JBS
Review
Journal of Biological Studies
eISSN: 2209-2560
V. 6, N. 4:282-298, 2023

Dental unit waterlines and health risks of pathogenic microbial contamination: An update review

Wafaa M. Hikal[1,2], Miroslava Kačániova[3,4*], Diaa Eldin E. Hussein[5], Amr Ghit[6,7], Slim Smaoui[8], Muhammad Tahir Aleem[9], Natália Čmiková[3], Hussein A.H. Said-Al Ahl[10]

[1]Department of Biology, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia, wafaahikal@gmail.com
[2]Environmental Parasitology Laboratory, Water Pollution Research Department, Environment and Climate Change Institute, National Research Centre (NRC), 33 El-Behouth St. Dokki, Giza 12622, Egypt.
[3]Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976 Nitra, Slovakia, n.cmikova@gmail.com
[4]School of Medical & Health Sciences, University of Economics and Human Sciences in Warsaw, Okopowa 59, Warszawa 01 043, Poland
[5]Department of Food Hygiene, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Port of Alexandria, Alexandria, Egypt, diaavet@hotmail.com
[6]Department of Biology and Biotechnology, University of Pavia, Pavia, Italy, amr.ghit@hotmail.com
[7]Department of Biotechnology, Institute of Graduate Studies and Research (IGSR), Alexandria University, Alexandria, Egypt.
[8]Laboratory of Microbial, Enzymatic Biotechnology and Biomolecules (LBMEB), Center of Biotechnology of Sfax, Road of Sidi Mansour Km 6, P. O. Box 1177, 3018, University of Sfax- Tunisia, slim.smaoui@cbs.rnrt.tn
[9]MOE Joint International Research Laboratory of Animal Health and Food Safety, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, P.R. China, dr.tahir1990@gmail.com
[10]Medicinal and Aromatic Plants Research Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre (NRC), 33 El-Behouth St. Dokki, Giza 12622, Egypt, shussein272@yahoo.com
*Corresponding author email: miroslava.kacaniova@gmail.com

Received 19 November 2023 | Accepted 30 December 2023 | Published 31 December 2023

Abstract

Recently, there has been great concern about the levels of oral diseases associated with inappropriate and unsanitary oral care methods. In this line, the most serious is the water contamination with pathogenic microbes. Owing to the water microbial contamination of dental units, patients' oral health is threatened as a result of systemic disorders. Patients receiving treatment in dental unit waterlines were found to be affected by dangerous bacteria. Since both clients and dental staff are exposed to aerosols and water from dental equipment, the water should be of great quality. Due to the fact that dental unit waterlines (DUWLs) create a suitable environment for microbial development and the formation of biofilms, the water is often contaminated with a high density of several microorganisms (e.g., bacteria, fungi, protozoa, viruses), causing a health problem for dentists, patients and elderly patients, especially those with immunocompromised. This has fueled interest in presenting the current situation to shed light on (i) the microbial contamination of DUWLs and (ii) the connected infectious risks. It is essential that an elevated degree of mouth care be combined with adequate water quality and preventive dental units to maintain oral comfort, prevent deterioration, and promote public health. The growing population of individuals with compromised immune systems and the latest technological advancements concerning biofilms, water quality, etc. necessitate the implementation of improved management practices and the application of decontamination protocols for waterlines.

Keywords: dental, waterlines, pathogenic microbiota, contamination

1. Introduction

HIV transmission to dental clinic patients during oral health care treatments made it critical to monitor the quality of water distributed through dental unit waterlines (DUWLs) as a public issue (Barbot et al., 2012). As a result, there is an increasing interest in focusing on the danger of contaminated water exposure in the dental office. Furthermore, increasing scientific paper outcomes revealed the presence of large amounts of potentially dangerous bacteria in dental treatment water. Besides, certain case reports that linked sickness to dental water pollution were reported (Barbot et al., 2012; Hikal et al., 2019).

As seen in Figure 1, modern dental chair units are made up of a network of interconnected narrow-bore plastic tubes known as DUWLs (CDC, 2021). The water provided by these dental unit waterline systems serves as a cooling agent for equipment and an irrigating agent during dental procedures. This water must be of high quality, as both patients and dental teams are routinely exposed to water and aerosols created by dental equipment (Barbot et al., 2012). DUWLs promote microbial growth and biofilm development because water is frequently polluted with high densities of microorganisms such as bacteria, fungi, protozoa, and viruses. These reasons endanger the health of dentists and patients (Barbot et al., 2012). The conditions in the ducts of the dental unit can promote the growth of microorganisms, which in turn leads to the formation of biofilm on the inner surface of the DUWL duct. The use of handpieces, especially those with a high rotation speed, releases a mist that contains microorganisms and biological material such as blood, saliva and dental plaque. This means that there can be a risk of infection for both dental practice staff and patients. Using a combination of measures to stop DUWL contamination can reduce the risk of cross-infections in dental facilities (Spagnolo et al., 2020).

2. The problems related to dental water quality

Microorganisms (Kumar et al., 2010) found in biofilms created by water stagnation in the DUWL water pipes may contaminate specific areas of dental chair units, potentially leading to cross-infection. In this sense, various bacteria, yeasts, fungi, viruses, protozoa, unicellular algae, and nematodes can grow and cause infection (Figueiredo-Filho et al., 2019). According to Garg et al. (2012), contaminated water may be swallowed by individuals or may come into contact with mucosal and/or tooth-permanent solvents. It provides direct access to connective tissue, twists possible absorption, and enters the circulatory system. It can also cause respiratory and ocular infections in patients and dentists (Arvind & Roma, 2020). O'Donnell et al. (2006) viewed the dental chair as a composite piece of medical equipment designed to provide the dentist with the essential characteristics for a wide range of dental procedures.

Water supply in these units can come from a reservoir that is disposable, independent, and in the shape of a bottle, or it might come from the water supply network (Ajami et al., 2012). Waterlines are pipelines that carry water and are made of inner faces of long lumen polyethylene (pipes of about 10 m in length and 0.5 to 1.0 mm in diameter). These qualities are generally connected with water stagnation, and tiny internal inadequacies provide an ideal habitat for the growth of microorganisms that were previously present in the water supply (Walker et al., 2003). The ensuing formation of biofilm is immediately responsible for the pollution of the water used in therapeutic procedures (Rodrigues et al., 2017; Walker et al., 2007; Walker et al., 2004; Szymańska et al., 2013; Sacchetti et al., 2007).

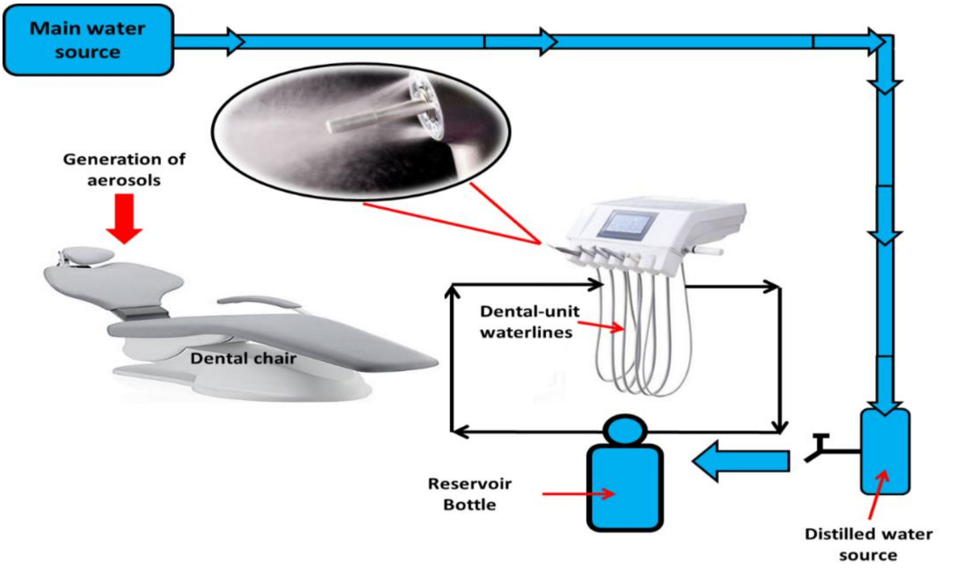

Figure 1. Dental Unit Waterline System.

3. Kind of microorganisms isolated from dental unit water

In terms of the microorganisms found in and separated from dental unit water, there are bacteria, yeasts, fungi, viruses, protozoa, unicellular algae, and nematodes (Kumar et al., 2010). These microorganisms isolated from water of dental unit waterlines are mentioned in Table 1.

4. Microbial Contamination of DUWLs and Biofilms

According to Barbeau et al. (1998), the water systems of dental units mimic an aquatic habitat, with various opportunistic microorganisms colonizing the internal surface of the pipes. Bacteria, fungi, and protozoa that settle and reproduce on the inside face of water pipes. This is frequently accomplished by the formation of a protective coating of viscous material (limo/ mucus) that allows them to live in difficult conditions and assault new places (De Oliveira et al., 2008), in particular fungi and protozoa or anaerobic bacteria (O'Donnell et al., 2007).
Figure 2 shows how DUWLs provide suitable circumstances for microbial proliferation and the formation of biofilms containing bacteria, fungi, and protozoa. DUWLs have been shown to have a significant number of microorganisms (Dogruöz et al., 2012; Walker et al., 2004; Göksay et al., 2008). Hazardous pathogens such as *Legionella*, *Pseudomonas*, and *Candida*

have also been discovered (Dogruöz et al., 2012; Walker et al., 2004; Göksay et al., 2008; Türetgen et al., 2009). These pathogens require special attention due to their ability to induce pneumonia and other respiratory illnesses in immunocompromised patients with wound problems. As a result, the dental unit's (DU) water feature is vital for both patients and dentists. Numerous investigations have been conducted on the high levels of bacterial pollutants (Walker et al., 2004; Göksay et al., 2009; Barbeau et al., 1996). *Candida* spp. induces superficial as well as systemic illnesses that have been resurrected from DUWLs on a sporadic basis (Walker et al., 2004; Szymańska et al., 2005; Genc et al., 1997).

Table 1. Types of the most common microorganisms isolated from water of dental unit waterlines (according to Barbot et al., 2012).

Bacteria species	*Achromobacter xyloxidans* *Acinetobacter* spp. *Arthrobacter* *Actinomyces* spp. *Alcaligenes denitrificans* *Bacillus* spp. *Bacteroides* spp. *Burkholderia cepacia*	*Caulobacter* spp. *Corynebacterium* *Flavobacterium* spp. *Fusobacterium* spp. *Klebsiella pneumoniae* *Moraxella* spp. *Lactobacillus* spp. *Legionella* spp. *Micrococcus* spp.	*Mycobacterium avium* *Nocardia* spp. *Pasteurella* spp. *Proteus vulgaris* *Pseudomonas aeruginosa* *Streptococcus* spp. *Staphylococcus aureus* *Xanthomonas* spp.
Fungi species	*Acremonium* spp. *Alternaria* spp. *Aspergillus* spp.	*Candida* spp. *Cladosporium* spp. *Phoma* spp.	*Penicillium* spp. *Scopulariopsis* spp.
Protozoa species	*Acanthamoeba* spp. *Cryptosporidium* spp.	*Microsporidium* spp. *Giardia* spp.	*Cryptosporidium* spp. *Giardia* spp.

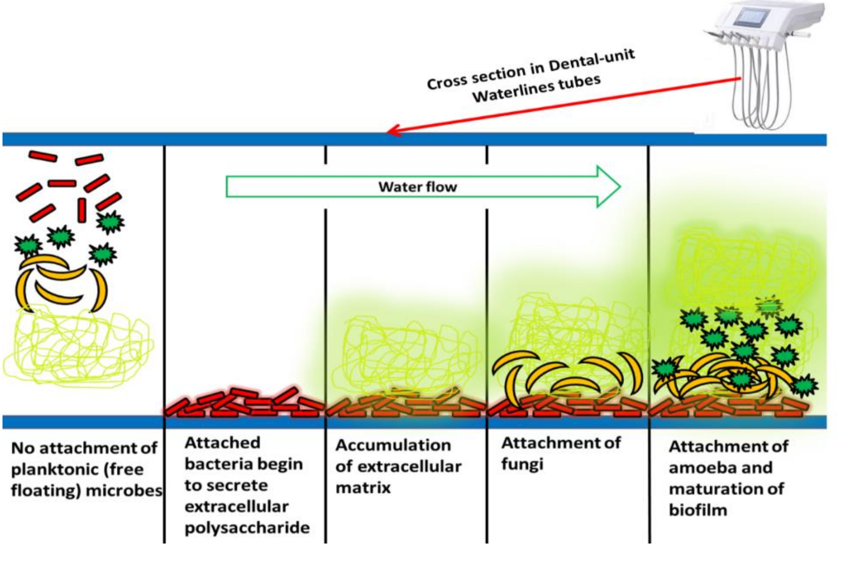

Figure 2. Stages of biofilm development in dental-unit waterline tubes. A complex biofilm includes bacteria, extracellular matrix, fungi and amoeba.

Gram-negative aerobic heterotrophic bacteria adhere to the inner surface of the tooth equipment, forming micro colonies that generate a variety of biofilm patterns. This biofilm is largely made up of highly hydrated bacterial polysaccharides that may communicate through pores and channels to form ordered, coordinated, and helpful colonies. Planktonic cells and their metabolites are released into the water instantaneously into patients' mouths during dental procedures when biofilm grows (Ajami et al., 2010; Wirthlin et al., 2015). Numerous microbe species in the biofilm do not survive in isolation but rather through a process known as quorum sensing. This communication strategy is based on the capacity to monitor not only the presence of other bacteria in the vicinity but also the performance and response to signaling molecules. Certain receptors identify these autoinducers and allow the cells to assess the population by signal concentration. When they reach a certain level, germs function as a single multicellular creature capable of organizing united responses to population survival (Davies et al., 1998; Sola et al., 2012).

The presence of a higher density of pathogens in the water exit on dental items poses a danger for diseases in patients and professionals. Previous research has revealed the presence of bacteria such as *Pseudomonas*, *Legionella*, and non-tuberculous (atypical or fast-growing) mycobacteria in water outlets, which pose a danger of infection, particularly in immunocompromised patients (Ajami et al., 2012).

Because of the ability of DUWL to contain numerous germs, the American Dental Association and the Centers for Disease Control and Prevention proposed a standard for dental-unit water system water as water with no more than 200 CFU/ mL. Salam et al. (2017) examined the microbiological contamination in two dental chairs' DUWLs and discovered that colony-forming units were greater than 500 CFU/ ml. The species found were predominantly gram-negative bacilli, such as *E. coli*, *Pseudomonas*, and *Klebsiella*, as well as gram-positive bacteria like *Enterococcus*. They came to the conclusion that DUWLs are significantly polluted with waterborne organisms from biofilm within the tubes, as well as human pathogens via back-siphonage from patients' mouth cavities. These organisms e.g., *Moraxella* spp., *Flavobacterium* spp., *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Mycobacterium* spp., *Candida* spp., *Actinomyces* spp., *Streptococcus* spp., and *Staphylococcus* spp. can cause significant systemic infections in individuals (Walker et al., 2000).

Dental unit water pollution occurs as a result of microbial biofilm growth and subsequent exfoliation from pipe surfaces inside dental unit water systems (Walker et al., 2000; Williams et al. 1996) (Table 2). Despite the fact that dental water has been linked to *Pseudomonas*, *Moraxella*, *Staphylococcus*, and *Legionella* (Williams et al., 1996). Not only are these organisms resistant to high temperatures and biocides by nature, but the biofilms in which they dwell further strengthen their resistance (Barbeau et al., 1998), posing medical hazards to immunocompromised patients. To decrease microbial burden, the Centres for Disease Control and Prevention (Kohn et al., 2003) suggests cleaning DUWLs at the start of the clinic day. However, research has revealed that this approach has little effect on biofilms in water lines and does not consistently enhance the quality of the water used during dental treatment (Anonymous, 2006). As a result, untreated or unfiltered dental-unit water is unlikely to fulfil drinking water regulations (500 CFU/mL); thus, one or more commercial devices and processes intended to enhance water quality should be used.

Salam et al. (2017) study revealed that DUWLs are substantially polluted with both waterborne and human harmful pathogens. The majority of the units contained colony-forming units exceeding 500 CFU/mL, which is not acceptable according to American Dental Association (ADA) guidelines. The significant amount of contamination of DUWLs reported in this investigation corroborated previous findings (Barbeau et al., 1996; Linger et al., 2001; Meiller et al., 2000; Gross et al., 1976). Salam et al. (2017) identified *Pseudomonas* in water samples from the handpiece and 3-way syringes, which was previously found in a Trabelsi investigation (Trabelsi et al., 2010).

Table 2. List of bacterial species that cause biofilms in water from dental units.

Name of Bacteria	Number of DUWLs included	Reference
Achromobacter	61	Abdouchakou et al. (2015)
Burkholderia cepacia	20	Uzel et al. (2008)
Alcaligenes faecalis, Actinomyces, Arthrobacter	107	Szymańska & Sitkowska (2013)
Bacteroides, Vibrio, Edwardsiella, Neisseria, Corynebacterium, Gradnerella	18 (Each of six specialties)	Fan et al. (2021)
Pseudomonas aeruginosa	-	Szymańska (2003)
Legionella	14 (Each in five hospitals)	Lizzadro et al. (2019)
Nontuberculosis Mycobacterium	9	Fotedar & Ganju (2015)
Serratia marcescens, Aeromonas spp., *Acinetobacter* spp.	31	Lal et al. (2015)
Streptococcus spp., *Staphylococcus* spp., *Enterococcoccus* spp.	-	Szymańska et al. (2008)
Klebsiella (Enterobacter), *Bacillus subtilis*	16	Wirthlin et al. (2003)
Flavobacterium spp., *Moraxella* spp.	24 (Clinics)	Alkhulaifi et al. (2020)

In previous research conducted by Agarwal et al. (2008) and Fotedar & Ganju (2015), flushed samples from the same source had a lower microbial load when compared to unflushed samples; nonetheless, the values remained high. Furthermore, it is possible that it will have no effect on a large range of species. As a result, flushing plays no important part in disinfection operations. Previous studies on the percentage decrease of viable counts and biofilm coverage following disinfection and flushing exposure have revealed only a 9.1 percent drop in viable count with flushing and a 0.5 percent reduction in biofilm with flushing (Alkhulaifi et al., 2020). Nevertheless, disinfectants such as chlorhexidine, betadine, sodium hypochlorite, alpron, sterilox, and oxygenal reduced the viable count by 100 percent (Alkhulaifi et al., 2020); however, the biofilm remained. Chemical disinfectants should not be harmful to patients or the dental-unit system (Cheng et al., 2021). There is a growing need for more research to determine the corrosive and poisonous nature of chemical disinfectants utilized, as well as an effective disinfection procedure within the DUWL system, resulting in a healthy dental unit for patients. As a result, disinfection of the water supply and DUWLs should be done on a regular basis in dental clinics and hospitals to ensure effective infection management (Salam et al., 2017).

Besides, Salam et al. (2017) suggested that future studies should focus on producing chemical disinfectants employing nanoparticles, which would decrease the downsides of the currently existing chemical disinfectants on the market. Salam et al. (2017) determined that the amount of colony-forming units in water samples is greater than the ADA suggested value. The presence of indicator organisms like *E. coli* and enterococci in water suggests faecal pollution and the development of other pathogenic species. The source of contamination might be the source of water or a back-sip on the age of organisms in patients' oral fluids. Gram-negative bacteria, such as *Pseudomonas aeruginosa*, can cause a variety of systemic infections, particularly in the immunocompromised, elderly, and young.

Moreover, the presence of fungus in these systems demands greater investigation. During dental treatment, direct contact with fungi such as *Candida*, *Aspergillus*, or inhalation of aerosols from high-speed drills can induce a variety of respiratory diseases, including asthma, allergies, and mucosal sores, especially in immunocompromised patients and dentists. As indicated in Table 3, the isolated fungi were *Penicillium waksmanii*, *Cladosporium* spp., *Penicillium* spp., *Candida famata*, *Cryptococcus laurentii*, *Candida guilliermondii*, *Penicillium verrucosum*, *Aspergillus pseudoglaucus*, *Penicillium decumbens*, and *Acremonium* spp. Some of these fungal genera are opportunistic infections that cause respiratory illnesses, including allergic rhinitis (Kadaifciler et al., 2013).

Table 3. List of fungal contamination of water from dental units detected.

Name of M.os isolated	Number of DUWLs included	Reference
Acremonium spp., *Aspergillus* spp., *Cladosporium* spp., *Phoma* spp., *Penicillium* spp., *Scopulariopsis* spp.	6 (Clinics)	Lisboa et al. (2014)
Candida spp., *Rhodotorula* spp., *Trichosporon* spp.	18	Mazari et al. (2018)
Cladosporium spp.	24 (Clinics)	Alkhulaifi et al. (2020)

Kadaifciler et al. (2013) exposed that when contaminated water passes through dental equipment, fungus spores and hyphal pieces are aerosolized into the air. Additionally, certain filamentous fungi are potential toxin-makers, and long-term exposure to modest levels of toxins may have a deleterious impact on the immune system (Szymańska 2005). Yeast-contaminated water presents a risk to humans in case of direct contact with open wounds. Several researchers found *Candida* sp. in DUWLs (Walker et al., 2000; Gencet al., 1997; Araujo et al., 2004; Szymańska 2005), and the opportunistic pathogenic yeast (genus *Candida*) can cause cutaneous disease and systemic disease. While the most pathogenic *Candida* species of this genus was *Candida albicans*, it has been cited as the causative agent of an increasing number of infections (Szymańska, 2005).

In the study of Kadaifciler et al. (2013), they isolated *Candida famata*, *Candida guilliermondii*, and *Cryptococcus laurentii* from DU water sites. Aerosols emanating from DU water with a diameter of less than 2.5 mm are considered dangerous. These small aerosols, which contain microorganisms, can induce asthma, rhinitis, allergic alveolitis, or other respiratory problems (Górny et al., 2004). In previous years, *Fusarium*, *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*, and *Scopulariopsis* were isolated from DU water (Göksay et al., 2005, Szymańska 2005). Moreover, the most prevalent genera known to produce allergy responses are *Penicillium* and *Aspergillus* (Asan et al., 2003). Also, in immunocompromised patients, *Cladosporium* and *Alternaria* cause respiratory and asthmatic symptoms.

Several studies have shown the isolation of fungi from DUs water contained several yeasts but mostly filamentous fungi (Walker et al., 2004; Göksay et al., 2008; Szymańska, 2005; Lisboa et al., 2014; Pankhurst et al., 1998; Pitt et al., 2000). *Penicillium* and *Aspergillus* are the prevalent genera that have been isolated in DUWLs and might induce allergic reactions, asthma, and other respiratory problems (Asan et al., 2004). Indeed, copious proof of DU water pollution has been collected since the 1960s (Blake, 1963). Many microorganisms (bacteria, viruses, fungi) have been built in water samples from DUWLs: *Staphylococcus cohnii*, *Staphylococcus warneri*, *Streptococcus salivarius*, *Enterococci*, *Streptococcus mitis*, *Klebsiella* (Enterobacter) *aerogenes*, *Bacillus subtilis*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Acinetobacter* spp., *Cladosporium* spp., *Flavobacterium* spp., *Aeromonas* spp., *Moraxella* spp., *Pseudomonas* spp., *Legionella* spp., etc. (Lal et al., 2015 ; Szymańska et al., 2008 ; Wirthlin et al., 2003 ; Alkhulaifi et al., 2020 ; Blake, 1963), and infection might be a risk to both dental workers and patients' health.

The United States Centers for Disease Control and Prevention (CDC) recommends that the grade of heterotrophic plate counts (HPCs) in dental unit water not exceed 500 CFU/mL (Kohn et al., 2003). Moreover, the American Dental Association (ADA) has set a restricted of ≤ 200 CFU/mL on the heterotroph bacterial load in water with dental unit waterlines (Anonymous, 1996). In the EU, however, there is no existing guideline regarding DUWLs, though in some countries drunk water standards (Szymańska et al., 2013).

Abdouchakour et al. (2015) noted that *Achromobacter* spp. and *P. aeruginosa* of the microbial contaminant of water lines in a dental healthcare center. Moreover, verification of the literature reports was granted by a recent study (Tuvo et al., 2020) which found *Legionella* and *Pseudomonas aeruginosa* contaminant of water samples from DUWLs. In addition, the existence of Gram-negative bacteria in DUWLs can guide the creativity of endotoxins (LPS) in the water and air of a dental surgery (Volgenant et al., 2018). Water from DU has also been discovered to contain fungi. In a study directed by (Mazari et al., 2018), 18 dental waterlines were assayed for the existence of yeasts in their internal areas. Of the 18 DUWLs studied, 10 were polluted (55.56%). *Candida albicans*, *Candida guilliermondii* and *Candida glabrata* as well as two species of non-*Candida, Trichosporon* spp. and *Rhodotorula* spp., were detected. In addition to bacteria, fungi and viruses, protozoa such as free-living amoebae have been separated from DUWLs (Castro-Artavia et al., 2017; Retana-Moreira et al., 2017)

Free-living amoebae could serve as a stock for microorganisms (e.g., *Legionella* spp. and *Pseudomonas* spp., etc.) or as germs in their own right (Hikal et al., 2017; Hikal et al., 2021; Hikal et al., 2020; Hikal et al., 2021; Spagnolo et al., 2019). In a recent investigation, the mean concentration of HPCs was determined by evaluating the amount of contamination by bacteria and amoebae in 30 DU at 22 and 36 °C, and was 1168.53 CFU/mL and 827.90 CFU/mL respectively (Spagnolo et al., 2019), while the denseness of *P. aeruginosa* tested to be of 25.13 CFU/100 mL. Of the 30 units, 26.67% had concentration of ≥ 103- 3 upper index

CFU/L of *L. pneumophila*; approximately 23% of samples involved L. pneumophila. The analysis revealed that the water in the DUWLs included much more microorganisms than the input water supply, supporting the involvement of the water system inside the dental unit in increasing microbial pollution.

Earlier studies have found an extensive range in the rate of the revival of *Legionella* contamination of DUWLs, from 0% to 100% of DUWL systems (Estrich et al., 2017; Lauritano et al., 2017) containing *Legionella pneumophila* sero group1 (Arvand et al., 2013), reaching levels as high as 105 colony-forming units per millilitre (Estrich et al., 2017; Dutil et al., 2006; Pankhurst et al., 2017). The existence and concentration of *Legionella* contaminants in DUWLs vary per the traits of the water supply system used, the design and model of the dental unit, and the methods of disinfection (Estrich et al., 2017).

5. Protozoans found in the water supplies

Protozoa are single-celled organisms that exist in a variety of forms and can live alone or as parasites. Examples of important protozoa in dental waterlines (DWL) include *Microsporidium*, *Giardia*, and *Cryptosporidium* (Dutil et al., 2006; Pankhurst et al., 2017; Chandler et al., 2002). Multiple protozoa are discovered in drinkable water and DWL, including *Giardia lamblia*, which lives in the guts of people and animals and causes the diarrheal illness giardiasis. *Giardia* is one of the most frequent waterborne diseases among humans in the United States and across the world. It is shielded by an exterior shell, allowing it to remain outside the body and in the environment for extended periods, and is therefore very difficult to eradicate with chlorine because of its capability to tolerate a cystic state (Chandler et al., 2002; Hikal et al., 2017; Hikal et al., 2020). On the other hand, cryptosporidiosis is a diarrheal illness caused by the tiny parasite *Cryptosporidium parvum*, which, like *Giardia*, has an exterior shell that permits it to survive outside the body for lengthy periods of time and renders it resistant to chlorine treatment. In Egypt, (Hassan et al., 2012) confirmed from the results of his study the verification of the contamination of dental irrigation systems with *Cryptosporidium* species in water samples collected from dental irrigation systems and their handpieces. Their results showed that *Cryptosporidium* spp., was found in 27.5% of the water samples taken (Collinet-Adler et al., 2010; Hikal et al.. 2021).

5.1. Genus *Acanthamoeba*

Acanthamoeba species from different water sources, like *A. castellanii*, *A. culbertsoni*, *A. hatchetti*, *A. healyi*, *A. polyphaga*, *A. rhysodes*, *A. astronyxis*, and *A. divionensis*, are being studied and isolated across the world. These microorganisms are the cause of many diseases, such as insidious, chronic, and mostly fatal disease granulomatous amoebic encephalitis (GAE), particularly in immune-compromised people with HIV/ AIDS or suffering from chronic illness, diabetes, organ transplantation, or debilitation without any previous contact with recreational freshwater (Marciano-Cabral et al., 2003; Schuster et al., 2004). *Acanthamoeba keratitis* is a potentially vision-threatening condition (Schuster, 2002). *Acanthamoeba* is ubiquitous, lives in a variety of water, air, and soil environments (Marciano-Cabral et al., 2003; Mergeryan, 1991), and is isolated from many hospital environments as well as dental irrigation methods (Dendana et al., 2008). Further compounding the risks of *Acanthamoeba* spp. is the fact that bacteria and viruses remain alive and multiplicate in *Acanthamoeba*, and some of them are potential pathogens of human disease (Fritsche et al., 2000; Greub et al., 2004; Berger et al., 2006). Apart from illness provoked by direct danger to *Acanthamoeba* spp., these amoebae might serve as shelter for highly virulent and antibiotic-resistant pathogenic microorganisms and water treatment (Huws et al., 2006). The increasing public health concern of *Acanthamoeba* worldwide is due to the existence of harmful *Acanthamoeba* in hospital water sources, DUWLs during oral health care procedures, tap water, and its public health hazards (Trabelsi et al., 2010; Lasjerdi et al., 2011; Hikal et al., 2015; Leduc et al., 2012; Khurana et al., 2015). Dendana et al. (2008) showed that the water at the entrance to the hemodialysis equipment is surrounded by *Acanthamoeba* as a result of prolonged stagnation of water, which can cause biofilms to form, providing favourable conditions for the development and spread of many microorganisms (Huws et al., 2006), besides the discovery of *Acanthamoeba* in dental water lines (Trabelsi et al., 2010; Barbeau et al. 2001).

Huws et al. (2006) reported the enlargement of methicillin-resistant *Staphylococcus aureus* existence, proliferation, and virulence in association with *Acanthamoeba polyphagia*. Cirillo et al. (1994) found the same result: an increased invasion of *Legionella pneumophila* grown in *Acanthamoeba* associated with those grown under regular laboratory conditions. A high-level of *L. pneumophila* resistance liberated from *A. polyphaga* to disinfectants and antimicrobials has (Kilvington et al., 1990; Barker et al., 1995) also been reported. When FLA and other pathogens coexist in healthcare environments, the problem is exacerbated by the fact that some pathogenic microorganisms, such methicillin-resistant *Staphylococcus aureus*, are more ferocious and highly resistant (Huws et al., 2006).

In a real hospital situation, Fukumoto et al. (2013) showed the coexistence of *Acanthamoeba* and *Parachlamydia acanthamoebae*, a possible germ that causes hospital-acquired pneumonia. As a result, they clarified that *Acanthamoeba* real influence on this pathogen's long-term tolerance could aid in the pathogen's spread to the healthcare setting (Marrie et al., 2001). Consequently, circumstances in health care establishments attended by immunocompromised or hemodialysis patients might be hazardous. It is possible that people in hospitals will also have *Acanthamoeba*-associated nosocomial diseases (Berger et al., 2006; Marrie et al., 2001; La Scola et al., 2002; La Scola et al., 2003).

A few investigations on the occurrence of *Acanthamoeba* spp. in freshwater sources have been conducted in Egypt (Marciano-Cabral et al., 2003; Hikal et al., 2015; Lorenzo-Morales et al., 2006; Hikal et al., 2021), canals, and discharges (Sadaka et al., 1994). *A. gruberi* and *A. rhysodes* were also isolated from the nasal passages of six healthy children who lived near the polluted canals (Sadaka et al., 1994). Hikal et al. (2015) discovered *A. lenticulata*, *A. griffin*, *Acanthamoeba castellanii*, and *A. hatchitti* in DUWLs. Also in Alexandria, (Hassan et al., 2012) investigated the hydraulic systems of hemodialysis and DU and isolated *Acanthamoeba* spp.

5. Conclusion

A large number of microorganisms were measured from biofilm collected in the dental units' waterlines. The contamination of the dental units' waterlines is authenticity, which can cause individual and cooperative disorders. Infectious risks associated with DUWLs have been identified. Along with the growing number of immunocompromised persons, as well as recent technical advancements in water quality and biofilm research, the management and ed version deployment of decontamination measures for dental waterlines should be improved.

Acknowledgments

We would like to thank the reviewers for their valuable comments and suggestions to improve paper quality

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflict of interests

The authors declare that they have no competing interests.

References

Abdouchakour, F., Dupont, C., Grau, D., Aujoulat, F., Mournetas, P., Marchandin, H., Parer, S., Gibert, P., Valcarcel, J., & Jumas-Bilak, E. (2015). *Pseudomonas aeruginosa* and *Achromobacter* sp. clonal selection leads to successive waves of contamination of water in dental care units. Applied and Environmental Microbiology Journal, 81, 7509-24.

Agarwal, D., Sunitha, S., & Reddy, C.V.K. (2008). Awareness and estimation of bacterial contamination of dental unit waterlines in dental clinics and dental institutions in Mysore City, Karnataka. Journal of Indian Association of Public Health Dentistry, 6, 46.

Ajami, B., Ghazvini, K., Movahhed, T., Ariaee, N., Shakeri, M., & Makarem, S. (2012). Contamination of a dental unit water line system by *Legionella pneumophila* in the mashhad school of dentistry in 2009. Iranian Red Crescent Medical Journal, 14, 376-8.

Alkhulaifi, M.M., Alotaibi, D.H., Alajlan, H., & Binshoail, T. (2020). Assessment of nosocomial bacterial contamination in dental unit waterlines: Impact of flushing. Saudi Dental Journal, 32, 68-73.

Anonymous (1996). ADA statement on dental unit waterlines. Journal of the American Dental Association, 127, 185-86.

Anonymous. (1996). ADA statement on dental unit waterlines. Adopted by the ADA Board of Trustees, December 13, 1995 and the ADA Council on Scientific Affairs, September 28, 1995. Northwest Dent, 75, 25-6.

Araujo, R., & Contreras, N. (2004). Microbiological contamination of dental unit water systems in general practices from Barcelona (Spain). Water Science and Technology: Water Supply, 4, 1-5.

Arvand, M., & Hack, A. (2013). Microbial contamination of dental unit waterlines in dental practices in Hesse, Germany: A cross-sectional study. European Journal of Microbiology and Immunology, 3, 49-52.

Arvind, R., & Roma M. (2020). Risk of eye infections in dental personnel and the need for its prevention: a case report. Journal of Ophthalamic Inflammation and Infection, 10 (21), 1-4.

Asan, A. (2004). Check list of *Aspergillus* and *Penicillium* species reported from Turkey. Turkish Journal of Botany, 24, 151-67.

Asan, A., Kirgiz, T., Sen, B., Camur-Elipek, B., Guner, U., & Guher, H. (2003). Isolation, identification and seasonal distribution of airborne and waterborne fungi in Terkos Lake (Istanbul-Turkey). Journal of Basic Microbiology, 43, 83-95.

Barbeau, J., & Buhler, T. (2001). Biofilms augment the number of free-living amoebae in dental unit waterlines. Research in Microbiology, 152, 753-60.

Barbeau, J., Gauthier, C., & Payment, P. (1998). Biofilms, infectious agents, and dental unit waterlines: a review. Canadian Journal of Microbiology, 44, 1019-28.

Barbeau, J., Tanguay, R., Faucher, E., Avezard, C., Trudel, L., Côté L., & Prévost, A. P. (1996). Multiparametric analysis of waterline contamination in dental units. Applied and Environmental Microbiology Journal, 62, 3954-9.

Barbot, V., Robert, A., Rodier, M.H., & Imbert, C. (2012). Update on infectious risks associated with dental unit waterlines. FEMS Immunology and Medical Microbiology, 65,196-204.

Barker, J., Scaife, H. & Brown, M.R. (1995). Intraphagocytic growth induces an antibiotic-resistant phenotype of *Legionella pneumophila*. Antimicrobial Agents and Chemotherapy, 39, 2684-8.

Berger, P., Papazian, L., Drancourt, M., La Scola, B., Auffray, J-P., & Raoult D. (2006). Ameba-associated microorganisms and diagnosis of nosocomial pneumonia. Emerging Infectious Diseases, 12, 248-55.

Blake, G.C. (1963). The incidence and control of bacterial infection in dental spray reservoirs. British Dental Journal, 115, 413-16.

Castro-Artavia, E., Retana-Moreira, L., Lorenzo-Morales, J., & Abrahams-Sandí, E. (2017). Potentially pathogenic *Acanthamoeba* genotype T4 isolated from dental units and emergency combination showers. Memórias do Instituto Oswaldo Cruz, 112, 817-21.

CDC. Oral Health (2021). Available: https://www.cdc.gov/oralhealth/infectioncontrol/summaryinfection-prevention-practices/dental-unit-water-quality.html 2021

Chandler, J. (2002). Special Report; Dental waterlines: Understanding and control long biofilms and other contaminants. Vista Research Group, LLC.

Cheng, L., Naibijiang, N., Hasenbai, A., Dong, H., & He, H. (2021). Bacteriostatic effects of nanometer silver disinfectant on the biofilms in dental unit water lines,Journal of Dental Sciences, 16(1), 327-332.

Cirillo, J.D., Falkow, S., & Tompkins, LS. (1994). Growth of *Legionella pneumophila* in *Acanthamoeba castellanii* enhances invasion. Infection and Immunity Journal, 62, 3254-61.

Collinet-Adler, S., & Ward, H.D. (2010). Cryptosporidiosis: environmental, therapeutic, and preventive challenges. European Journal of Clinical Microbiology and Infectous Disaeses, 29, 927–935.

Davies, D.G., Parsek, M.R., Pearson,J.P., Iglewski, B.H., Costerton,J.W., & Greenberg, E.P. (1998). The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science, 280, 295-8.

De Oliveira, A.C., Maluta, R.P., Stella, A.E., Rigobelo, E.C., Marin, J.M., & de Ávila, F.A. (2008). Isolation of *Pseudomonas aeruginosa* strains from dental office environments and units in Barretos, state of São Paulo, Brazil, and analysis of their susceptibility to antimicrobial drugs. Brazilian Journal of Microbiology, 39, 579-84.

Dendana, F., Sellami, H., Jarraya, F., Sellami, A., Makni, F., Cheikhrouhou F, Dogruöz, N., Ilhan-Sungur, E., Göksay, D., & Türetgen, I. (2012). Evaluation of microbial contamination and distribution of sulphate-reducing bacteria in dental units. Environmental Monitoring and Assessment, 184: 133-9.

Dutil, S., Tessier, S., Veillette, M., Laflamme, C., Mériaux, A., Leduc, A., Barbeau, J., & Duchaine, C. (2006). Detection of Legionella spp. by fluorescent in situ hybridization in dental unit waterlines. Journal of Applied Microbiology, 100, 955-63.

Estrich, C.G., Gruninger, S.E., & Lipman, R.D. (2017). Rates and predictors of exposure to *Legionella pneumophila* in the United States among dental practitioners: 2002 through 2012. Journal of the American Dental Association, 148, 164-71.

Fan, C., Gu, H., Liu, L., Zhu, H., Yan, J., & Huo, Y. (2021). Distinct microbial community of accumulated biofilm in dental unit waterlines of different specialties. Frontiers in Cellular and Infection Microbiology, 11, 670211.

Figueiredo-Filho, A.O., Bem, J.S.P., Sobrinho, C.R.W., & Souza, F.B. (2019). Microbiological water evaluation from biofilm adhered to dental unit waterlines. International Journal of Odontostomatology, 13, 357-62.

Fotedar, S., & Ganju, S. (2015). Microbial contamination of dental unit water lines in HP Government Dental College, Shimla. Saudi Journal for Dental Research, 6, 129-32.

Fritsche, T.R., Horn, M., Wagner, M., Herwig, R.P., Schleifer, K.H., & Gautom, R.K. (2000). Phylogenetic diversity among geographically dispersed *Chlamydiales endosymbionts* recovered from clinical and environmental isolates of Acanthamoeba spp. Applied and Environmental Microbiology Journal, 66, 2613-9.

Fukumoto, T., Matsuo, J., Hayashi, Y., Oguri, S., Nakamura, S., Mizutani, Y., Yao, T., Akizawa, K., Suzuki, H., Shimizu, C., Matsuno, K., & Yamaguchi, H. (2013). Impact of free-living amoebae on presence of *Parachlamydia acanthamoebae* in the hospital environment and its survival in vitro without requirement for amoebae. Journal of clinical microbiology 48, 3360-3365.

Garg, S.K., Mittal, S., & Kaur, P. (2012). Dental unit waterline management: historical perspectives and current trends. Journal of Investigative and Clinical Dentistry, 3, 247-52.

Genc, A., Kadir, T., Ercalik, S., Erdem, H., & Demirbas, B. (1997). The probability of microbial contamination by the internal water/air lines of turbines. Turkish Dental Association IV International Dental Congress, Istanbul. TR.

Göksay, D., Cotuk, A., & Zeybek, Z. (2008). Microbial contamination of dental unit waterlines in Istanbul, Turkey. Environmental Monitoring and Assessment, 2008, 147, 265-9.

Górny, R.L. (2004). Filamentous microorganisms and their fragments in indoor air. a review. Annals of Agricultural and Environmental Medicine, 11, 185-97.

Greub, G., & Raoult D. (2004). Microorganisms resistant to free-living amoebae. Clinical Microbiology Reviews, 17, 413-33.

Gross, A., Devine, M.J., & Cutright, D.E. (1976). Microbial contamination of dental units and ultrasonic scalers. Journal of Periodontology, 47, 670-3.

Hachicha, J., & Ayadi, A. (2008). Free-living amoebae (FLA): detection, morphological and molecular identification of *Acanthamoeba* genus in the hydraulic system of an haemodialysis unit in Tunisia. Parasite, 15, 137-42.

Hassan, A., Farouk, H., Hassanein, F., Abdul-Ghani, R., & Abdelhady, A.H. (2012). *Acanthamoeba* contamination of hemodialysis and dental units in Alexandria, Egypt: a neglected potential source of infection. Journal of Infection and Public Health, 5, 304-10.

Hikal, W.M., & Said-Al Ahl, H.A.H. (2017). Anti-cryptosporidium activity of essential oil: A review. American Journal of Food Science and Health, 3, 35-40.

Hikal, W.M., & Said-Al Ahl, H.A.H. (2019). Influence of *Plectranthus amboinicus* essential oil on potentially pathogenic *Acanthamoeba* isolated from water tanks in Tabuk, Saudi Arabia. Ecology, Environment and Conservation, 25, 1137-45.

Hikal, W.M., & Said-Al Ahl, H.A.H. (2021). Dental unit water lines and risk of pathogenic parasitic contamination. Asian Basic and Applied Research Journal, 3, 1-13.

Hikal, W.M., & Said-Al, Ahl, H.A.H. (2019). Cysticidal activity of *Trachyspermum ammi* essential oil against Acanthamoeba isolates from dental clinic and hospital water networks. Pakistan Journal of Zoology, 51, 799-1806.

Hikal, W.M., Alhawiti, L.S.E, Said-Al Ahl, H.A.H., & Kačániová, M. (2021). Potential dental unit water lines contaminants and their impacts: A parasite perspective. Bulletin of Environment, Pharmacology and Life Sciences,10, 201-208.

Hikal, W.M., Bratovcic, A., Baeshen, R.S., Tkachenko, K.G., & Said-Al Ahl H.A.H. (2021). Nanobiotechnology for the detection and control of waterborne parasites. Open Journal of Ecology, 11, 203-23.

Hikal, W.M., Said-Al Ahl, H.A.H., & Tkachenko, K.G. (2020). Present and future potential of antiparasitic activity of *Opuntia ficusindica*. Tropical Journal of Natural Products Research, 4, 672-9.

Hikal, W.M., Tkachenko, K.G., Said-Al Ahl, H.A.H., Sany, H., Sabra, A.S., Baeshen, R.S., & Bratovcic, A. (2021). Chemical composition and biological significance of thymol as antiparasitic. Open Journal of Ecology, 11, 240-266.

Hikal, W.M., Zaki, B., & Sabry, H. (2015). Evaluation of ozone application in dental unit water lines contaminated with pathogenic *Acanthamoeba*. Iranian Journal of Parasitology, 10, 410-9.

Hikal, WM. (2020). Parasitic contamination of drinking water and Egyptian standards for parasites in drinking water. Open Journal of Ecology, 10, 1-21.

Huws, S.A., Smith, A.W., Enright, M.C., Wood, P.J., & Brown, M.R.W. (2006). Amoebae promote persistence of epidemic strains of MRSA. Environmental Microbiology, 8, 1130-3.

Kadaifciler, D.G., Ökten, S., & Sen B. (2013). Mycological contamination in dental unit waterlines in Istanbul, Turkey. Brazilian Journal of Microbiology, 44, 977-81.

Khurana, S., Biswal, M., Kaur, H., Malhotra, P., Arora, P., Megha, K., Kilvington, S., & Price, J. (1990). Survival of *Legionella pneumophila* within cysts of *Acanthamoeba polyphaga* following chlorine exposure. Journal of Applied Microbiology, 68, 519-25.

Kohn, W.G., Collins, A.S., Cleveland, J.L., Harte, J.A., Eklund, K.J., & Malvitz, D.M. (2003). Guidelines for infection control in dental health-care settings--2003. MMWR Recomm reports, Morbidity and Mortality Weekly Report, 52, 1-61.

Kumar, S., Atray, D., Paiwal, D., Balasubramanyam, G., Duraiswamy, P., & Kulkarni, S. (2010). Dental unit waterlines: source of contamination and cross-infection. Journal of Hospital Infection, 74, 99-111.

La Scola, B., Boyadjiev, I., Greub, G., Khamis, A., Martin, C., & Raoult, D. (2003). Amoeba-resisting bacteria and ventilator-associated pneumonia. Emerging Infectious Diseases, 9, 815-21.

La Scola, B., Mezi, L., Auffray, J-P., Berland, Y., & Raoult, D. (2002). Patients in the intensive care unit are exposed to amoeba-associated pathogens. Infection Control & Hospital Epidemiology, 23, 462-5.

Lal, S., Singhrao, S.K., Achilles-Day, U.E.M., Morton, L.H.G., Pearce, M. & Crean, S. (2015). Risk assessment for the spread of *Serratia marcescens* within dental-unit waterline systems using *Vermamoeba vermiformis*. Current Microbiology, 71, 434-42.

Lasjerdi, Z., Niyyati, M., Haghighi, A., Shahabi, S., Biderouni, F.T., Taghipour, N., Eftekhar, M., & Nazemalhosseini Mojarad, E. (2011). Potentially pathogenic free-living amoebae isolated from hospital wards with immunodeficient patients in Tehran, Iran. Parasitology Research, 109, 575-80.

Lauritano, D., Nardone, M., Gaudio, R.M., Candotto, V., & Carinci, F. Risk assessment of colonization of Legionella spp. in dental unit waterlines. Journal of Oral Implantology, 10, 283-8.

Leduc, A., Gravel, S., Abikhzer, J., Roy, S., & Barbeau, J. (2012). Polymerase chain reaction detection of potentially pathogenic free-living amoebae in dental units. Canadian Journal of Microbiology, 58, 884-6.

Linger, J.B., Molinari, J.A., Forbes, W.C., Farthing, C.F., & Winget, W.J. (2001). Evaluation of a hydrogen peroxide disinfectant for dental unit waterlines. Journal of the American Dental Association, 132, 1287-91.

Lisboa, G.M., Lisboa, Y.R.M., Pinheiro, T.M.L., Stegun, R.C., & da Silva-Filho, E.A. (2014). Microbial diversity in dental unit waterlines. Acta odontologica latinoamericana, 27, 110-4.

Lizzadro, J., Mazzotta, M., Girolamini, L., Dormi, A., Pellati, T., & Cristino, S. (2019). Comparison between two types of dental unit waterlines: How evaluation of microbiological contamination can support risk containment. International Journal of Environmental Research and Public Health, 16, 328.

Lorenzo-Morales, J., Ortega-Rivas, A., Martínez, E., Khoubbane, M., Artigas, P., Periago, M.V., Foronda, P., Abreu-Acosta, N., Valladares, B., & Mas-Coma, S. (2006). Acanthamoeba isolates belonging to T1, T2, T3, T4 and T7 genotypes from environmental freshwater samples in the Nile Delta region, Egypt Acta Tropica, 100, 63-9.

Marciano-Cabral, F., & Cabral, G. (2003). Acanthamoeba spp. as agents of disease in humans. Clinical Microbiology Reviews, 16, 273-307.

Marrie, T.J., Raoult, D., La Scola, B., Birtles, R.J., & de Carolis, E. (2001). Legionella-like and other amoebal pathogens as agents of community-acquired pneumonia. Emerging Infectious Diseases, 7, 1026-9.

Mazari, W., Boucherit-Otmani, Z., El Haci, I.A., Ilahi, A., & Boucherit, K. (2018). Risk assessment for the spread of *Candida* sp. in dental chair unit waterlines using molecular techniques. International Dental Journal, 68, 386-92.

Meiller, T.F., Kelley, J.I., Baqui, A.A., & De Paola LG. (2000). Disinfection of dental unit waterlines with an oral antiseptic. Journal of Clinical Dentistry, 11, 11-5.

Mergeryan, H. (1991). The prevalence of Acanthamoeba in the human environment. Reviews of Infectious Diseases, 13, S390-1.

O'Donnell, M.J., Shore, A.C., & Coleman, D.C. (2006). A novel automated waterline cleaning system that facilitates effective and consistent control of microbial biofilm contamination of dental chair unit waterlines: a one-year study. Journal of Dentistry, 34, 648-61.

O'Donnell, M.J., Shore, A.C., Russell, R.J., & Coleman, D.C. (2007) Optimisation of the long-term efficacy of dental chair waterline disinfection by the identification and rectification of factors associated with waterline disinfection failure. Journal of Dentistry, 35, 438-51.

Pankhurst, C.L., & Coulter, W.A. (2017). Do contaminated dental unit waterlines pose a risk of infection?. Journal of Dentistry ,35, 712-20.

Pankhurst, C.L., Johnson, N.W., & Woods, R.G. (1998). Microbial contamination of dental unit waterlines: the scientific argument. International Dental Journal, 48, 359-68.

Pitt, J.I. (2000). A laboratory guide to common *Penicillium* species. North Ryde, N.S.W.: Food Science Australia.

Retana-Moreira, L., Abrahams-Sandí, E., Castro-Artavia, E., Fernández-Sánchez, A., Castro-Castillo, A., Reyes-Batlle, M., & Lorenzo-Morales, J. (2015). Isolation and molecular characterization of *Acanthamoeba* strains from dental units in Costa Rica. Journal of Eukaryotic Microbiology, 62, 733-6.

Rodrigues, S., Suvarna, S., Suvarna, J., Saralaya, V., Saldanha, S., & Shenoy V.K. (2017). Microbial assessment of dental unit waterlines in an institutional setup in Karnataka, South India. Indian Journal of Dental Research, 28, 555-9.

Sacchetti, R., De Luca, G., & Zanetti, F. (2007). Influence of material and tube size on DUWLs contamination in a pilot plant. New Microbiologica, 30, 29-34.

Sadaka, H.A., El-Nassery, S.F., Abou Samra, L.M., & Awadalla, H.N. (1994). Isolation and identification of free-living amoebae from some water sources in Alexandria. Journal of the Egyptian Society of Parasitology, 24, 247-57.

Salam, N., Mulamoottil, V.M., & George B. Assessment of Microbial Contamination in Dental-Unit Water Lines: An Analytical Study, 15(1), 97-101

Salam, N., Mulamoottil, V.M., & George, B. (2017). Assessment of microbial contamination in dental-unit water lines: an analytical study. Journal of the Indian Association of Public Health Dentistry, 15, 97.

Schuster, F.L. (2002). Cultivation of pathogenic and opportunistic free-living amebas. Clinical Microbiology Reviews, 15, 342-54.

Schuster, F.L., & Visvesvara, G.S. (2004). Amebae and ciliated protozoa as causal agents of waterborne zoonotic disease. Veterinary Parasitology, 126, 91-120.

Sola, M.C., de Oliveira, A.P., Feistel, J.C., & Rezende, C.S.M. (2012). Mecanismos de quorum sensing e sua relevância na microbiologia de alimentos. Enciclopedia Biosfera, 8, 2012.

Spagnolo, A.M., Sartini, M., & Cristina, M.L. (2020). Microbial contamination of dental unit waterlines and potential risk of infection: A narrative review. Pathogens, 9, 651.

Spagnolo, A.M., Sartini, M., Cave, D.D., Casini, B., Tuvo, B., & Cristina, M.L. (2019). Evaluation of microbiological and free-living protozoa contamination in dental unit waterlines. International Journal of Environmental Research and Public Health, 16, 2648.

Szymanska, J. (2003). Biofilm and dental unit waterlines. Annals of Agricultural and Environmental Medicine, 10, 151-7.

Szymańska, J. (2005). Evaluation of mycological contamination of dental unit waterlines. Annals of Agricultural and Environmental Medicine, 12, 153-5.

Szymańska, J. & Sitkowska, J. (2013). Bacterial contamination of dental unit waterlines. Environmental Monitoring and Assessment, 185, 3603-11.

Szymańska, J., Sitkowska, J., & Dutkiewicz, J. (2008). Microbial contamination of dental unit waterlines. Annals of Agricultural and Environmental Medicine, 15, 173-9.

Taneja, N. & Sehgal, R. (2015). Free living amoebae in water sources of critical units in a tertiary care hospital in India. Indian Journal of Medical Microbiology 33, 343-8.

Trabelsi, H., Sellami, A., Dendena, F., Sellami, H., Cheikh-Rouhou, F., Makni, F, Ben Dhiaa, S. & Ayadi, A. (2010). Free-living amoebae (FLA): morphological and molecular identification of *Acanthamoeba* in dental unit water. Parasite, 17, 67-70.

Türetgen, I., Göksay, D., & Cotuk, A. (2009). Comparison of the microbial load of incoming and distal outlet waters from dental unit water systems in Istanbul. Environmental Monitoring and Assessment, 158, 9-14.

Uzel, A., Cogulu, D., & Oncag, O. (2008). Microbiological evaluation and antibiotic susceptibility of dental unit water systems in general dental practice. International Journal of Dental Hygiene 6, 43-7.

Tuvo, A., Privitera, G., & Casini, B. (2020). Prevention and control of *Legionella* and Pseudomonas spp. colonization in dental units. Pathogens, 9, 305.

Volgenant, C.M.C., & de Soet, J.J. (2018). Cross-transmission in the dental office: Does this make you ill? Current Oral Health Reports, 5, 221-8.

Walker, J.T, Bradshaw, D.J., Bennett, A.M., Fulford, M.R., Martin, M.V., & Marsh, P.D. (2000). Microbial biofilm formation and contamination of dental-unit water systems in general dental practice. Applied and Environmental Microbiology Journal, 66, 3363-7.

Walker, J.T., & Marsh, P.D. (2004). A review of biofilms and their role in microbial contamination of dental unit water systems (DUWS). International Biodeterioration and Biodegradation, 54, 87-98.

Walker, J.T. & Marsh, P.D. (2007). Microbial biofilm formation in DUWS and their control using disinfectants. Journal of Dentistry, 35, 721-30.

Walker, J.T., Bradshaw, D.J., Finney, M., Fulford, M.R., Frandsen, E., Stergaard E, Ten Cate, J. M., Moorer, W. R., Schel, A. J., Mavridou, A., Kamma, J. J., Mandilara, G., Stösser, L., Kneist, S., Araujo, R., Contreras, N., Goroncy-Bermes, P., O'Mullane, D., Burke, F., & Marsh, P. D. (2004). Microbiological evaluation of dental unit water systems in general dental practice in Europe. European Journal of Oral Sciences, 112, 412-8.

Walker, J.T., Bradshaw, D.J., Fulford, M.R., & Marsh, P.D. (2003). Microbiological evaluation of a range of disinfectant products to control mixed-species biofilm contamination in a laboratory model of a dental unit water system. Applied and Environmental Microbiology Journal, 69, 3327-32.

Williams, J.F., Molinari, J.A., & Andrews, N. (1996). Microbial contamination of dental unit waterlines: origins and characteristics. Compendium of continuing education in dentistry 6, 538-40, 542 passim; quiz 558.

Wirthlin, M.R., & Roth, M. (2015). Dental unit waterline contamination: a review of research and findings from a clinic setting. Compendium of continuing education in dentistry, 36, 216-9.

Wirthlin, M.R., Marshall, G.W.J., & Rowland, R.W. (2003). Formation and decontamination of biofilms in dental unit waterlines. Journal of Periodontology, 74, 1595-609.