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Distribution and Genetic Diversity of *Legionella pneumophila* Serotype in Kuwait Environment

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Abstract

Background: *Legionella pneumophila* is a waterborne organism that is increasingly recognized to cause community acquired and nosocomial pneumonia in humans. Domestic water systems have often been implicated as the source in outbreaks of Legionnaires' disease. Legionella can survive under various conditions in various water sources and acquired antibiotic resistance to many routinely prescribed antibiotics. Routine monitoring of environmental water for *Legionella* species is proving helpful in reducing disease outbreak.

Methods: A total of 260 of *Legionella pneumophila* isolates were isolated from environmental water sources from building facilities in Kuwait. The genetic diversity of the 102 isolates were analysis by the SBT method according to the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Legionella. The distribution of Legionella isolates was investigated according to geographical region.

Results: The Legionella isolates were discriminated into 11 distinct SBT profiles, of which six (ST1223, ST1436, ST1555, ST1604, ST1718, and ST1719) were new to the ESGLI SBT database. *L. pneumophila* sg 7 was distributed broadly through Kuwait, accounting for 38.2% of the isolates and predominated in cooling towers water with unique sequence type to Kuwait. The second most dominant strain *L. pneumophila* sg 3 (32.4%), predominated in the bathroom. *L. pneumophila* sg 1 (18.3%), *L. pneumophila* sg 10 (6.9%), and *L. pneumophila* sg 4 (3.9%), predominated in the cooling towers. In SBT analysis, *L. pneumophila* sg 7 isolates were differentiated into 2 sequence types (STs), ST1718 (37.3 %) is the dominant ST in cooling tower. The unique allelic profile of ST1718, obtained from the cooling tower, was not found in the ESGLI SBT database.

Conclusions: The findings of this study highlight the importance of understanding the epidemiology and ecology of *L. pneumophila* from public facilities in terms of public health. Furthermore, provide useful information for future epidemiological investigation of local and regional outbreaks of Legionnaires' disease.

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Introduction

Legionnaires' disease (LD), usually is acquired by inhalation or aspiration of aerosol contaminated by Legionella species from environmental water sources, such as hot water supplies, cooling towers, and evaporative condensers. Potable water is considered as an important infection source in community-acquired, nosocomially acquired, and travel-associated LD cases [1-8]. *Legionella* spp. are gram-negative bacteria that normally occupy natural aquatic environments, where they can survive as intracellular parasites of protozoa. It includes 52 species and over 70 different serogroups. Over 20 species have been proven to be causative agents of LD. However, *Legionella pneumophila* species accounts for approximately 90% of confirmed cases of legionellosis. The majority of community-acquired cases are caused by strains belonging to *Legionella pneumophila* serogroup 1, other non-*L. pneumophila* sg 1 strains, sg 2 to 15, accounted for 7.4% of cases [9,10]. The genetic diversity of *L. pneumophila* was related to horizontal gene transfer of mobile genetic elements among *L. pneumophila* strains, and between different *Legionella species*. The potential health risk of *Legionella* to humans is theoretically associated with cells densities above 10^4 to 10^5 CFU per liter of water [11,12].

Commonly used method for environmental surveillance of Legionella is the standard culture technique [13,14]. *Legionella* culture is required for epidemiological typing of isolated strains to detect the source of the infection [15,16,17]. However, the fastidious nature of these bacteria, effect the culture technique's sensitivity to be 30 to 60% and require 3 to 7 days to grow visible colonies. Therefore, numerous phenotypic and genotypic typing methodologies have been developed and applied to the epidemiological typing of *L. pneumophila* [18]. These methods included monoclonal antibody (MAb) subgrouping method [19] and genotyping methods, such as restriction fragment length polymorphism (RFLP) analysis [20], amplified fragment length polymorphism (AFLP) [21], and pulsed field gel electrophoresis (PFGE) [22,23]. Recently, a sequence-based typing (SBT) scheme of *L. pneumophila* that uses sequences from seven genes has been used in typing *L. pneumophila* serogroup 1 strains, and strains belonging to others serogroup [16]. The SBT method has the potential for excellent type ability, reproducibility, and epidemiologic concordance [16, 22-24] and now is established as the standard sub typing technique within the European Working Group for *Legionella* Infections (EWGLI). The strains are assigned an SBT pattern number by EWGLI (EWGLI: www.ewgli.org) based on the sequence of the seven target genes. The method is now considered the gold standard for epidemiological typing [16, 24].

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In Kuwait, research related to *Legionella* is scarce and no active surveillance program exists [25-27]. Furthermore, no reports are available on the current status of the prevalence Legionnaires' disease and associated cases in Kuwait. However, annual reports presented by the Ministry of Planning show that the death of a percentage of the population ($27.6/10^4$ of population) is due to respiratory diseases without specifying the etiological agent [28].

Due to the following factors, it is likely that the prevalence of *Legionella* is underestimated; Kuwait's a hot temperate climate; the absence of water safety regulations for *Legionella* monitoring and decontamination; and the increased use of cooling towers within recreational and health care facilities may increase the risk of legionellosis. In addition, water temperatures in water tanks during the summer in residential compounds may be favorable for *Legionella* multiplication (50°C). Owing to the possibility of environmental exposure to *Legionella*, this is the study aimed at assessing the current distribution of *Legionella* species from environmental water sources from public facilities such as buildings, public baths, hospitals, throughout Kuwait. Furthermore, the molecular typing of *L. pneumophila* sg isolates was conducted using sequence-based typing (SBT) to assess the genetic diversity among the isolates.

Material and Methods

Legionella pneumophila

A total number of 102 environmental isolates of *L. pneumophila* were used in this study. These isolates were previously isolated from domestic water system samples from different residential sites [29] and from 38 cooling towers in 12 sites in Kuwait [30]. *L. pneumophila* was isolated on buffered charcoal yeast extract medium using standard methods AS/NZS 3896 [31].

Sero and subgrouping of *Legionella pneumophila*

The Oxoid Legionella Latex test was used to identify and differentiate between sero group 1 and serogroups 2-14 (code DR0800; Oxoid; UK). The isolates were sero grouped and sub grouped, when applicable using the Dresden panel of monoclonal antibodies aspreviously described [9,10].

Sequence based typing (SBT)

The genomic DNA was extracted from the isolates using the QIAamp DNA Mini kit (Qiagen). *L. pneumophila* isolates were genotyped using the seven gene protocol sequence-based typing (SBT) scheme developed by ESGLI as previously described [16,24]. Trace files with the obtained sequences were analyzed by using the Legionella SBT quality tool. New alleles and STs encountered for the first time in this study were submitted to the database.

Whole genome sequencing (WGS)

The genomic DNA was extracted from four representatives isolates using the QIAamp DNA Mini kit (Qiagen) and was subjected to whole genome sequencing using the Illumina MiSeq platform with 250 bp paired-end reads according to the manufacturer's instructions. The flaA genes were extracted from the de novo assemblies (CLC-bio vers. 8.0). The four isolates were selected among isolates that failed to give aflaA PCR product and showed one of the following four allelic profiles(1) F,14,16,16,15,13,2; (2) F,14,16,25,7,13,24; (3) F,14,16,65,7,13,217; or (4) F,14,16,19,15,13,215.

Results

Serological distribution of Legionella species

A total of 271 isolates of *Legionella* were isolated from hospital cooling tower (49%), public buildings cooling tower (24%), public bath (14%), kitchen (8%), tanks (3%) and swimming pool (1.5%). Seventy three percent of the total samples were collected from cooling tower water, and the rest of the samples were collected from hot water. Among the 271 isolates, the 260 *L. pneumophila* isolates statistically predominated (96%), whereas Legionella species other than *L. pneumophila* accounted for 4% of the total. Among the 102 sequenced *L. pneumophila*, the sg 7 strain accounted for 39 (38%), whereas strains sg 3, sg 1, sg 10, and sg 4 accounted for 32%, 19%, 7%, and 4%, respectively (Figure 1).

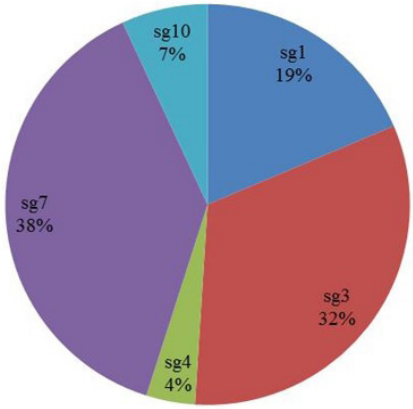

Figure 1: Distribution of *Legionella pneumophila* serotypes from environmental water sources in Kuwait for 2013.

Analysis of geographic distribution of *Legionella pneumophila*

L. pneumophila sg 1 was prevalent in 4 governorates throughout Kuwait, and was represented in Al Asimah (10%), Mubarak Al-Kabeer (7.7%), Al Ahmadi (1.3%) and Al Farwaniyah (2.5%). *L. pneumophila* sg 3 and sg 10 were represented only in Al Asimah governorate (36.3%) and (6.3%), respectively. *L. pneumophila* sg 7 was detected in 3 governorates, Al Asimah(17.5%), Hawalli (5%), Al Jahra (5%). *L. pneumophila* sg 4 was detected in 2 governorates, Al Asimah (3.8%) and Al Ahmadi (1.3%)(Figure 2).

Legionella pneumophila serotype according to facility type

L. pneumophila sg 7 accounted for 38.2% (39/102) of the total isolates and predominated in facilities such as buildings and cooling towers (hospitals), although not in public baths. The distribution rates of *L. pneumophila* strains other than sg 7 depended on the facility types; *L. pneumophila* sg 3 (32.4%) prevailed in public baths, buildings and cooling towers (hospitals), *L. pneumophila* sg 1 (18.6%) in public baths, buildings and cooling towers (hospitals), and *L. pneumophila* sg 4 in buildings and cooling towers (hospitals)(3.9%). *L. pneumophila* sg 10 (6.9%) predominated in in public baths and buildings (Figure 3).

Legionella pneumophila sero type in cooling tower water and hot water

In order to determine whether the distribution of *Legionella pneumophila* depended on the sample type, the species and sero group distributions were compared between the 72 isolates from the cooling tower and the 30 isolates from hot water (Figure 4). The predominant

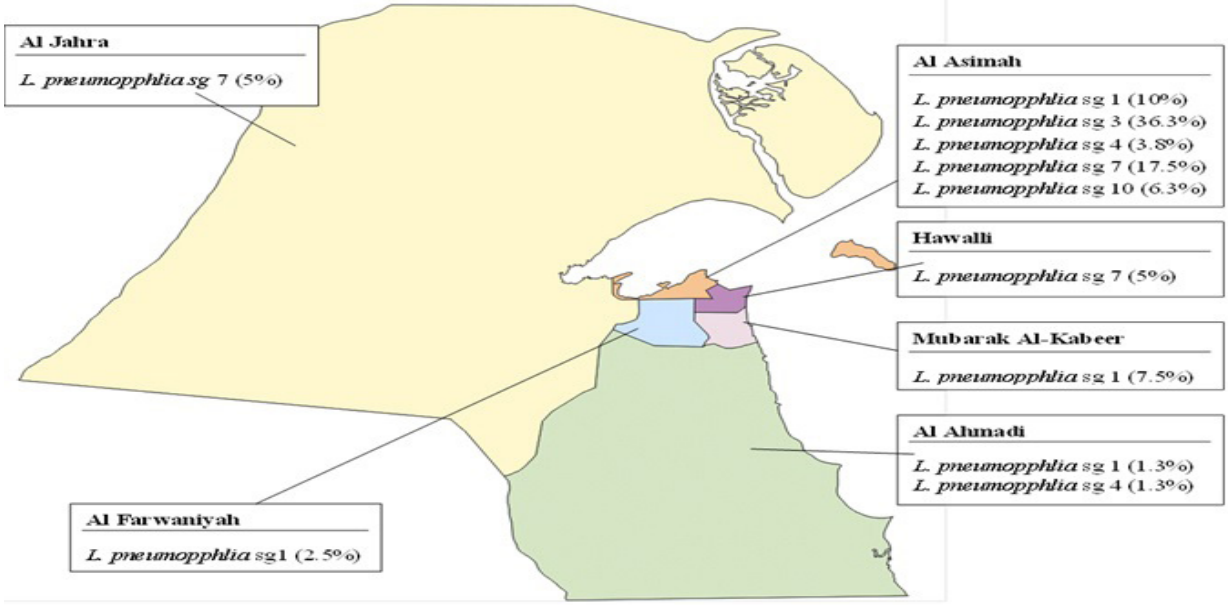

Figure 2: Distribution of *Legionella pneumophila* isolated from environmental water sources from 6 geographic regions in Kuwait.

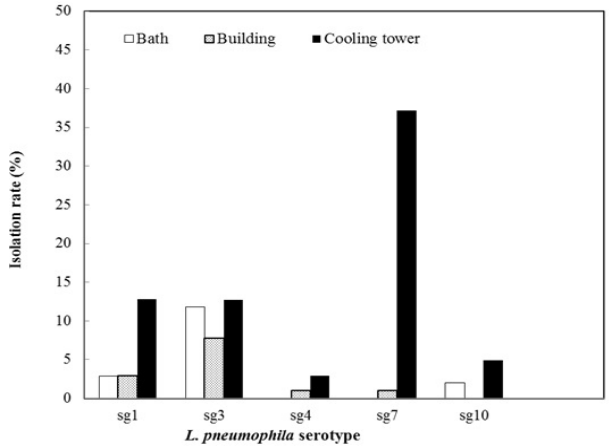

Figure 3: Comparison of Isolation rates of *L. pneumophila* sero type from public bath, building and cooling tower water.

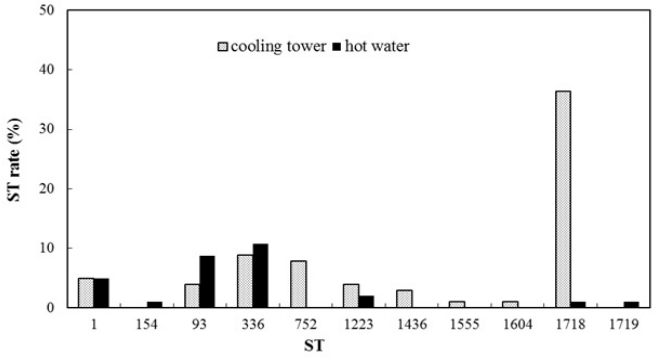

Figure 4: Comparison of ST profiles of Isolates from cooling tower water (n=72) and hot water (n= 30).

strain in the cooling tower was *L. pneumophila* sg 7 accounted for 52.8% of isolates collected from cooling towers. The secondarily dominant strains depended on the sample type was *L. pneumophila* sg 3 accounted for 66.7% of isolates collected from hot water. In our comparative analysis of distribution between the cooling tower and the hot water, *L. pneumophila* sg 1 (18% and 20%, respectively), sg 4 (4.2% and 3.3%, respectively) and sg 10 (7% and 6.7%, respectively) were the predominant strain in both sample types.

Genetic diversity

For SBT, among 260 isolates of *L. pneumophila*, 102 isolates were selected randomly, and these isolates were differentiated by SBT into 11 different sequence types (STs). ST1718 of *L. pneumophila* sg 7, as the predominant type, accounted for 37.3%, and ST1555 (1%) was found only in one of the cooling tower samples. The profile for the two STs could not be found in the EWGLI SBT database. For *L. pneumophila* sg 4 (los Angeles), ST1719 (1%) was found only in one sample of hot water, whereas ST1436 (2.9%) of *L. pneumophila* sg 4 (Portland) was found only in the cooling tower. Both ST profiles could not be found in the EWGLI SBT database. ST154 (1%) of *L. pneumophila* sg 1 (Oxford/OLDA) was found only in one of the hot water samples, ST 752 (7.8%) only found in cooling tower samples and ST1 (9.8%) was commonly distributed. ST1604 (1%) of *L. pneumophila* sg 10 was found only in one of the cooling tower samples, ST 1223 (5.9%) was commonly distributed. Both ST profiles could not be found in the EWGLI SBT database. ST93 (12.7%) and ST336 (19.3%) of *L. pneumophila* sg 3 were commonly distributed (Table 1).

Discussion

This study, part of it has been published, is the first study that provides the distribution and genetic diversity of environmental *L. pneumophila* serotype isolated from water in cooling towers and hot water systems in Kuwait. Among the cooling tower systems and hot water systems, the predominant species, *L. pneumophila*, accounted for 96.4% and 94.6% of the total isolates, respectively. *L. pneumophila* have been demonstrated by other studies to be the predominant

ST	Serogroup, mAb subtype	Alleic profile (flaA, pilE, asd, mip, mompS, proA, neuA)	No. of isolates (%)
1	1, Oxford/ OLDA	1,4,3,1,1,1,1	10 (9.8)
154	1, Oxford/ OLDA	11,14,16,16,15,13,2	1 (1)
752	1, Oxford/ OLDA	22,4,3,1,1,1,1	8 (7.8)
93	3	3,10,1,28,14,9,13	13 (12.7)
336	3	11,14,16,25,7,13,24	20 (19.6)
1719*	4, Los Angeles	11,14,16,65,7,13,217	1 (1)
1436*	4, Portland	6,10,19,67,19,4,48	3 (2.9)
1718*	7	11,14,16,19,15,13,215	38 (37.3)
1555*	7	16,21,12,19,82,21,215	1 (1)
1223*	10	1,4,3,5,1,1,213	6 (5.9)
1604*	10	1,4,3,10,1,1,209	1 (1)

Table 1: Distribution of serogrop from 11 SBT profile for *L. pneumophlia* isolates (n=102) in Kuwait.
*New sequence types.

	No. (%) Legionella Isolates			No. (%) of *L.pneumophila* isolates		
Samples Source	Total	*L. pneumophlia*	Non-*L. pneumophlia*	Total	sg1	Non-sg1
Cooling tower water	197	190 (96.4%)	7 (3.6%)	190	51 (27%)	139 (73%)
Hot water	74	70 (94.6%)	4 (5.4%)	70	18 (25.7%)	53 (75%)
Total	271	260(96.3%)	11 (4.2%)	260	69 (26.5%)	192 (73.6%)

Table 2: Comparative distribution of Legionella species between water in cooling towers and hot water (n=271)

species in water of cooling systems and hot water systems [3,19,32,33]. The ecology of *L.pneumophila* serotype was found differed between the water in cooling towers and the hot water samples collected from public facilities. *L. pneumophila* sg 7 was identified as a major strain (52.8%) in water of cooling towers and *L. pneumophila* sg 3 as a major strain (66.7%) in the hot water samples. *L. pneumophila* sg 1, compared to only 27% of the isolates collected from water in cooling towers and 25.7% of the isolates collected from hot water (Table 2). The results of this study differed from those reported in previous studies of public facilities. Specifically to *L. pneumophila* strain, sg 1 was the most frequently detected strain in cooling towers and hot water [3,19,32,33].

In the comparative analysis of the SBT distribution of the isolates according to sample type, ST1718 was the predominant type in the isolates from the cooling tower water and ST336 was the dominant type in the hot water samples. However, STs 752, 1436, 1555, and 1604 were found only in isolates from the cooling tower water, and STs 154 and 1719 were found only in the hot water isolates(Figure 4).The predominant profile for sg1 in this study was ST1 (1, 4, 3, 1, 1, 1), which is broadly distributed throughout the world [22,34,35,36]. STs 1719, 1436, 1718, 1555, 1223, 1604 were not detected in the EWGLI SBT database or in any other studies. Our results are similar to those observed by Kozak et al. (2009) [37], who reported that 58% of the STs found were unique to the United States.

Conclusion

In conclusion, the results shown that the comparative populations of environmental isolates of *L.pneumophila* stains isolated from public facilities varied according to the types of facility as well as the geographic allocations of the facilities. Also, the results revealed several unique allelic profiles of STs and showed that ST1718 of *L. pneumophila* sg 7 was the prevalent sequence type in Kuwait. This study highlight the significance of understanding the epidemiology and ecology of *L. pneumophila* strain from public facilities in terms of public health and provide useful information for future epidemiological investigation of local and regional outbreaks of LD. Thus, routine monitoring of environmental water for *Legionella* species is an auxiliary implement to reduce the bacterial contamination of water systems and to assist the development of a more active prevention strategy. Further study will require the focus on correlation analysis by clustering between environmental and clinical isolates of *Legionella* strains.

Competing Interests

The author declares that he has no competing interest.

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