ARTICLE IN PRESS

International Journal of Hygiene and Environmental Health xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

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

Legionella contamination in warm water systems: A species-level survey

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ARTICLE INFO

Keywords:
Biodiversity
Drinking water quality
ISO 11731
MALDI biotyper

ABSTRACT

Legionellae constitute a frequent contamination of warm water systems and can lead to serious infections. Therefore, in many countries it is mandatory to monitor warm water systems for their presence. The method of examination in Germany is regulated by guideline ISO 11731 and DIN EN ISO 11731-2, and the results are reported as concentration of *Legionella* spp. Only limited information is available on the presence of individual species of Legionellae in the examined systems, since most investigations and research focus solely on *Legionella pneumophila* as the most important human pathogen. In this study 76,220 samples obtained from 13,397 warm water systems originating from 24 different zip code districts covering an area of more than 71,000 km^2 in southern Germany were examined. This resulted in the identification of 47,924 *Legionella* isolates to the species level using a MALDI-TOF mass spectrometry-based method. *Legionella* species distribution was analyzed with respect to warm water system type, geographic region (defined as zip code district) and temperature during sample taking.

Overall, 20.7% of the samples were found positive for *Legionella* species and 14 different species of *Legionella* were recovered. These were not equally present throughout the geographic area investigated, but instead an individual regional diversity of *Legionella* species was observed for the examined zip code districts. Although *Legionella pneumophila* represented 84% of all contaminations found, depending on the geographical region its proportion varied substantially between 57.5% and 91.2%. The occurrence of other species was also of importance since they accounted for up to 42% of contaminations regionally, with *Legionella londiniensis* being most prominent representing up to 38.8% of recovered colonies. In addition, the influence of temperature on the individual species was disparate, but the temperature range between 50 °C and 59 °C was identified as the optimal condition for facilitating emergence of the majority of recovered *Legionella* species.

The identification of *Legionella* to the species level by MALDI-TOF allowed for a more concise depiction of the regional distribution and the ecology of this genus, and may be of additional value when counter measures need to be initiated.

1. Introduction

Ever since their discovery in 1976 (McDade et al., 1977; Winn, 1988), legionellae have become a common focus of public health concerns. Outbreak situations, often even resulting in related deaths, have been repeatedly reported in the headlines, and also increased the public's attention (e.g. recently Maisa et al., 2015; Essig et al., 2016). The key role of domestic warm water systems, as the most important reservoir for *Legionella* infections, has long been recognized (e.g. Wadowsky et al., 1982). As in many other countries around the world, drinking water legislation was adopted in Germany in 2011 to require general testing for *Legionella* contamination in public buildings and private homes exceeding a certain minimal size, as a preventive measure to improve health safety for their inhabitants (Anon, 2012a).

Clinically, the species most frequently reported as the cause of legionellosis in Germany, as well as in most other countries, is *Legionella pneumophila.* According to the annual infection report published by the Robert Koch Institute, 85% of the *Legionella* infections in Germany were caused by *L. pneumophila* in 2012, and 84% in 2013 (Robert Koch-Institut, 2013, 2014). However, in recent years a number of other publications have also pointed to the importance of non-L. pneumophila species in the clinic as well as in the field of drinking water safety (Stallworth et al., 2012; Svarrer and Uldum, 2012; Anderson et al., 2016; Isenman et al., 2016; Vaccaro et al., 2016). Remarkably, the vast majority of reported legionellosis cases were infected outside of outbreak events in private or professional surroundings and are not

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http://dx.doi.org/10.1016/j.ijheh.2017.10.011
Received 14 August 2017; Received in revised form 23 October 2017; Accepted 24 October 2017
1438-4639/

nosocomial (Robert Koch-Institut, 2015).

For the examination of potable water, the legally mandatory testing method according to guideline DIN EN ISO 11731-2 (Anon, 2008b) aims to capture solely the presence of *Legionella* spp. in general, without closer specification, and to count the number of colony forming units (CFU). Numerous publications, however, have already reported the identification of the exact species of *Legionella* from drinking water samples (for example but not exclusively: Ehret et al., 1987; Frahm and Obst, 1994; Bartie et al., 2003; Brooks et al., 2004; Helbig et al., 2006; Svarrer and Uldum, 2012). Available publications examining the prevalence of *Legionella* contaminations to date have primarily focused on warm water systems in hospitals and public buildings such as hotels (e.g. Leoni et al., 2005; Wright, 1985; Habicht and Müller, 1988; Borella et al., 2005; Erdogan and Arslan, 2007; Fragou et al., 2012), whereas private homes investigated were either limited in number and geographic scope, or represented relatively uncommon settings (Habicht and Müller, 1988; Mathys et al., 2008; Barna et al., 2016; Kruse et al., 2016). It is also well documented that temperature influences growth and concentration of legionellae in warm water systems (e.g. Rhoads et al., 2015; Dilger et al., 2016a). Nonetheless, thus far data on the influence of system temperatures on the growth of different *Legionella* species in drinking water installations is very limited.

In a previous examination we were able to demonstrate that a modification of the workflow required by the ISO 11731 (Anon, 1998) and DIN EN ISO 11731-2 (Anon, 2008b) regulations, introducing MALDI-TOF mass spectrometry as the method for species identification, allows for the identification of *Legionella* isolates to the species level (Moliner et al., 2010). Beyond the limitations of the original protocol, this provides a very fast and simple mode of identification, and facilitates a clear depiction of the exact species of legionellae and other bacteria occurring in warm water systems (Dilger et al., 2016b).

The current study aims to harness and assess the additional information gained when this modified protocol is applied on a large scale. It's intention is to provide a concise survey, covering a large geographic area (> 71,000 km^2; Fig. 1), considering both a large number and wide variety of warm water systems. During the course of this investigation, a total of 76,200 samples originating from 13,397 different warm water systems located in 24 adjacent zip code districts situated in southern Germany were tested. The species identifications of 47,924 *Legionella* colonies recovered from these samples were analyzed according to the general prevalence of *Legionellae* and individual *Legionella* species in the considered geographic region, the occurrence of multiple *Legionella* species in the same warm water system, the regional abundance of the different species, and the influence of temperature in the various warm water systems on the occurrence of the different *Legionella* species. The large number of specimens collected over such a wide geographic area should help to build a more comprehensive picture of *Legionella* contaminations affecting warm water systems than what is currently possible with data from the previously published literature mentioned above.

2. Materials and methods

2.1. Sample collection

Sample collection was performed in accordance with ISO 5667-5:2006 (Anon, 2006a) and ISO 19458:2006 (Anon, 2006b). In brief: Aerator and similar fittings were removed, the tap was disinfected by flaming or with 70% isopropanol. After disinfection, one liter of warm water was discarded and 250 ml warm water were collected in a sterile container containing 200 mg sodium thiosulfate. This amount of sodium thiosulfate is sufficient to inactivate the potentially present legally allowed concentrations of chlorine disinfectants. For each warm water system at least the boiler inlet, outlet and risers were examined according to the legal regulations. (Anon., 2012b)

The majority of samples collected came from private homes (ca. 93.7%). To a lesser extent also public buildings such as schools (2.5%), town halls (0.5%), sport facilities (1.0%), hotels (1.0%), hospitals and retirement homes (1.6%) in the southern part of Germany were included.

2.2. Culturing

Enumeration was done according to the technique prescribed by the German environmental protection agency (Umweltbundesamt; UBA) (Anon., 2012b), which is based on ISO11731-2:2008. A 1 ml sample is directly spread onto 2 GVPC (Becton Dickinson) agar plates. One hundred milliliters of the same sample are filtered through a 0.45 μm

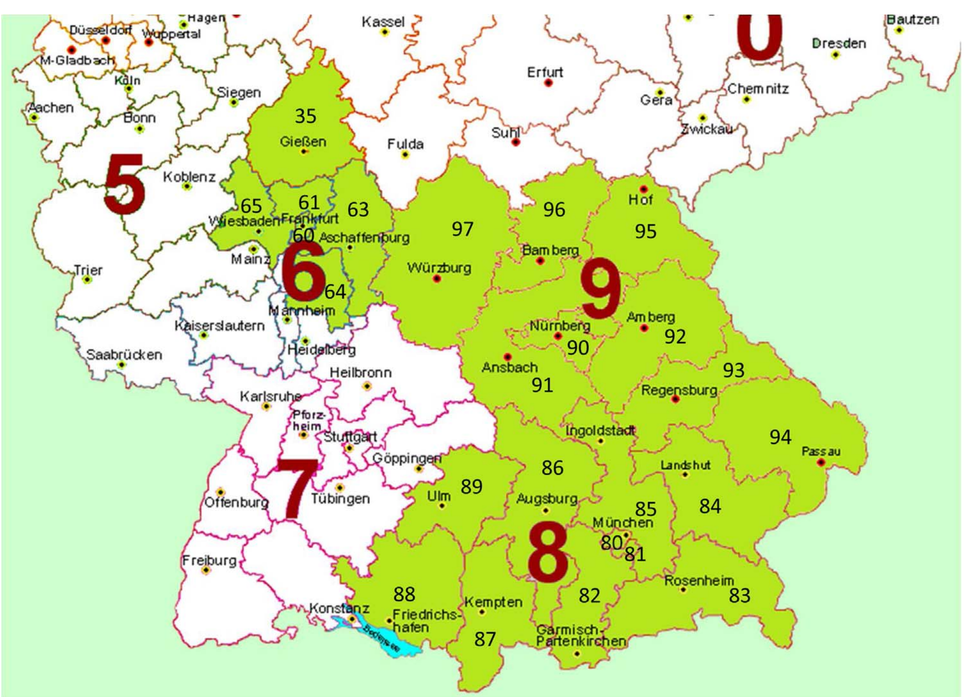

Fig. 1. Geographic area and partitioning of the zip code districts where samples originated from. The 24 zip code districts fulfilling the requirements for examination are shaded green. German zip codes consist of a five-digit number that is hierarchically structured by geography. Zip code districts are presented in this analysis based on the leading two digits of each zip code.