Water Research 90 (2016) 71–78

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Prevalence of *Legionella* in premise plumbing in Hungary

Zsófia Barna, Mihály Kádár, Emese Kálmán, Anita Scheirich Szax, Márta Vargha*

Department of Water Hygiene, National Public Health Center, Directorate of Environmental Health, Albert Flórián út 2-6, H-1097 Budapest, Hungary

ARTICLE INFO

Article history:
Received 12 July 2015
Received in revised form
30 November 2015
Accepted 3 December 2015
Available online 10 December 2015

Keywords:
Legionella spp.
Water quality
Premise plumbing
Hot water
Water temperature
Source water

ABSTRACT

Legionella is one of the emerging concerns of water quality in built water environments. Premise plumbing systems are among the recognised sources of infection. In the present study, colonisation of hot water networks in health care facilities, schools, hotels, private residences, office and industrial buildings was investigated. Data was analysed in connection with building and premise plumbing characteristics. Over 60% of all buildings were colonised by *Legionella*; counts were over 1000 CFU/L in 49%. The most prevalent type was *Legionella pneumophila* serogroup 2–14, isolated from 75% of the positive samples. Centrally produced hot water was found to be a key risk factor (46% of the samples were positive vs. 16% in individual systems); within this group the type of the building was less relevant. Colonisation levels in schools were similar to hotels or hospitals, representing a previously underestimated risk setting. Systems supplied by water from deep groundwater sources were significantly less likely to be colonised than more vulnerable sources (bank-wall filtration, surface water abstraction or karstic water; 28% vs. 51% positive), regardless of the type of treatment applied, including the presence of disinfection. The aggravating effect of larger, more complex and older buildings on colonisation was also confirmed.

The present study represents the first baseline analysis, pre-empting regulation or monitoring requirements for *Legionella*. The prevalence of legionellae and the identified risk factors are indicative for other settings lacking targeted interventions. The statistically confirmed risk factors can serve as indicators for preliminary risk assessment and the prioritisation of interventions.

1. Introduction

Legionellae are one of the emerging water-borne pathogens, causing respiratory infections including severe pneumonia known as Legionnaire's disease. Legionellae are present in most natural water environments. However, they predominantly pose a risk of infection in built water systems where conditions are favourable for their proliferation and *Legionella*-containing aerosol is generated. Though the largest outbreaks reported to date were associated with cooling towers and spa pools, premise plumbing is also an important source of infection (den Boer et al., 2002; Shivaji et al., 2014). In Hungary, majority of the reported cases were linked to the hot water network of large buildings, mainly hospitals and hotels (ECDC, 2015).

Premise plumbing installations in these buildings are complex structures, where the distance between the boiler room and the distant taps can amount to hundreds of meters. Successive reconstruction works tend to leave dead end pipelines behind, providing optimal growth conditions for legionellae. Most reports on *Legionella* prevalence in premise plumbing systems concentrate on the health care facilities, accommodation sites or private residences, while educational institutions and industrial settings receive limited attention (Leoni et al., 2005; Mouchtouri et al., 2007; Mathys et al., 2008; Bargellini et al., 2011).

Previous studies suggest that the contamination of premise plumbing systems is mostly driven by water temperature (Zacheus and Martikainen, 1994; Straus et al., 1996). Various other parameters, such as pipe materials, flow circumstances, stagnation, pipe corrosion, some trace elements and the presence of other microorganisms are well characterised factors influencing the growth of legionellae (Stout et al., 1985; Rogers et al., 1994; Serrano-Suarez et al., 2013), though the outcomes are often ambiguous. Other potential factors in *Legionella* colonisation, such the drinking water source, have received limited attention so far (Vickers et al., 1987; Zacheus and Martikainen, 1994). Though it is a general belief that

* Corresponding author.
E-mail addresses: barna.zsofia@oki.antsz.hu (Z. Barna), kadar.mihaly@oki.antsz.hu (M. Kádár), kalman.emese@oki.antsz.hu (E. Kálmán), szax.anita@oki.antsz.hu (A. Scheirich Szax), vargha.marta@oki.antsz.hu (M. Vargha).

http://dx.doi.org/10.1016/j.watres.2015.12.004
0043-1354/

large plumbing networks provide optimal circumstances for the growth of *Legionella,* only few studies have established a link between the building characteristics (e.g. size and age of the building or the water network) and *Legionella* prevalence (Alary and Joly, 1991; Borella et al., 2004; Leoni et al., 2005).

Currently, there is no regulation for environmental *Legionella* monitoring in Hungary, thus most buildings are operated without the recognition and management of *Legionella* risk. Consequently, there is no national limit of intervention. International guidelines usually define 1000 colony forming units (CFU)/L as a limit value of public health concern (EWGLI Technical Guidelines, 2011). In premise plumbing systems, colonisation is generally characterised as a combination of the rate of samples positive for *Legionella*, and the counts detected in the positive samples. Counts below 1000 CFU/L indicate that the system is under control; if it exceeds this value in over half of the samples, intervention is necessary. Immediate action is generally proposed in case of *Legionella* counts over 10,000 CFU/L (EWGLI Technical Guidelines, 2011). The above parameters, however, are only preliminary indicators of the risk of infection, complemented by the virulence of the *Legionella* strains present in the system, the potential exposure routes and the immunostatus of the exposed population.

The aim of the present study was dual: (1) assess *Legionella* prevalence – including species and subtype distribution – in the hot water system of various buildings (such and health care facilities, accommodation sites, schools, office buildings, factories and private residences) in Hungary and (2) to investigate those parameters (including building characteristics and basic water quality data) which are known or easily available to building operators and thus can feed into an initial risk assessment. Such a primary screening exercise, if based on statistically evidenced risk factors, would facilitate the recognition of the scope of the problem and the prioritisation of risk management efforts.

2. Material and methods

2.1. Sample collection and study sites

In total, 1809 water samples were taken from hot water storage tanks, showerheads and faucets of different type of buildings (168), such a health care facilities (22), accommodation sites (21), educational institutions (26), office buildings (10), industrial buildings (35) and private residences with central (26) and individual hot water supply (26) from diverse geographic locations in Hungary between 2006 and 2013. Water samples were collected under normal operation (i.e. during the school year for educational facilities, or during working days in office buildings). The sampling scheme within the buildings was designed to represent the entire plumbing system (sampling at hot water storage tanks, return loops and distal outlets, including showerheads and taps).

Water samples were collected according to the standards ISO 5667–5:2006 and ISO 19458:2006. 250 ml samples were drawn without flaming after 1 min flushing in sterile bottles with 0.1% $Na_2S_2O_3$ to neutralise residual free chlorine and transported immediately to the laboratory for bacteriological analysis (ISO, 19458:2006). Temperature of the water at the time of sampling was measured with an electronic calibrated thermometer (testo-735, Testo Ltd., Lenzkirch, Germany).

Water sampling was part of an on-site sanitary investigation including the visual inspection of the boiler rooms, premise plumbing and hygiene conditions. It was complemented by a questionnaire survey collecting information on the building, potable water source, hot water production and premise plumbing characteristics. The presence of additional risk sources (e.g. solar collector, decorative fountain, cooling tower, hydrotherapy pool, air-conditioning, humidifiers) was also investigated, but as answers were almost uniformly negative, further investigation was directed only to the premise plumbing system.

2.2. Microbiological analysis

The water samples were analysed for *Legionella* sp. by standard culture technique according to ISO 11731–2:2004; briefly as follows: 100 mL aliquot was filtered on 0.45 μm pore size black cellulose nitrate membrane (Sartorius Stedim Biotech Ltd., Göttingen, Germany). A 5-min acid wash (pH 2.2) was applied to suppress background microbiota. *Legionella* sp. was cultured on GVPC (Oxoid Ltd., Basingstoke, Hampshire, UK) at 36 ± 1 °C for 10 days and results were read on day 3, 5 and 10 under a dissecting microscope. Presumptive *Legionella* colonies (at least 3 colonies per plate) were subcultured on BCYE with and without cysteine (Oxoid Ltd., Basingstoke, Hampshire, UK) to test for cysteine auxotrophy; cultures were incubated at 36 ± 1 °C for 2 days. Presumptive legionellae were identified by seroagglutination (*Legionella* latex test, Oxoid Ltd., Basingstoke, Hampshire, UK). The test allows for the differentiation of *Legionella pneumophila* serogroup 1 and 2–14 and the detection of seven species of non-*pneumophila* legionellae. Further typing was performed using microagglutionation (Denka-Seiken Co. Ltd, Tokyo, Japan), which differentiates serotypes 1 to 15 of *L. pneumophila*.

Pseudomonas aeruginosa was determined according to ISO 16266:2006 by membrane filtration on cetrimide agar (Merck KGaA, Darmstadt, Germany). Heterotrophic plate count was enumerated on yeast extract agar according to ISO 6222:1999 standard using the pour plate method (Oxoid Ltd., Basingstoke, Hampshire, UK).

2.3. Data management and statistical analysis

Only well-characterised hot water networks were included in the analysis, where samples from multiple representative points were available. All systems were sampled under normal operating conditions (i.e. without any targeted intervention to reduce *Legionella* colonisation, such as shock disinfection).

Chemical characteristics of the supplied water were derived from the National Drinking Water Database. Yearly average value of each parameter for the water supply zone where the sampled facility was located was taken into account. Only water-source derived parameters (i.e. which do not change significantly in the distribution system) were assessed.

Statistical analysis was performed using SPSS (SPSS Inc. Chicago, Il., USA). Mann–Whitney test (MW) and Kruskal–Wallis test (KW) were performed to compare the mean values of the measured variables in connection with *Legionella* spp. colonisation. The following variables were taken into consideration: drinking water source, age and size of the building, age of the premise plumbing system, hot water temperature, temperature decrease within the hot water network, production and storage of hot water (including the number and position of storage tanks).

Colonisation was characterised by two different categorisation: presence/absence of *Legionella* in the sample (absence indicates <10 CFU/L), or titre above or below 1000 CFU/L. Correlation of *Legionella* counts and chemical parameters was assessed using regression analysis for the determination of significance levels.

3. Results

3.1. Legionella colonisation

Legionella spp. was isolated from 61% of the 168 investigated