AKADÉMIAI KIADÓ

European Journal of Microbiology and Immunology

12 (2022) 4, 93–99

DOI:
10.1556/1886.2022.00023

REVIEW PAPER

Contamination of dental unit water lines (DUWL) with *Legionella pneumophila* and *Pseudomonas aeruginosa*; A Middle East systematic review and meta-analysis

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Received: November 10, 2022 • Accepted: December 12, 2022
Published online: January 10, 2023

ABSTRACT

This review aimed to evaluate the contamination rate of dental unit waterlines (DUWL) with *Pseudomonas aeruginosa* and *Legionella pneumophila* in several countries in the Middle East.

Literature search was conducted in databases such as PubMed, Scopus, Web of Science, and Google Scholar to gather studies published from the beginning of 2000 to 30th April 2020. Medical Subject Headings (MeSH) terms were; "Legionellosis"; "Legionnaire", "Legionellosis", "*L. pneumophila*", "dent", "dental", "dentistry", "Dental Unit Waterlines", "dental water", "DUWL", "Middle East", "*P. aeruginosa*", "Iran", "Turkey", "Iraq", and "Jordan". The search was independently conducted by two of the authors. Data was analyzed using Comprehensive Meta-Analysis software.

Almost all studies included in this review reported a high rate of bacterial contamination of DUWL, which exceeded the current standard bacterial contamination level of <200 (CFU) mL^{-1} recommended by the American Dental Association (ADA). The combined prevalence of *L. pneumophila* from four countries (Iran, Jordan, Turkey, and Iraq) was 23.5% (95% Cl: 6.5–57.7), and the combined prevalence of *P. aeruginosa* was reported 21.7% (95% Cl: 7.1–50.1%).

This study showed a high bacterial contamination rate of DUWL with opportunistic pathogens. So, it is recommended to prevent biofilm formation in DUWL, some measures should be extended by practical approaches allowing for water quality control and improvement on-site in the dental practices such as mobile filtration units, chlorination and disinfection chemicals.

KEYWORDS

contamination, dental unit water lines, *Pseudomonas aeruginosa*, *Legionella pneumophila*, Middle East

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INTRODUCTION

The microbial quality of water in dental unit water systems (DUWL) is highly important to prevent the exposure of dentists, dental staff, and patients to contaminated water aerosols produced by these units [1]. Dental unit water systems consist of several long nylon

or polyvinyl chloride pipes with a small diameter. When water flow becomes stagnant in these pipes for a long time, biofilm formation on the inner surface of water lines is facilitated [2], leading to bacterial contamination and infections [3]. According to the guidelines of the American Dental Association (ADA), DUWL should contain no more than 200 colony-forming units (CFU) mL^{-1} [4].

Pseudomonas aeruginosa and *Legionella pneumophila* are commonly found in dental unit waterlines [5]. According to reports, dentists, dental staff, and the patients referring to dental clinics are exposed to a higher risk of bacterial and viral infections and consequently higher rates of respiratory disorders compared to the general public [6]. It is believed that a significant ratio of respiratory infections in dentists is due to the large production of aerosols during the scaling process [6, 7].

It appears that the hospital water system contaminated with endemic *Legionella* is the main source of nosocomial legionellosis resulting from the inhalation of contaminated water aerosols from water sources [8, 9]. *Legionella* spp. are responsible for about 3–8% of all community-acquired pneumonias (CAP), and particularly, 85% of these pneumonias are attributed to *L. pneumophila* [10].

Generally, *Pseudomonas* spp. are not causative agents for oral infections; however, patients with cystic fibrosis and immunodeficiency have a higher susceptibility to pulmonary infections caused by *P. aeruginosa*, which an important route of its transmission is via the aerosols produced during dentistry procedures [11, 12]. Hence, water quality monitoring in dental clinics is vital for the early identification of *Legionella* and other microorganisms and preventing the infections raised by these microorganisms.

Objective

There is no comprehensive review on the contamination rate of DUWL with microbial agents in the Middle East. Considering the critical importance of this issue, we decided to explore the contamination rate of DUWL with *P. aeruginosa* and *L. pneumophila* in a number of Middle East countries through systematic review and meta-analysis.

METHODS

Data sources and search strategy

Literature search was conducted in the databases of PubMed, Scopus, Web of Science, and Google Scholar to gather the studies published from the beginning of 2000 to 30th April 2020. Medical Subject Heading (MeSH) terms and text words were; "Legionellosis", "Legionnaire", "Legionnaires disease", "Legionellosis", "Legionella", "*L. pneumophila*", "dent, dental", "dentist", "dentistry", "Dental Unit Waterlines", "dental water", "DUWL", "Middle East", "*P. aeruginosa*", "*P. aeruginosa*", "Iran", "Turkey", "Iraq", and "Jordan". The search was independently conducted by two of the authors.

Study eligibility criteria

We focused on the studies reporting the contamination rate of DUWL with *L. pneumophila* and *P. aeruginosa*, published from the beginning of 2000 to 30th April 2020. Only the studies that used standard diagnostic methods for the microorganisms were included. The studies conducted before 2000 and those employing substandard methods were excluded. Case reports, case series, conference papers, and abstracts were also excluded.

Quality assessment

For quality assessment of the studies, in addition to eligibility criteria, was used the Critical Appraisal Skills Programme (CASP) checklist for cross-sectional studies (www.casp-uk.net) [13].

Data extraction

Two authors extracted the data, including the following items: the family name of the first author, publication year, time of study conduction, setting (s), sample size, the prevalence of *Legionella* spp., *L. pneumophila*, *Pseudomonas* spp., and *P. aeruginosa*, and diagnostic techniques.

Statistical analysis

Statistical analysis was performed using Comprehensive Meta-Analysis software (Version 3.3.070). The random effect method was used to estimate the overall prevalence of *Pseudomonas* and *Legionella*. Statistical heterogeneity among the selected studies was explored using the Q2 test and the I^2 statistic. The I^2 value of >50% or a *P*-value of <0.05 were considered as a sign of significant heterogeneity among the studies. As well, the Egger regression test and funnel plot were used for assessing publication bias.

RESULTS

Characteristics of the studies included

In total, 1738 studies were obtained through literature searching. After removing duplicates, 245 additional studies were excluded upon reading the abstracts, titles, and full-texts. Finally, 10 studies were selected for systematic review and meta-analysis. Four out of the 10 studies were from Turkey, three from Iraq, two from Iran, and one from Jordan. No studies from other Middle Eastern countries met our inclusion criteria. The studies included in the present review used phenotypic methods, such as buffered charcoal yeast extract (BCYE), cetrimide agar, the oxidase test, molecular testing such as polymerase chain reaction (PCR), and also, direct fluorescent antibody assay and the ELISA technique to identify microorganisms (Table 1).

Total viable count (TVC). *Legionella* was present at the concentration of 312 CFU/100 mL or greater [B Ajami et al. [14]], and bacteria were present at a concentration between

Table 1. Characteristics extracted from included studies

First author (s)	Study time	Publication	Location	Sample size	*Legionella* spp.	*Legionella pneumophila*	*Pseudomonas* spp.	*Pseudomonas aeruginosa*	Technique
P. Ghalyani [6]	–	2015	Iran	50	–	5	–	4	PCR, Culture in Cetrimide agar, and oxidase test
B. Ajami [14]	2009	2012	Iran	52	–	19	–	–	ELISA test
A.A. Taher [7]	2015–16	2017	Iraq	94	34	–	–	–	BCYE, PCR, Culture in Cetrimide agar, and oxidase test
Z. S. Alsehlawi [33]	2016	2016	Iraq	94	–	9	–	–	BCYE, PCR, Culture in Cetrimide agar, and oxidase test
S. R. Oleiwi [15]		2017	Iraqi	52	–	–	–	11	Culture
SY. Ma'ayeh [16]	–	2008	Jordan	30	–	26	–	–	BCYE, Culture in Cetrimide agar, and oxidase test
A. Uzel [1]	2007	2008	Turkey	20	–	0	76	22	BCYE
D. Goksay [3]	–	2008	Turkey	59	0	–	–	14	BCYE, Culture in Cetrimide agar, and oxidase test
D. Gungor [18]	–	2014	Turkey	100	0	–	13	3	BCYE, latex agglutination Kit, Culture in Cetrimide agar, and oxidase test
E. Bodrumlu [17]	–	2007	Turkey	71	0	–	–	–	BCYE, Direct fluorescent antibody assay

Abbreviations: PCR; polymerase chain reaction, BCYE; buffered charcoal yeast extract

50 and 90 CFU/100 mL [RS Oleiwi et al. [15]]. The count of *L. pneumophila* in the samples obtained from DUWL ranged between 0 and 8.3×10^3 (CFU) mL^{-1} [SY Ma'ayeh et al. [16]]. Also, samples from DUWL were contaminated with bacteria beyond 200 CFU mL^{-1} [E Bodrumlu et al. [17], Dogruöz Güngör et al. [18], Duygu Göksay et al. [3], and A Uzel et al. [1]]. The detailed microbial contamination data of DUWL have been summarized in Table 2.

Overall effects

***Combined prevalence of* L. pneumophila.** The prevalence of *L. pneumophila* reported by the studies reviewed varied between 0 and 86.7%. The combined prevalence of *L. pneumophila* in four countries (Iran, Jordan, Turkey, and Iraq) was obtained at 23.5% (95% Cl: 6.5–57.7, $Z = 1.5$,

Table 2. Total viable count (TVC) used in the studies included in the present review

First author	Explanations
P. Ghalyani [6]	–
B. Ajami [14]	• *Legionella* was present at concentrations of 312 CFU/100 mL or greater
A.A. Taher [7]	–
Z. S. Alsehlawi [33]	–
S. R. Oleiwi [15]	• 50–90 CFU/100 mL
SY. Ma'ayeh [16]	• The counts of *Legionella pneumophila* obtained from the DUWL samples ranged between 0 and 8.3×10^3 (CFU) mL^{-1}
A. Uzel [1]	• All three types of water samples obtained from 20 units were found to have higher TVC values than EU guidelines
D. Göksay [3]	• All of high-speed drill (range 370–52,240 (CFU) mL^{-1}) and 90% of oral rinsing cup (range 183–119,117 (CFU) mL^{-1}) exceed American Dental Association (ADA) standard for dental unit water
D. Güngör [18]	• It was found that 37 out of 50 output waters (range 2–58,533 (CFU) mL^{-1}) and 18 out of 50 input waters (range 1–28,111 (CFU) mL^{-1}) exceeded the ADA's limit of 200 (CFU) mL^{-1} in DUWL
E. Bodrumlu [17]	• 27% of the dental unit water samples were contaminated with bacteria above 200 (CFU) mL^{-1}

Abbreviations: CFU; colony forming unit, ADA; american dental association, TVC; total viable count

$P = 0.001$, $Q = 53.6$, $I^2 = 92.5$, $t = 0.13$, $P = 0.89$) (Fig. 1, Table 3). The visual assessment of the relevant funnel plot showed the presence of publication bias among the studies (Fig. 2), but the Egger regression test revealed no publication bias ($P = 0.89$).

***Combined prevalence of* P. aeruginosa.** The prevalence of *P. aeruginosa* in the selected studies varied between 3% and 23.7%. The combined prevalence of *P. aeruginosa* in four countries (Iran, Jordan, Turkey, and Iraq) was obtained at 21.7% (95% Cl: 7.1–50.1%), $Z = 1.95$, $P = 0.051$, $Q = 42{,}105$, $I^2 = 90.5$, $t = 0.16$, $P = 0.87$) (Fig. 1, Table 3).

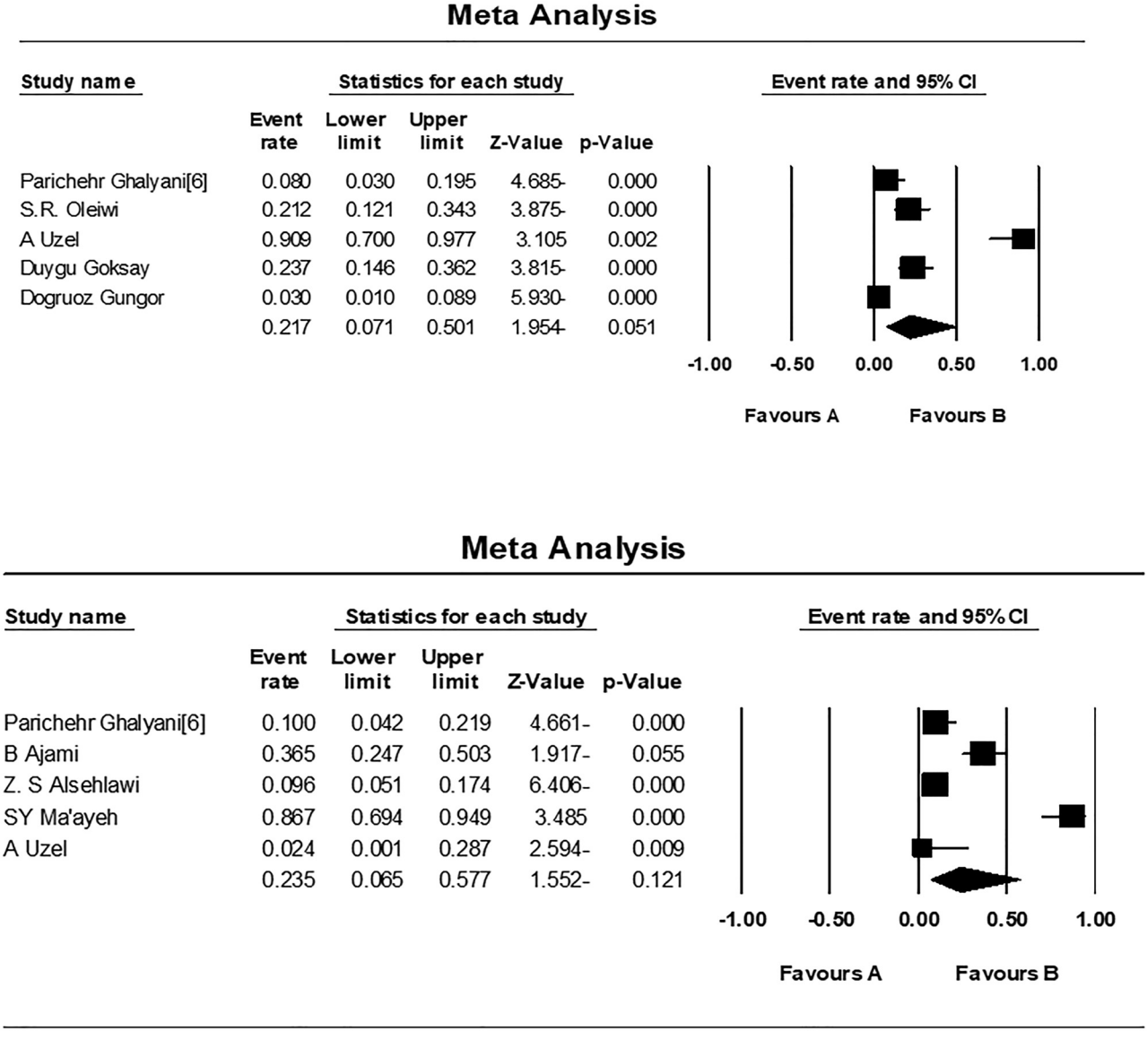

Fig. 1. Forest plot of meta-analysis on the *Legionella pneumophila* (top image), and *Pseudomonas aeruginosa* (below image) isolated from dental unit waterlines

Table 3. Overall effects of resulted from included studies

Overall effects	Number of studies	Heterogeneity test			Egger's test			Random model	
		Prevalence (95% CI) (%)	Z	*P*	Q	*P*	I^2	T	*P*
Legionella pneumophila	5	23.5% (6.5–57.7)	1.5	0.00	53.6	0.00	92.5	0.13	0.89
Pseudomonas aeruginosa	5	10.3 (4.7–20.9)	5	0.00	28.5	0.00	85.9	0.02	0.98

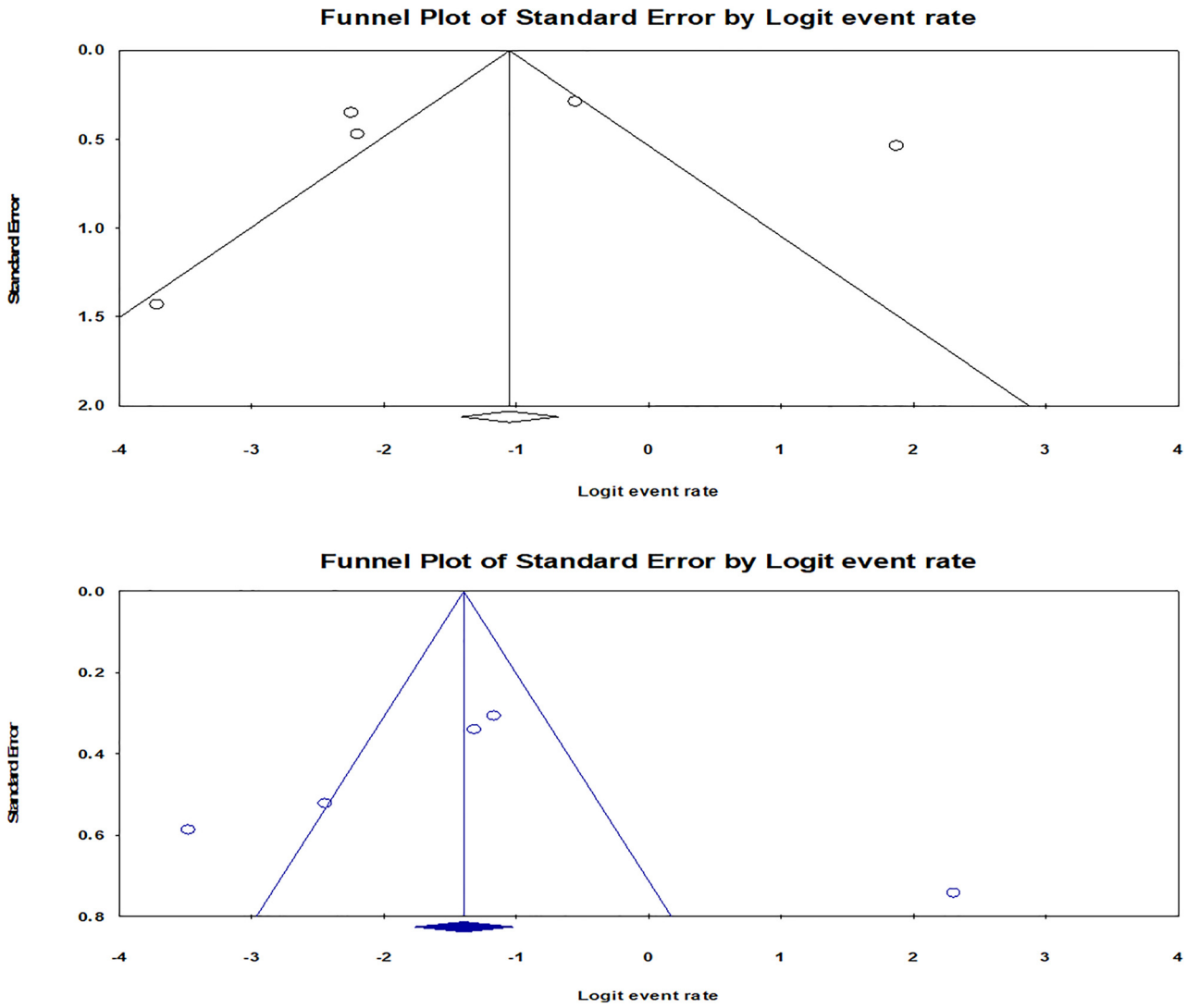

Fig. 2. Funnel plot of meta-analysis on the *Legionella pneumophila* (top image), and *Pseudomonas aeruginosa* (below image) isolated from dental unit waterlines

The results of funnel plot analysis suggested the presence of publication bias among the studies (Fig. 2); however, this was not confirmed by the Egger regression test ($P = 0.87$).

DISCUSSION

The ADA has recommended an allowable standard for the contamination of DUWL with aerobic mesophilic bacteria as no more than 200 (CFU) mL^{-1} [4]. In the present study, *Legionella* was detected at the concentration of 312 CFU/100 mL or greater [14], and the concentration of 50–90 CFU/100 mL in one of studies included in the present review [15]. In another study, the count of *L. pneumophila* in DUWL samples ranged between 0 and 8.3×10^3 (CFU) mL^{-1} [16]. As well, all of the three studies conducted in Turkey showed that DUWL samples were contaminated with bacteria at concentrations >200 (CFU) mL^{-1}[1, 3, 17]. These results were in line with the findings of a study conducted in seven European countries evaluating the microbiological profile of DUWL in general dentistry offices reporting that the microbial content of the water supplied by 51% of DUWL exceeded the current ADA-recommended bacterial contamination threshold (i.e. <200 (CFU) mL^{-1}) [19]. Compared with our findings, different studies have reported high microbial contamination rates above the ADA's standard who reported the contamination rates of 96%, and 63.1%, respectively [20, 21].

Our systematic review and meta-analysis on the studies included showed that the prevalence of *L. pneumophila* in DUWL varied from 0 to 86.7%. The combined prevalence of *L. pneumophila* in DUWL samples from four countries (Iran, Jordan, Turkey, and Iraq) was obtained at 23.5%. The prevalence of *P. aeruginosa* in DUWL samples varied between 3% and 23.7%, and its combined prevalence from the studies conducted in four countries (Iran, Jordan, Turkey, and Iraq) was obtained at 21.7%. This broad variation in the prevalence of *Legionella* and *Pseudomonas* in the present

review can be possibly attributed to variabilities in geographical locations, water sources [16], diagnostic methods, the type and quality of water supplying systems [19], chlorine concentration in water lines [17], the materials used in the manufacturing of tubes [16], the duration of the use of tubes, infection control measures in dental offices, and the age of the waterline system [6].

As mentioned, the diagnostic method is an important parameter in detecting bacterial contamination. Some *Legionella* spp. cannot be easily identified by routine culture methods and need molecular-based techniques [3]. Also, the DFA technique has a relatively low sensitivity and specificity for detecting *Legionella*, which may even interfere with the detection of other bacteria [17]. Two studies reported that the loads of microorganisms were significantly higher in the output water of dental units compared to input water, showing biofilm formation in DUWL [3, 18]. These results were inconsistent with the findings of two studies conducted in another region of the world [22, 23]. Due to their complex nature, established biofilms are difficult to be removed [24] even by hydrogen peroxide and iodine [25]. The presence of sludge, sediment, and some materials associated with biofilm formation may play a significant role in the persistence of *L.* spp. [26]. The input water of DUWL is typically free of pathogenic bacteria, but the detachment of microorganisms from biofilms causes the bacterial contamination of the output water [27]. In accordance with our study, other studies have reported the presence of *Legionella* in DUWL [22]. A study conducted in seven European countries showed a low level of contamination of DUWL with *Legionella* spp. (i.e. 9% in Danish and Spanish samples and zero in samples from the UK (United Kingdom), the Netherlands, Greece, Germany, and Ireland), which was lower compared to the value obtained in this review[19]. Regarding *P. aeruginosa* contamination, the results of the recent study were comparable with our findings, where *P. aeruginosa* was isolated from 6, 5, 7, and 10% of samples from Greece, the Netherlands, Germany, and Spain, respectively [19]. Similar to our results, several studies have reported the high prevalence of *L. pneumophila* in dental units with the frequencies of 58, 33.3 [28], and 30% [29].

It is known that *P. aeruginosa* is an opportunistic pathogen that more frequently causes infections in immunocompromised patients [4]. Consistent with our observation in this review, Others found that *P. aeruginosa* was the most prevalent bacteria in the samples collected from DUWL [30, 31]. Accordingly, another one in 2002, declared that dentists' offices due to contaminated aerosols were a high-risk place for the transmission of *P. aeruginosa* to dentists, dental staff, and patients [32]. Overall, the high contamination rate reported by almost all the studies included in the current review seems alarming in terms of infection control measures in Middle Eastern countries.

Therefore, in the light of available guidelines, the quality of water in DUWL should be regularly checked to prevent biofilm formation and the excessive growth of pathogenic microorganisms in these tubes and lines. Moreover, DUWL must be constantly chlorinated, and dental units' reservoirs and tanks should be fed with sterile and high-quality water. Also, water used for dental units should have a total colony count of <200 CFU mL^{-1} and fulfil standards of drinking water certain bacteria. Sterile water or saline should be provided from a separate, and reasonably single use source for surgical procedures. Anti-retraction valves should be fixed on all handpieces and must be frequently checked and kept.

CONCLUSIONS

The present review and meta-analysis showed a high contamination rate of DUWL with *L. pneumophila* and *P. aeruginosa* in some Middle Eastern countries. Therefore, it is recommended to use high-quality water, implant filters in water reservoirs, and regularly monitor water resources, as the best measures that can be taken, to prevent bacterial colonization and biofilm formation in DUWL and avoid many infections caused by opportunistic pathogens.

Declaration of competing of interest: All authors – none to declare.

Authors' contributions: All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

Funding: None to declare.

Ethics statement: Not applicable.

ACKNOWLEDGMENTS

The authors thank their colleagues for their help in this study.

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