al., our study also cultured the presence of Gram-negative nonfermenting bacteria in the outlet water. Of Gram-negative bacteria *Burkholderia cepacia*, *Pseudomonas* spp. and *Achromobacter* spp. were detected.

Hot-water piping in health care centres is very frequently contaminated with *Legionella* spp. (in the form of plankton or biofilm), which may lead up to lethal pneumonia – hospital-acquired legionellosis in immune-suppressed patients. As cold water is not usually treated (physically, chemically) it may become a very adequate vehicle for *Legionella* spp. survival. This fact is pointed out e.g. in the paper by Johanson et al. [17]. Our study also established the presence of *Legionella* spp. in cold water, untreated against the presence of *Legionella* spp. in the water system of the Olomouc University Hospital. Temperatures of the samples taken in the sanitary facilities were within range 11,2 to 13,6 °C. Temperature of the sample taken in the changeroom was 14,4 °C. Surveillance of hospital water systems is needed. During the last decade more patients with hemato-oncologic disorders and low or intermediate risk of febrile neutropenia have been discharged from the hospital before leukocyte recovery. In the domestic environment, tap water poses a new risk of infection [18]. *L. pneumophila* sg 1 causes 95 to 98 % of community-acquired LD [2]. *L. pneumophila* sg 6 is the second most common serogroup according to the frequency of isolation from clinical samples. Moreover, *L. pneumophila* sg 6 remains an important aetiology of nosocomial LD and may cause an infection through contaminated water system or medical instruments [19]. LD is normally acquired by the inhalation or aspiration of *Legionella* spp. from contaminated environmental source.

Our investigation was carried out in the first half of 2011 at the Bone Marrow Transplant Unit, Department of Hemato-Oncology, Olomouc University Hospital. When comparing the results of water sample testing while using the formerly used technology and currently used technology for the treatment of water designated for the transplant unit patients, *Legionella* spp. failed to occur in any one of the cases. In contrast, *Legionella* spp. was cultured during the epidemiology investigation held in 2011 in the cold water and the

biofilm in the toilets of sanitary facilities in patient cleanrooms. The unit uses untreated cold water from the water system. The system supplying cold potable water is not subjected to regular disinfecting. However, the results of our study show it may be contaminated with *Legionella* spp. Flushing the toilet generates a micro aerosol that may contain the above bacteria, therefore leading to an increased risk of nosocomial legionellosis. Water used for personal hygiene of the patients, leads through the device Aqua Osmotic 100 kompakt (Aqua Osmotic Systems, CZE). Throughout our investigation no case of pneumonia caused by *L. pneumophila* was recorded. We recommended application of a chlorinated disinfectant (Presept tablets) in the flushing container water, regular mechanical cleaning of the inside walls of the water tank to remove the biofilm and regular control of the water in the flushing container for the presence of *L. pneumophila*. Health centres that provide medical care for immunocompromised and transplant patients should routinely monitor their water system for the presence of *L. pneumophila* [20]. Another measure to prevent the occurrence of *L. pneumophila* in the water systems of health care centres is heating the circulating hot water in the system to 70 °C because this temperature kills *Legionella* spp. in three minutes [21]. A disadvantage of this method is the risk of scalding. In addition, increasing the hot-water temperature may warm up the cold water because of the heat exchange between the two systems. The result could be an increase in cold-water-associated *Legionella* spp. transmission. We believe that temperature of approximately 50 °C, typically recommended to inactivate *Legionella* spp. in the hot water system is absolutely insufficient [22]. The use of air humidifiers in health care centres represents one of the riskiest factors for the transfer of *L. pneumophila* [23]. The Bone Marrow Transplant Unit at the Olomouc University Hospital is air-conditioned with the help of a sanitary air-conditioning unit HYD HKBCA 0150 (Nickel, Prague, CZ). This unit works exclusively with fresh air. Air humidity is conditioned by the CONDAIR MATIC MC (Condaire, SUI) steam generator, where water steam is heated under increased pressure to above 100 °C, which guarantees the absence of any bacteria in the incoming air.

The dental unit waterlines are a source of healthcare acquired infection in the dental medicine. Transmission of infection from contaminated dental unit waterlines is by aerosol droplet inhalation in susceptible individuals. A small number of studies described infection in susceptible hosts with *Legionella* spp. or *Pseudomonas* spp. [24]. The problem of legionellosis globally has an increasing tendency, with the risk group including persons above the age of 65 [7]. In the past 10 years the USA alone recorded a rise by 217 % [25]. The

Czech Republic has likewise noted an upward trend. While in 2010 the Czech Republic reported a total of 43 cases of legionellosis, between the years 2001 and 2010 there was a total of 163 cases of legionellosis recorded [8].

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