The prevalence of Legionella pneumophila in different water systems: A global systematic review and meta-analysis

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Abstract

The presence of Legionella pneumophila (LP) in aquatic habitat is a global concern. The current study was undertaken to estimate the prevalence of LP in water systems with the aid of a systematic review and meta-analysis. The searching was performed among some international databases, including Scopus, PubMed, and Embase to retrieve the related articles between 1/January/1983 and 25/July/2017. Therefore, thirty-six articles (with 43 studies) out of 1,541 articles collected, were included in the meta-analysis. The overall prevalence of LP in water systems was determined as 20% (95%CI: 15-25). Also, the lowest and highest pooled prevalence of LP was observed in Poland (4% (95%CI: 0-13%)) and Kuwait (98% (95%CI: 90-100%)), respectively. The lowest and highest prevalence of LP-based on water resources subgroups was a water reservoir (15% (95%CI: 2-37%) and well (40% (95%CI: 26-50%), respectively. The number of studies that used polymerase chain reaction (PCR) for detection of LP was 16/43 (37.3%) while the culture method was 27/43 (62.7%). Generally speaking, the relatively high prevalence of LP among the investigated water systems was demonstrated, which should be reduced by performing appropriate control actions.

Introduction

Legionella pneumophila (LP) as a heterotrophic bacterium is a gram-negative, aerobic, non-sporogenous, and a mobile [1, 2] which can be isolated from various environments such as soil and water systems, including water used in cooling towers and ventilation, surface water, tap water, and spring water [3–6].

The tolerance to high temperature is among the features, which could stimulate the presence, and proliferation of LP in water systems [7–9]. Although the range of optimal temperature for proliferation was indicated as 29–40 °C, rarely, it can survive in water at temperatures ranging from 0 to 63 °C [10]. Also, the efficiency of chemical or thermal disinfection can be reduced in the presence of biofilm or amoebae; which can be resulted in further contamination of water systems by LP11.

The droplets of contaminated water with LP could convey the bacterium into the lungs and as a result of swallowing by macrophages [12, 13]. Therefore, the approaching of efficient and appropriate methods is crucial to decreasing as well as controlling the prevalence of LP water systems. In this regard, according to the guideline of World Health Organization (WHO), the acceptable limit of LP in the drinking water and cooling tower systems were defined as 1 CFU/L (WHO 2008) and 1000 CFU/L [14], respectively.

Legionnaires’ disease are among issues caused by these water-borne pathogens, commonly known as Legionella. Twenty-five out of 59 species in the Legionella genus, have been correlated with human disease [15]. According to Cazalet et al. (2008), almost 20 kb of 33-kb locus carrying the genes for the proteins in lipopolysaccharide biosynthesis in LP is highly specific for L. Pneumophila serogroup 1 (LP-Sg1) [16]. Also, multigenome via comparative hybridization detected 3 genes, including lpp0837/wzm, lpp0831, and lpp0838/wzt strains only LP-Sg1 that used for real-time PCR method for identifying LP-Sg1 [16].
Moreover, *Legionella pneumophila serogroup 1* (LP) is responsible for the majority of reported cases of Legionnaires’ disease (about 90%) [17]. Legionnaires’ disease, for the first time, was diagnosed as pneumatic form [10]. The incidence of LD is increasing, especially in Europe (EU) United States of America (USA). In this context, only in 2010 and 2012, 6,305 and 4,486 cases of the Legionnaire’s disease were reported in Europe [18] and the USA [19], respectively. Some signs and symptoms include coughing, shortness of breath, high fever, muscle pains, and headaches, Nausea, vomiting, and diarrhea were associated with Legionnaires’ disease [10]. Due to the significant health effect of LP contamination in water systems, some investigations with different outcomes were performed [6, 20–22]. In this regard, the prevalence of LP in the cooling tower systems in Kuwait and Saudi Arabic were determined as 98(95%CI: 89–100) and 2(95%CI: 1–4), respectively [23, 24].

The culture medium and polymerase chain reaction (PCR) techniques are used to isolate the LP in water systems. The culture techniques based on ISO 11731 can be considered as gold-standard for detecting LP in water systems [14]. In the PCR method, the limit of detection (LOD) is $2 \times 10^2$ GU/100 mL for LP (*mip* gene), while the LOD for culture methods is 1 CFU/100 mL [25, 26]. Although culture techniques are used widely, these techniques have several limitations including a long time needed to obtain results, weak recovery, weak sensitivity, inability to detect viable but non-culturable cells (VBNC) [27]. In another hand, the polymerase chain reaction (PCR) techniques due to reproducibility, sensitivity, specificity, high-throughput and reducing in the required time to less than 24 hours are widely approached to detect LP in water systems [28–30].

However, no systematic review and meta-analysis was performed regarding the prevalence of *Legionella pneumophila* in different water systems. Therefore, for the first time, the current study was undertaken to perform a meta-analysis to investigate the prevalence LP in the water systems based on geographical, quality of research as well as the type of water.

**Material And Methods**

In this study, the systematic review was conducted in accordance with the Cochrane protocol and Prisma protocol [31].

**2.1. Search strategy**

The search strategy was performed on the *Legionella pneumophila* studies in water systems. Searching was performed in the main international databases, including Scopus, PubMed, and Embase.

The following terms were used: (a) PubMed: Search ((((((Legionella[Title/Abstract]) OR *Legionella pneumophila*[Title/Abstract])) OR microbe[Title/Abstract]) OR bacteria[Title/Abstract])) AND (((water[Title/Abstract]) OR drinking water[Title/Abstract]) OR tap water[Title/Abstract]) OR water system[Title/Abstract])) AND (“Legionella”[Mesh] AND “Legionella pneumophila”[Mesh]); (b) Scopus: ((title-abs-key (legionella and pneumophila) or title-abs-key (legionella) or title-abs-key (pneumophila) or...
The references articles were reviewed to obtain more articles. Seventeen years (1/January/1983 and 25/July/ 2017) was chosen as the searching period.

2.2. Inclusion and exclusion criteria

Full-text articles were downloaded and then reviewed carefully to check if they meet the proposed criteria [32–34] such as (1) original study; (2) cross-sectional data; (3) published in English; (4) published online between 1/January/1983 and 25/July/ 2017; (5) full-text available articles; (6) existence of exact total sample size and positive samples; (7) the defined type of water was examined; and (8) accurate methods including culture or PCR techniques were mentioned. The articles were excluded when not meet our criteria.

2.3. Data extraction

The obtained information from each study can be summarized as study characteristics; (the first author, year of study); total sample size; the number of positive samples; the geographical study (countries); study methodology (Culture or PCR); type of water systems (tap drinking water, hot water, water reservoir, cold water, well and spring).

2.4. Meta-analysis of data

The ratio of the positive samples \( p_i \) to the total sample \( n_i \) defined as prevalence \( P = p_i / n_i \), which is between 0 to 1 value [32, 35–37]. Estimation of the prevalence of LP in water systems was performed using the binomial distribution model [38]. The heterogeneity \( (\hat{I}^2) \) statistics was used to determine the variation between the prevalence of *Legionella pneumophila* among the included studies [39]. In the current study, when the heterogeneity was higher than 50%, the random effect model (REM) was used to estimate prevalence based on the defined subgroup. The Begg’s and Egger’s test was used to estimate publication bias [21, 40]. A meta-analysis of data was conducted using Stata 12.2 intercooled version (Stata Corp, College Station, TX). All statistical analysis was significant at P-values< 0.05.

Results

A total number of 1541 of articles were screened in the initial screening through databases. Finally, 36 articles (43 studies) were included in the conducted systematic review and meta-analysis (Table 1, Figure 1). Given the geographical analysis, the lowest and highest pooled prevalence of LP was observed in Poland (4 (95%CI: 0–13%)) and Kuwait (98 % (95%CI: 90–100%)), respectively (Figure 2). Also, the global pooled prevalence of LP in water systems was determined as 20% (95%CI: 15–25%) (Figure 2). While, the
lowest and highest prevalence of LP-based on water resources subgroups was water reservoir (15% (95%CI: 2–37%) and well (40% (95%CI: 26–50%), respectively (Figure 3).

According to Begg's (p-value = 0.029) and Egger's (p-value = 0.024), significant publication bias among studies was noted (Figure 4A-B); Hence, to remove the effect of publication bias, the metatrim test was performed. The pooled prevalence of LP in a water system based on metatrim in the random effect model (REM) was 21% (95%CI: 15–26%) (Figure 5).

Discussion

The prevalence of LP in the water systems was obtained as an average of 20% (95%CI: 15–25%). The prevalence of LP in the different countries considerably was difference (Figure 2), which can be associated with variation in the quality of disinfection of water, the average life of water facilities, the chemical quality of water and the type of exploitation in each country.

Some factors such as water temperature, stagnation of water in pipes, the formation of biofilms on the interior walls of pipes, the presence of protozoa, the chemical quality of water and pH could affect Legionella growth in aquatic systems [71, 72]. However, the proposed temperature for growth of LP is 29–40 °C with an optimum of 35 °C; it could survive in aqueous systems at temperatures of 0–63 °C [10]. The increase in temperature above 55 °C can be considered as an efficient technique for the prevention of bacterial growth and consequently, lower incidence of legionellosis [73]. According to Leoni et al. (2001), no LP growth was observed at temperatures above 41 °C [74]. However, the growth of LP decreased with increase in temperature [75].

Moreover, LP quickly reproduces in hot water systems such as showerheads (45–50 °C) [61]. Based on one recent study, the highest prevalence of LP in hot spring was 17.6% in the 40–48.6 °C [7]. Findings showed that the prevalence of LP in cold water was higher than hot water (Figure 4), which can be correlated with the inhibition effect of increase in temperature on LP growth. Additionally, based on ecological data, protozoans and amoeba can protect this bacteria from disinfectant, osmolality, and pH variations [61, 76].

The influence of metallic elements such as iron and manganese on the growth of Legionella previously also has been demonstrated [77, 78]. Due to organophilic properties of LP, its growth is limited in the water with low iron concentrations. Considering to findings of Portier et al. (2016), the iron pyrophosphate and ferric iron chelator did not affect the persistence of LPin the biofilms, but ferrous iron chelator showed a positive effect because of higher bioavailable ferrous ion. Hence, the growth of LP in the pipes that are made of iron could be stimulated. Also, manganese has an indirect effect on Legionella growth by enhancing the growth of biofilms and plantations [79].

Moreover, the protective role of manganese in enzymatic activity and increasing in resistance to oxidative stress has been confirmed [80]. In this regard, the positive correlation between Mn concentration (> 3µg/L) with a prevalence of LP in water was demonstrated [81]. The presence of a copper ion in water
due to its antibacterial nature can resulted in further decline in the prevalence of Legionella [82]. However, biofilms in water systems protect Legionella against disinfection [83]. Therefore, in order to reduce the prevalence of Legionella, washing of pipes and tanks in addition to exhausting of water facilities with chlorinated hot water can be recommended. According to Oberdorfer et al. (2008), the prevalence of Legionella in the old water system of the hospital was higher than new ones [84]. Nowadays, the use of polyethylene (PE) and Polyvinyl chloride (PVC) materials in water facilities is continuously expanding. The growth of biofilm was increased with the releasing of volatile organic compounds in PVC and PE pipes [85]. Therefore, using PE and PVC in water facilities may increase the prevalence of LP in water systems.

pH is another chemical parameter that affects the growth and survival of LP in water systems. The highest prevalence of LP was reported in weakly acid (pH 5–7, 37.5%) [7]. In a report by Ohno et al. (2003), the optimal range of pH for LP in water systems was defined between 6.0 and 8.0 [8]. However, other data showed that LP had not been explored in extremely acidic water [60, 86].

Considering the frequency of detection techniques, PCR (37.3%) approximately was similar to the culture method (62.7%). While in culture techniques, only living bacteria can be detected [87], in the PCR in addition to living bacteria, the bacteria that damaged by the disinfection which their DNA remains also can be detected [88]. Moreover, in the culture technique, the incubation period of 3–14 days is recommended to facilitate the growth of bacterial colonies, while the bacteria can be damaged by used acid treatment in culture technique. In another hand, the observed limitation regarding detection sensitivity (50–60%) is another disadvantage of culture techniques [89]. Due to Culturable None but Viable (CNBV) properties, LP can survey in water for a long time without being identified by culture technique. Likewise, data have shown that Legionella prevalence in amoeba-water samples was 25–50% higher than other samples because of symbiosis survival with the amoeba [90, 91]. Moreover, one of the most significant limitations of PCR techniques is bias due to the presence of inhibitors such as polysaccharide and chlorophyll in the samples [92].

Since the culture and PCR techniques have different ranges of detection limits, however, by using PCR technique, higher level of contamination in pathological samples can be detected quickly and accurately, approaching of both methods in the case of environmental issues such as LP might be recommended.

**Conclusion**

In the current study, the prevalence of LP in water systems in the defined subgroups were meta analyzed. A high prevalence of LP in water systems worldwide was demonstrated by the current study as a first systematic review in this field. Likewise, the higher prevalence of *Legionella pneumophila* was considerable in the water systems, particularly in cold water. In this context, approaching of control actions such as avoiding stagnation of water in water systems, use of high-quality water, and continuous purification of water using disinfectants factors can be recommended. Continuous monitoring of water
quality to assess the effectiveness of measures to control and prevent the prevalence of legionellosis is crucial.

**Abbreviations**

LP: Legionella pneumophila; WHO: World Health Organization; PCR: polymerase chain reaction; LOD: limit of detection; VBNC: Non-culturable cells; PVC: Polyvinyl chloride; CNBV: Culturable None but Viable.

**Declarations**

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Not applicable

**Authors’ contributions**

Searching in international database was performed by Z.GH and MJ.NA; screening of papers based on title and abstract was performed by Y.FA, M.AZ and A.AS; Data extraction and data preparation was performed by AM.MK and AZ.AL. Meta-analysis of data and preparation of manuscript was performed by Y.FA and AM.MK.

**Conflict of Interest**

There is no conflict of interest.

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**Availability of data and materials**

Not applicable

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**
Not applicable

Competing interests

The authors declare that they have no competing interests

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**Table 1**

Table 1. Characteristics included of studies
| Year | Study | Country       | Water resource         | Total sample size | Positive sample size | Technique                  | Ref |
|------|-------|---------------|------------------------|-------------------|----------------------|----------------------------|-----|
| 2011 | Iran  | Tap drinking water | 32                     | 6                 | BCYE[2]              | [20]                       |     |
| 2003 | Iran  | Hot water tank | 30                     | 11                | BCYE                 | [41]                       |     |
| 2008 | Iran  | Water reservoir | 33                     | 5                 | BCYE                 | [42]                       |     |
| 1997 | Iran  | Water reservoir | 210                    | 14                | BCYE                 | [43]                       |     |
| 2011 | Iran  | Tap drinking water | 110                    | 29                | BCYE                 | [44]                       |     |
| 2012 | Iran  | Tap drinking water | 140                    | 8                 | PCR[3]               | [45]                       |     |
| 2005 | Iran  | Tap drinking water | 113                    | 30                | BCYE                 | [46]                       |     |
| 2012 | Iran  | Tap drinking water | 140                    | 12                | PCR                  | [47]                       |     |
| 2008 | Iran  | Water reservoir | 240                    | 100               | BCYE/GVPC[4]        | [48]                       |     |
| 2010 | Iran  | Cold water tank | 77                     | 14                | PCR                  | [49]                       |     |
| 2015 | Iran  | Cold water tank | 78                     | 18                | PCR                  | [50]                       |     |
| 2015 | (Iran (Kerman) | Cold water tank | 50                     | 7                 | PCR                  | [50]                       |     |
| 2012 | Iran  | Cold water tank | 140                    | 10                | PCR                  | [51]                       |     |
| 2011 | Saudi Arabia | Cold water tank | 300                    | 5                 | BCYE                 | [24]                       |     |
| 2013 | Jordan | Hot water tank | 200                    | 17                | BCYE and BAP[5]     | [52]                       |     |
| 2010 | Taiwan | Tap drinking water | 706                    | 148               | BCYE and BAP        | [53]                       |     |
| 2005 | Morocco | Hot water tank | 128                    | 25                | BCYE                 | [54]                       |     |

[1] LOD methods for PCR: $2 \times 10^2$ GU/100 mL for LP (mip gene) and culture methods is 1 CFU/100 ml.

[2] Buffered charcoal yeast extract: culture techniques

[3] Polymerase chain reaction technique

[4] Glycine-Vancomycin-Polymyxin-Cycloheximide technique

[5] Blood agar plates

Figures
Figure 1

Flowchart describing the study design process based on PRISMA
Figure 2

Forest plot of the prevalence of LP in the water resources based on geographical.
Figure 3

Forest plot of the prevalence of LP in the water resources based on the type of water resources.
Figure 4

Evaluate publication bias based on Beggs (A) and Eggers (B) tests

Figure 5
Sensitivity analysis (Metatrim analysis) of the prevalence of LP in the water systems