

# Prevalence of *Legionella* Species in Water Resources of Iran: A Systematic Review and Meta-Analysis

CME Article

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## What's Known

- The most common *Legionella* species in the world is *L. pneumophila*.

## What's New

- For the first time, it is determined that the combined prevalence of *Legionella* species in water resources of Iran is 27.3%.
- The combined prevalence of *Legionella* spp. in hospital water, dental settings water, and other water resources are 28.8%, 23.6%, and 29.6%, respectively.
- The most common *Legionella* species is *L. pneumophila* with 60.5% prevalence.

## Abstract

**Background:** *Legionella* species are ubiquitous and naturally found in lakes, rivers, streams and hot springs, and other water resources. The present study aimed to investigate the prevalence of *Legionella* species in water resources of Iran by a systematic review and meta-analysis.

**Methods:** In search of papers relevant to the prevalence of *Legionella* in water resources of Iran, the scientific information database in both English and Persian languages was used. The search was limited to studies between the year 2000 and end of July 2016. Each cohort and cross-sectional study that reported the contamination of water with *Legionella* was included in the present study. For data analysis, comprehensive meta-analysis software with Cochran's Q and I<sup>2</sup> tests were used. P values less than 0.05 were considered statistically significant.

**Results:** The prevalence of *Legionella* species in water resources of Iran was 27.3% (95% CI: 25.3-29.3). The prevalence of *Legionella* spp. in hospital water, dental settings water, and other water resources were 28.8% (95% CI: 26.4-31.2), 23.6% (95% CI: 16.1-33.2), and 29.6% (95% CI: 25.6-33.8), respectively. The most common *Legionella* species was *L. pneumophila* with a prevalence of 60.5% (95% CI: 53.3-67.2) and the prevalence of all other species was 52.5% (95% CI: 44.7-60.2). The highest prevalence was reported in Isfahan with 55.7% (95% CI: 48.0-63.0).

**Conclusion:** Based on the results, the prevalence rate of *Legionella* species in water resources of Iran was high and the most common *Legionella* species was *L. pneumophila*.

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**Keywords** • *Legionella* • Water Resources • Iran

## Introduction

*Legionella* spp. are ubiquitous and naturally found in lakes, rivers, streams and hot springs, swimming pools, water tanks, water piping systems, cooling towers and air conditioning systems.<sup>1</sup> So far, 52 species of this bacterium have been detected among which *Legionella pneumophila* is the most important pathogenic species to humans.<sup>2</sup> Of the 52 species and 71 serogroups of *Legionella* family that can contaminate water sources, at least 20 species are pathogenic to humans, especially in those individuals with underlying medical conditions such as chronic

respiratory disease, immunocompromised, undergone surgery requiring general anesthesia, or undergone kidney transplantation.<sup>3</sup> High age, gender, smoking, alcohol consumption, and underlying diseases such as chronic lung disease, heart and kidney failure, type 2 diabetes, inadequate antibiotic treatment, immunity defects, and prolonged hospitalization have been identified as being the highest risk factors for diseases associated with *Legionella*.<sup>4</sup> Certain species of *Legionella* are associated with asymptomatic disease (Legionnaires) or disease with a mild cough, fever, and sore throat (Pontiac fever).<sup>5</sup> The disease occurs subsequent to exposure to the aquatic environment, when the water is stagnant and warm (25-42 °C) and the bacteria are inhaled into the lungs accompanied by aerosolized droplets. The most frequent route transmission is through inhalation or microaspiration of *Legionella* from contaminated water sources, including hot water systems and water from cooling towers. It has also occurred through nebulizers and showers.<sup>6</sup> Based on CDC's estimates, hospitalization rate caused by legionellosis accounts for 8,000 to 18,000 people in the United States each year.<sup>7</sup> Common habitats for this bacterium are hospitals that provide susceptible conditions for people to contract the disease.<sup>8</sup> The first outbreak of the disease was reported in 1957.<sup>9</sup> The prevalence range of legionellosis outbreaks in hospitalized patients has been reported at 0% to 47%.<sup>7</sup> According to reports, 3% to 8% of all community-acquired pneumonias (CAP) are likely produced by *Legionella* spp. and 85% of those result from *L. pneumophila*.<sup>10</sup> *Legionella* nosocomial infection prevalence is often associated with the contamination of hospital water resources.<sup>11</sup> Biofilm formation of *Legionella* in water piping systems will ensure the survival of this bacterium and thus it could resist the biochemical effects of chlorine and other disinfectants.<sup>12</sup> Epidemiological data show that the epidemic with the highest numbers of *Legionella* occurs in water.<sup>13</sup> The important point is that *Legionella* in certain circumstances includes encounters with poor diet, oxidative stress, stress of osmotic pressure, and water chlorination convert to the mode that is still viable but is not culturable.<sup>14</sup> Water stagnation, temperatures between 25 °C to 42 °C, organic contamination, and the presence of protozoa are suitable and susceptible conditions for the growth of *Legionella* species in water.<sup>15</sup> Despite many reports pertaining to *Legionella* and its prevalence in water resources of different countries, there are few reports about this bacterium in Iran and in fact, at present, there

is no meta-analysis.<sup>16</sup> Therefore, the present study aimed to investigate the prevalence of *Legionella* species in water resources of Iran by a systematic review and meta-analysis.

## Materials and Methods

### Search Strategies

According to the Prisma protocol (PRISMA, <http://www.prisma-statement.org>) for searching papers, various databases were used to select articles in both English and Persian languages. Targeted databases were PubMed, Scopus, Web of Science, Cochrane Library, ScienceDirect, MEDLINE, Google Scholar, the Iranian Scientific Information Database ([www.sid.ir](http://www.sid.ir)), Iranmedex ([www.iranmedex.com](http://www.iranmedex.com)), Magiran ([www.magiran.com](http://www.magiran.com)), and Irandoc ([www.irandoc.ac.ir](http://www.irandoc.ac.ir)). The search was limited to studies between the year 2000 and end of July 2016. The applied keywords included *Legionella* and Iran in combination with words such as epidemiology, hospital water, tap water, and cooling water. Two investigators, one with a background in bacteriology and the other in epidemiology, independently searched the relevant studies.

### Inclusion and Exclusion Criteria

Each cohort and cross-sectional study that reported contamination with *Legionella* in Iran was included in the present study. The search was restricted to environmental studies. The exclusion criteria were clinical trials, review articles, letters to the editor, congress and meeting abstracts, short communication articles, papers presented in languages other than English or Persian, animal studies, meta-analysis or systematic reviews, abstract forms of studies, case report articles, duplicate publication of the same paper, unpublished studies, confusing studies, and studies with sample size less than 20. To include all relevant and potential studies, the references of systematic reviews and meta-analysis papers were surveyed.

### Data Extraction

Following a careful and detailed study of full-text articles, information such as the name of first author, study period, publication year, location of the study, sample size, sampling location, number of positive samples, diagnostic methods and the prevalence rate, number of *Legionella* spp., and detection methods were extracted. The information was then entered into Microsoft Excel software.

### Data Synthesis and Analysis

Data analysis was performed using comprehensive meta-analysis software

version 3.3.070. In case of heterogeneity, random effects model was used. To calculate the possibly of heterogeneity between studies, the Cochran Q and  $I^2$  tests were used. The prevalence of contamination of water resources with *Legionella* was reported with 95% confidence intervals (CIs).

## Results

### Characteristics of Selected Studies

As shown in figure 1, 3,886 studies were identified by searching the English and Persian databases out of which 680 papers were excluded because of duplication and 3,203 articles were included for eligibility evaluation. Then, 380 articles were excluded due to the study design, book, congress, publication prior to the year 2000, thesis, or were in other languages. In the next step, the abstracts of 2,823 papers were screened and 2,608 papers were excluded due to subject irrelevance. Subsequently, 215 papers with full texts were evaluated out of which 189 papers were deleted owing to the incomplete report of prevalence data, confusing data, or sample size less than 20. Finally, 26 studies were included in the meta-analysis (16 studies related to hospital water, 3 studies related to dental settings water, and the remaining 7 studies were associated with other water resources). The selected studies were from different geographical regions of Iran and approximately covered the country as a whole, but mostly focused on the central region, especially in Tehran (n=8) followed by Isfahan (n=5). The largest sample size belonged to the study dated 2015 by Yazdanbakhsh et al.

Phenotypic and molecular methods have been used for the detection and identification of *Legionella* species. The detection of phenotypic methods included morphology, culture on BCYE-a medium and smear microscopy as well as molecular techniques and biochemical tests such as PCR, nested PCR, real-time PCR, DFA, ELISA, and latex agglutination. Six studies used both phenotypic/biochemical tests and molecular methods for detection and identification and 11 studies only applied molecular techniques (table 1). Based on the selected studies (figure 2), the prevalence of *Legionella* species in water resources varied from 0% (95% CI: 0.0-1.4) to 70% (95% CI: 57.3-80.2).

### Overall Effects

Based on the heterogeneity test, there were heterogeneities between the studies ( $Q^2=173.623$ ,  $I^2=91.42$ ,  $t=291.3$ ,  $P<0.001$ ). Thus the random model was used to combine

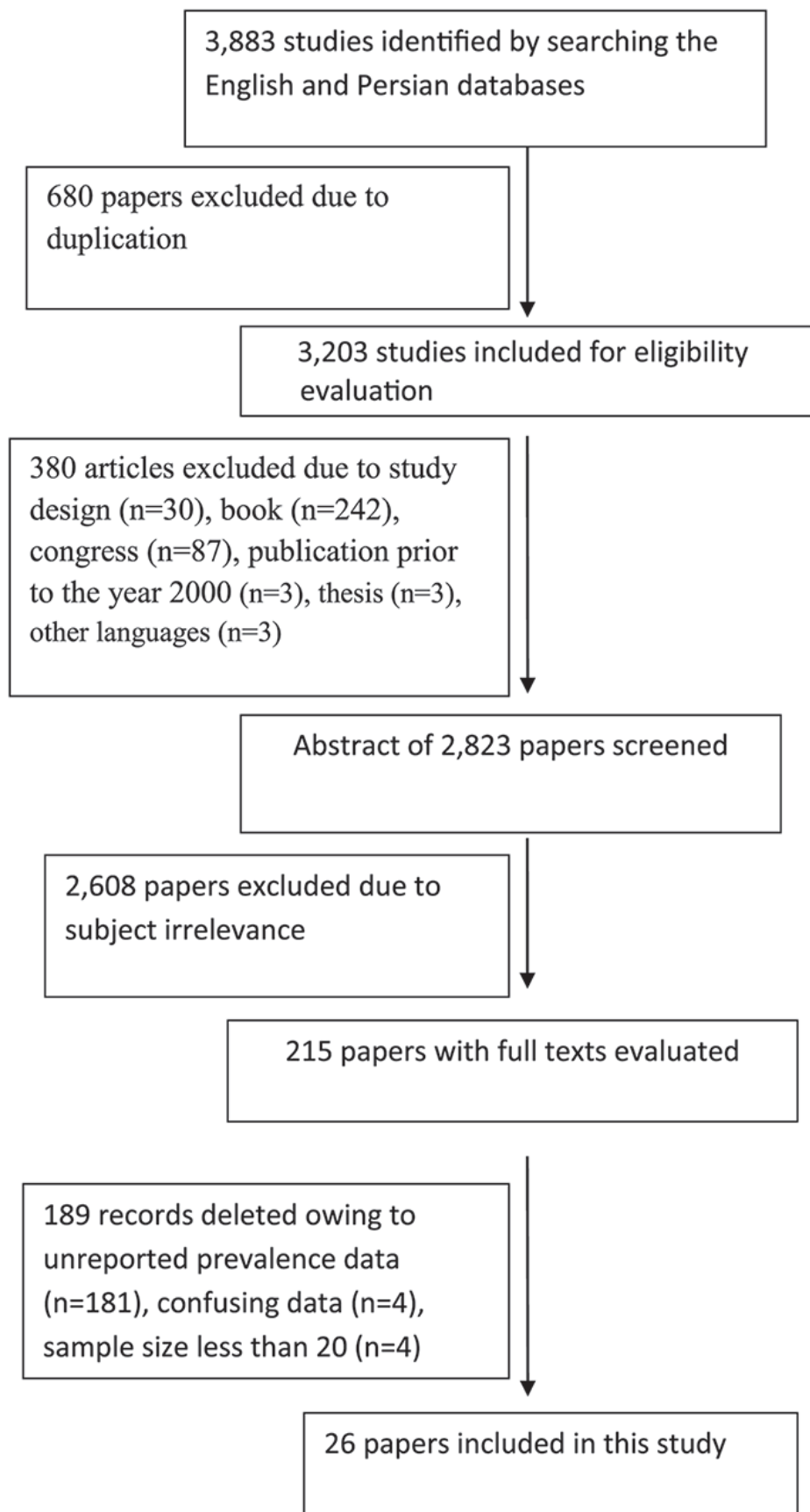
the prevalence of *Legionella* species in water resources. Table 2 shows that the combined *Legionella* species prevalence in water resources of Iran was 27.3% (95% CI: 25.3-29.3). To evaluate the weight of each study in this meta-analysis, the forest plot test was used (figure 2).

### Subgroups Analysis for *Legionella* Species of Water Resources

According to the subgroups analysis, the prevalence of *Legionella* spp. in hospital water, dental settings water and other water resources were 28.8% (95% CI: 26.4-31.2), 23.6% (95% CI: 16.1-33.2), and 29.6% (95% CI: 25.6-33.8), respectively (table 2). As evident in table 2, the most common species of *Legionella* was related to the *L. pneumophila* with a prevalence of 60.5% (95% CI: 53.3-67.2) and the prevalence of all other species was 52.5% (95% CI: 44.7-60.2). Based on the location, the highest prevalence was reported in Isfahan with a rate of 55.7% (95% CI: 48.0-63.0) followed by Tehran with the prevalence of 27.9% (95% CI: 24.5-31.5).

## Discussion

Overall, the combined prevalence of *Legionella* species in water resources of Iran was 27.3%. This prevalence is low compared to other countries which might be due to the fastidious growth of this bacterium, the need for special skills to grow, and the presence/lack of inhibitors to control the samples. As mentioned in our results, the prevalence varied from 0% to 70%. Such significant variation in prevalence is possibly related to the sampling from different geographical locations, number of samples, the sample size for concentration and filtration, type of water systems (hot and cold), and water quality.<sup>43</sup> Based on the report of the CDC, the prevalence of legionellosis disease in hospitals is between 25% to 45%,<sup>44</sup> and the death rate from this disease in hospitals is 30%.<sup>45</sup> Our results showed that the prevalence of *Legionella* spp. in hospital water, dental settings water, and other water resources were 28.8%, 23.6%, and 29.6%, respectively. Since all hospitals in Tehran, and probably throughout the country, obtain their water supply from the municipal water suppliers, the presence of *Legionella* in hospital water system indicates a high resistance of this organism to adverse environmental conditions. Therefore, water treatment operations and disinfection with chlorine must be used by modern methods (e.g. simultaneous ozonation and disinfection combination method) to remove *Legionella*.<sup>46</sup> The results of several studies show



**Figure 1:** Flow diagram of the study process according to the inclusion and exclusion criteria.

**Table 1:** Characteristics of the included studies for meta-analysis

Study	Year of study	Publication (year)	Location	Sample size	Number of <i>Legionella</i> spp.,	Detection methods
Hosseini Doust <sup>17</sup>	-	2003	Tehran	32	6	PCR
Hosseini Doust et al. <sup>18</sup>	2006	2008	Tehran	132	30	Morphology, PCR
Mohabati Mobarez <sup>19</sup>	-	2007	Tehran	110	29	Morphology, PCR
Eslami <sup>20</sup>	2011	2012	Tehran	32	11	Morphology, PCR
Rafiee <sup>21</sup>	2011-2012	2014	Tehran	45	13	Morphology
Esmaeili <sup>22</sup>	-	2008	Tehran	113	30	Real-time PCR
Yaslianifard <sup>23</sup>	2010-2011	2012	Tehran	52	5	Morphology
Mirmohamadlou <sup>24</sup>	2013-2014	2016	Tehran	150	56	Morphology, PCR
Baghal Asghari <sup>25</sup>	-	2012	Isfahan	33	23	Nested PCR
Baghal Asghari et al. <sup>26</sup>	-	2012	Isfahan	60	42	Morphology, Nested PCR
Baghal Asghari and Nikaeen <sup>27</sup>	-	2013	Isfahan	44	29	Morphology, DFA
Movahedian Attar <sup>28</sup>	2003	2004	Isfahan	30	11	PCR
Ghalyani <sup>29</sup>	-	2015	Isfahan	50	5	Morphology, Latex agglutination
Motaharrynia <sup>30</sup>	-	2010	Zanjan	120	25	Morphology, PCR
Ghotaslou <sup>31</sup>	-	2013	Tabriz	140	10	Morphology
Mirhossaini <sup>32</sup>	-	2009	Khorrman-abad	240	100	Morphology
Yazdanbakhsh <sup>33</sup>	2015	2016	Shahroud	562	0	ELISA
Ajami <sup>34</sup>	2009	2012	Mashhad	52	19	PCR
MAG Moghadam <sup>35</sup>	2011-2012	2013	Rasht	140	12	PCR
MAG Moghadam et al. <sup>36</sup>	2014	2015	Guilan	135	12	PCR
MAG Moghadam and Honarmand <sup>37</sup>	2014	2016	Guilan	63	6	Morphology
Moosavian and Khosro Shahi <sup>38</sup>	-	2004	Ahvaz	210	14	Morphology, PCR
Moosavian and Dashti <sup>39</sup>	-	2011	Khuzestan	150	33	PCR
Ahmadinejad <sup>40</sup>	2006	2011	Kerman	77	30	Nested real-time PCR
Ahmadrajab <sup>41</sup>	2015	2016	Kerman and Bam	128	29	Morphology
Alipour <sup>42</sup>	2011	2013	Bandar Abbas	66	15	PCR

Meta Analysis

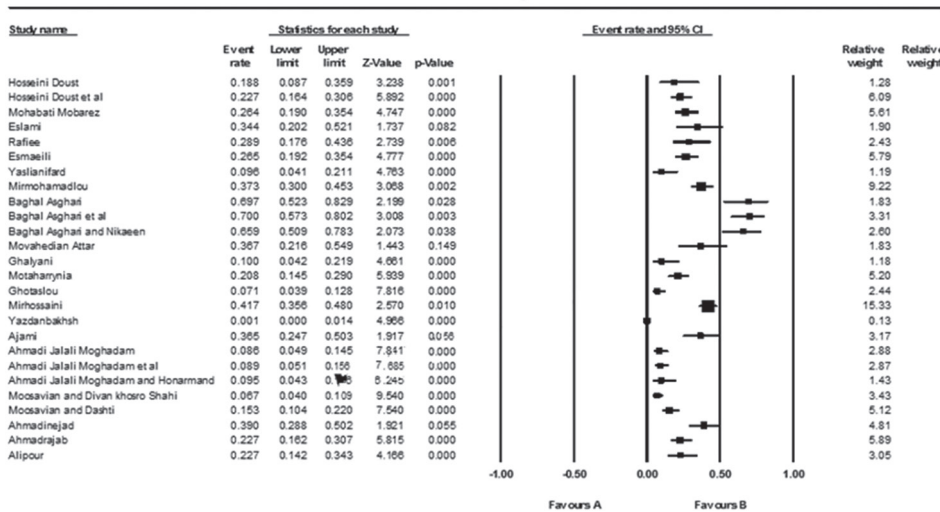


Figure 2: Forest plot of the meta-analysis for *Legionella* species prevalence of water resources. It shows the assortment of studies based on the criterion that have the best effect and indicate the weighted average and effect of each study with respect to the total study.

**Table 2:** Subgroups analysis for *Legionella* of water resources

Subgroups	Number of studies	Heterogeneity test			Egger's test		Random model		
		NTM prevalence (95% CI (%))	Z	P value	Q	P value	I <sup>2</sup>	t	P value
Overall effect	26	27.3 (25.3-29.3)	19.1	<0.001	291.3	<0.001	91.4	1.9	0.05
Hospital water	16	28.8 (26.4-31.2)	15.03	<0.001	139.5	<0.001	89.2	1.9	0.07
Dental settings water	3	3.6 (16.1-33.2)	4.8	<0.001	26.4	<0.001	92	4.9	0.01
Other water resources	7	29.6 (25.6-33.8)	8.6	0.06	134.4		95.5	0.2	0.84
Based on <i>Legionella</i> species									
<i>L. pneumophila</i>	19	60.5 (53.3-67.2)	2.8	0.004	75.5	<0.001	76.1	17	<0.001
Other species	7	52.5 (44.7-60.2)	63.6	0.52	9.1	0.16	34.5	5	0.35
Based on location									
Tehran	8	27.9 (24.5-31.5)	1.45	<0.001	18.02	0.12	61.1	1.7	0.12
Isfahan	5	55.7 (48.0-63.0)	7.5	0.14	39.8	<0.001	89.9	2.7	0.07
Zanjan	1	20.8 (14.4-29.0)	5.9	-	-	-	-	-	-
Tabriz	1	7.1 (3.9-12.8)	7.8	-	-	-	-	-	-
Guilan	3	8.9 (6.3-12.4)	12.17	<0.001	0.05	0.97	0.00	2.17	0.27
Khorram-abad	1	41.7 (35.6-48.0)	2.57	0.01	0.00	1.00	0.00	-	-
Shahrud	1	0.001 (0.0-0.014)	4.9	<0.001	0.00	1.00	0.00	-	-
Ahvaz	2	11.1 (8.1-14.9)	11.8	<0.001	6.8	0.01	85.2	-	-
Kerman	2	29.4 (23.4-36.1)	5.6	<0.001	6.1	0.01	83.6	-	-
Bandar Abbas	1	22.7 (14.2-34.3)	4.1	<0.001	0.00	1.00	0.00	-	-
Mashhad	1	36.6 (24.7-50.3)	1.9	0.05	-	1.00	0.00	-	-

that even the concentration of trace elements (iron, manganese, zinc, and copper), water hardness, and alkalinity in hospital water is effective against the presence of *Legionella* and its density.<sup>15,24</sup> As stated, 23.6% of dental settings water is contaminated with *Legionella*, which is an alarming rate. As shown previously, dental staff had a higher degree of positivity for *L. pneumophila* serum antibodies.<sup>47</sup> Turetgen et al. (2009) showed that the microbial contamination of water in dental units was high for *L. pneumophila*.<sup>48</sup> The use of waterline system coated with disinfectants, use of separate water source in dental settings for easy disinfection, and designing biofilm removal systems should all be considered for the reduction of *Legionella* spp.

Our meta-analysis, consistent with all studies conducted in other regions of the world, revealed that *L. pneumophila* had the most frequent occurrence in all water resources of Iran. Studies have shown that in America and Europe, 70% to 90% of legionellosis infection incidences are related to *L. pneumophila*.<sup>49</sup> Based on the studies in the United States, from 2003 to 2005, an average of 2,000 people were infected each year by contaminated water.<sup>50</sup>

No difference between detection by PCR and conventional culture was observed in the Iranian studies while theoretically, PCR should provide higher sensitivity than the culture method.<sup>51</sup> However, sometimes PCR is inhibited

by inhibitors and unknown materials and the report of *Legionella* is based on CFU in the culture method but PCR does not comply with this rule.<sup>52</sup> A standard method for the detection of *Legionella* species in environmental water is to use culture techniques. However, these have a number of limitations such as the slow growth rate of *Legionella* and commonly 3-10 days is necessary for incubation. Furthermore, only culturable isolates are detectable in this method and treatment with acid may cause damage and leads to stress in *Legionella*.<sup>53</sup> However, the PCR technique detects both the living and dead cells,<sup>54</sup> and probably for this reason no isolate was detected in one of the studies conducted by Yazdanbakhsh et al. (2015).

France, Italy, and most European countries determined and confirmed 10,000 CFU/l as the risk threshold for water contamination with *Legionella* bacterium in water distribution network systems. If the pollution exceeds this amount, control measures are necessary.<sup>55,56</sup> Concentrations above this rate of culturable *Legionella* may increase the risk of human infection.<sup>57</sup> Regarding the importance of *Legionella* bacterium in water resources, particularly in hospital water, and its transfer to patients in sensitive wards such as ICU and CCU, control measures such as treatment operation and disinfection with chlorine and modern methods (ozonation and simultaneous disinfection combination method) are necessary.

The strength of this study is that an extensive search of multiple databases was conducted to identify appropriate articles. Additionally, article selection was carried out independently by two researchers. In case of disagreement between researchers, this was resolved through internal discussions. The meta-analysis was performed in accordance with the available published guidelines (PRISMA, <http://www.prisma-statement.org>) and subgroups analysis was also performed to reduce heterogeneity. The present study has some limitations which have to be pointed out. We were not aware of past studies with unpublished results and hence they were not included in the present study. Additionally, we did not contact the authors of the included studies to obtain further data in cases where clarification was required. Thus, the meta-analysis was performed only based on the available information in selected studies.

### Conclusion

Based on the results of the present study, the prevalence rate of *Legionella* species in water resources of Iran was high and the most common *Legionella* species was *L. pneumophila*.

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**Conflict of Interest:** None declared.

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