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Dental Unit Water Lines and Risk of Pathogenic Parasitic Contamination

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Review Article

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ABSTRACT

Mouth care is of great importance for patients in dental care units, for provision of oral health care, and there is great concern about the levels of oral diseases associated with inappropriate and unhygienic oral care practices, the most important of which is the contamination of water with microbes. Oral health is at risk due to systemic diseases transmitted to patients due to microbial contamination of the dental unit waterlines (DUWLs) with pathogenic microbes. Reports of cases infected with pathogenic microbes were published of patients receiving treatment in dental unit waterlines. The quality of water is of considerable importance because both patients and dental team are regularly exposed to water and aero-sols generated by dental equipment. Studies have demonstrated that DUWLs provide a favorable environment for microbial proliferation and biofilm formation, and that water is consequently often contaminated with high densities of various microorganisms (bacteria, fungi, protozoa, viruses). The presence of high levels of microbial contamination may be a health problem for dentists and patients, especially those who are immuno-compromised. The current status of knowledge on microbial contamination of DUWLs is presented, with an emphasis on the infectious risk associated with DUWLs. It is essential that a high standard of mouth care combined with adequate and water quality, and preventive dental units in order to maintain oral comfort and prevent a deterioration and public health.

Keywords: Microorganisms; water; dental unit waterlines; biofilm; health.

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1. INTRODUCTION

1.1 Dental Unit Waterlines

The water being delivered through dental unit waterlines (DUWLs) during oral healthcare procedures is suddenly of public concern by the aftermath of the Florida HIV transmission case several years ago [1]. So, there is a growing interest in focusing on the risk of exposure to contaminated water in the dental office. Also, increasing of the scientific reports results, which prove the high levels of potentially pathogenic microorganisms in the dental treatment water. Moreover, some case reports that associated illness with dental water contamination were published [1,2].

Modern dental chair units consist of a network of interconnected narrow-bore plastic tubes called dental unit waterlines (DUWLs). The water delivered by these DUWLs acts as both a coolant for a range of instruments and an irrigant during dental treatments. The quality of water is of considerable importance because both patients and dental team are regularly exposed to water and aerosols generated by dental equipment [1,3]. Studies have demonstrated that DUWLs provide a favorable environment for microbial proliferation and biofilm formation, and that water is consequently often contaminated with high densities of various microorganisms (bacteria, fungi, protozoa, viruses). The presence of high levels of microbial contamination may be a health problem for dentists and patients, especially those who are immuno-compromised [1].

1.2 The Problems Related to Dental Water Quality

The quality of dental unit water is of considerable importance since patients and dental staff are regularly exposed to water and aerosols generated from the dental unit. Microbial contamination of specific component parts in dental chair units is an important potential source of cross-infection [4]. This water hosts a diverse microflora of bacteria, yeasts, fungi, viruses, protozoa, unicellular algae and nematodes which may be contaminated with micro-organisms found in the biofilm formed due to water stagnation in the narrow-bore dental unit waterline (DUWL) tubings [4]. The contamination of the dental units' waterlines is a reality, which can develop individual and collective infections [5].

According to Garg et al. [6] contaminated water may be ingested by patients or may contact mucosal and / or teeth continuity solutions. It provides direct access to connective tissue and turns possible absorption and reach of circulatory system. It can serve as a source of respiratory and ocular infection for patients and dentists. O'Donnell et al. [7] evaluated the dental chairs a complex medical device designed to provide the dentist with necessary features for a wide variety of dental procedures. In these units, the water supply can arise from a reservoir, replaceable, independent, in the form of a bottle or from the water supply network [8]. The pipes through which the water passes are called waterlines and are made up of internal surfaces of long lumen plastic (pipes of about 10 m in length and 0.5 to 1.0 mm in diameter). These characteristics when associated to the stagnation of water for most of the time, and microscopic internal imperfections constitute a favorable environment for the establishment of microorganisms already present in the water supply [9]. The consequent formation of biofilm is directly responsible for the contamination of water used in clinical procedures [9-14].

1.3 Types of Organisms Isolated from Dental Unit Water

Regarding the microorganisms present and isolated from dental unit water, which are bacteria, yeasts, fungi, viruses, protozoa, unicellular algae and nematodes [4], including *Achromobacter xyloxidans*, *Acinetobacter* spp., *Actinomyces* spp., *Alicaligenes dentrificans*, *Bacillus* spp., *Bacteroides* spp., *Caulobacter* spp., *Flavobacterium* spp., *Fusobacterium* spp., *Klebsiella pneumoniae*, *Lactobacillus* spp., *Legionellas* pp., *Micrococcuss* pp., *Moraxella* spp., *Mycobacterium avium*, *Nocardia* spp., *Pasteurella* spp., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Streptococcus* spp., *Staphylococcus aureus*, *Mycobacterium* spp., *Xanthomonas* spp., *Phoma* spp., *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp. *Scopulariopsis* spp., *Acanthamoeba* spp., *Cryptosporidium* spp., *Microsporidium* spp. and *Giardia* pp., as well as the many microorganisms.

2. MICROBIAL CONTAMINATION OF DUWLs AND BIOFILMS

Barbeau et al. [15], reported that the water systems of dental units resemble an aquatic ecosystem in which various opportunistic pathogens colonize the inner surface of the

pipes. These pathogens consists of microorganisms, including bacteria, fungi and protozoa, which colonize and replicate on the inner surface of water pipes, often forming a protective layer of viscous substance (limo/mucus) that allows them to survive in adverse environments and in vade new locations [5,16], such as fungi and protozoa or anaerobic bacteria [17].

DUWLs provide a suitable environment for microbial multiplication and the formation of biofilm which may be comprised of bacteria, fungi, and protozoa. A high number of microorganisms have been found in DUWLs [18-20]. Potential pathogens such as *Legionella*, *Pseudomona*s, and *Candida* also have been detected [19,21]. These microorganisms are of particular concern because of their ability to cause pneumonia, and other respiratory infections while wound infections are immuno-compromised. Consequently, the quality of dental unit water is extremely important both for patients and dentists. There have been many studies concerning the high levels of bacterial contamination [19,21-26]. *Candida* spp. which can cause both superficial and systemic diseases have been recovered occasionally from DUWLs [19,22,23].

A Gram-negative aerobic heterotrophic bacterium which attaches the inner surface of dental equipment tubing creating micro colonies that will lead to multiple biofilm patterns. This biofilm consists primarily of highly hydrated bacterial polysaccharides capable of communicating through pores and channels forming structured, coordinated, and functional communities. With the development of biofilm, planktonic cells and by-products are released into the water directly into patients' mouths during dental procedures [8,27].

Different species of microorganisms in the biofilm do not survive in isolation, but through a system called quorum sensing. This signaling system is based on the ability of not only monitoring the presence of other bacteria around it, but also the production and response to signaling molecules. These auto-inducers are detected by specific receptors that allow the cells to evaluate the population contingent by signal concentration. Upon reaching a critical level, microorganisms act as a single multicellular organism, being able to organize unified responses favorable to population survival [28,29].

The presence of a high density of microorganisms in the water exit on dental equipment is a potential risk for infections in patients and professionals, not to mention its role on cross-infections. Previous studies have reported the presence of bacteria such as *Pseudomonas*, *Legionella* and non-tuberculosis (atypical or fast-growing) mycobacteria in the water outlets what is a risk for infection, especially in immuno-compromised patients [8].

Dental-unit water line (DUWL) containment of many microorganisms helped the American Dental Association and the Centers for Disease Control and Prevention have suggested a standard for dental-unit water system water as water not having more than 200 cfu/ml. Salam et al. [30], evaluated the microbial contamination in DUWLs from two dental chairs, and found that colony-forming units were above 500 cfu/ml. The organisms identified were mostly gram-negative bacilli particularly *Escherichia coli*, *Pseudomonas* and *Klebsiella*, as well as gram-positive bacteria such as *Enterococci*. They concluded that, DUWLs are heavily contaminated with waterborne organisms from biofilm within the tubes as well as human pathogens as a result of the back-siphon age from oral cavity of patients. These organisms may cause serious systemic infections to patients, especially the children, the elderly and the immuno-compromised. The range of microorganisms includes both environmental organisms (e.g. *Moraxella* sp. and *Flavobacterium* sp.) and opportunistic and true human pathogens (e.g. *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Mycobacterium* sp., *Candida* sp., *Actinomyces* sp., *Streptococcus* sp. and *Staphylococcus* sp.) [24].

The most common cause of dental-unit water contamination is believed to be the formation and subsequent sloughing off of microbial biofilms from the surfaces of tubing within dental-unit water systems [24,31].

Although exposure to *Pseudomonas*, *Moraxella*, *Staphylococcus* and *Legionella* has been linked to dental water, the medical risk of dental-unit water line (DUWL) contamination is most significant to immune-deficient individuals [31]. Not only are these organisms intrinsically resistant to high temperatures and biocides, but also the biofilms they inhabit enhancing their resistance [32].

In 1993, Centers for Disease Control and Prevention [33] recommended that dental water

lines be flushed at the beginning of the clinic day to reduce the microbial load. However, studies have demonstrated this practice does not affect biofilm in the water lines or reliably improve the quality of water used during dental treatment [34]. Dental-unit water that remains untreated or unfiltered is unlikely to meet drinking water standards (<500 cfu/ml); therefore, one or more commercial devices and procedures designed to improve the quality of water should be employed. The results of Salam et al. [30] study indicated that DUWLs are heavily contaminated with waterborne as well as human pathogenic organisms. Most units had colony-forming units above 500 cfu/ml, which is not acceptable as per American Dental Association (ADA) recommendations. The high level of contamination of DUWLs demonstrated in this study confirmed the results of others [26,35-37].

Salam et al. [30] detected *Pseudomonas* in the water samples from hand piece and 3-way syringe, which was also similar to a study conducted in Mangalore [38]. Some previous studies in India, Agarwal et al. [39] and Fotedar and Ganju [40] showed that flushed samples from the same source had reduced microbial load to some extent when compared to the unflushed samples; however, still the values were high. In addition, it might not affect the wide variety of organisms. Therefore, flushing does not play any significant role in disinfection processes. Previous studies across the globe on percentage reduction of viable counts and biofilm coverage after exposure to disinfectants and flushing showed only 9.1% reduction in viable count with flushing and only 0.5% reduction in biofilm with flushing. However, exposure to disinfectants such as chlorhexidine, Betadine, sodium hypochlorite, Alpron, Sterilox and Oxygenal showed 100% reduction in viable count; however, still the biofilm persisted. The chemical disinfectants should not cause harm to patients as well as the dental-unit system. There is increased need to conduct further studies to find out the corrosive and toxic nature of chemical disinfectants used as well as to find out effective disinfection process within the DUWL system, thereby creating a healthy dental unit to the patients. Hence, disinfection of the water source and DUWLs should be done regularly for proper infection control in dental clinics and hospitals [30].

Salam et al. [30] also, concluded that, future research should focus on developing chemical disinfectants using nanoparticles, which would limit the draw backs of the currently available chemical disinfectants in the market. Salam et al., [30] concluded that, the colony-forming units in water samples are higher in number than the ADA recommended value. Presence of indicator organisms such as *E. coli* and *Enterococci* indicates fecal contamination of water and growth of other pathogenic organisms. The source of contamination may be the source of water or back-siphonage of organisms from the oral fluids of patients. Gram negative bacteria such as *Pseudomonas* may cause several systemic infections, mainly to the immuno-compromised, the elderly and children.

However, the existence of fungi in the systems requires more attention. During dental treatment, direct contact with water contaminated with fungi such as *Candida*, *Aspergillus*, or inhalation of aerosols from high-speed drill may cause various respiratory infections, such as asthma, allergies, and wounds on mucosae membranes, especially on immuno-compromised patients and dentists. The isolated fungi were identified as *Penicillium waksmanii*, *Cladosporium* spp., *Penicillium* spp., *Candida famata*, *Cryptococcus laurentii*, *Candida guilliermondii*, *Penicillium verrucosum*, *Aspergillus pseudoglaucus*, *Penicillium decumbens*, and *Acremonium* sp. Some of these fungal genera are known as opportunistic pathogens that led to respiratory diseases such as allergic rhinits [41].

Kadaifciler et al. [41] carried out their study to investigate the distribution of fungi in 41 DUWLs in 21 private and 20 public dental clinics. Also, they suggested that fungal spores and hyphal fragments may be aerosolized into the air when contaminated water passes through dental equipment's. In addition, several filamentous fungi are also potential toxin producers, and exposure to small amounts of toxins over several years may have negative effects on the immune system [22].

Water contaminated with yeast can be a risk if there is direct contact with open wounds. Many researchers have found the species *Candida* in DUWLs [23,24,42,43]. Opportunistic pathogen yeast in the genus *Candida* can cause skin and systemic disease. While, *Candida albicansis* generally considered to be the most pathogenic of the *Candida* species, a variety of other members of this genus have been cited as the causative agents of an increasing number of infections [43].

In the study of Kadaifciler et al. [41] *Candida guilliermondii*, *Candida famata* and *Cryptococcus laurentii* were isolated from the different water points of DUs. Aerosols originating from DU water be-low 2.5mm are considered dangerous. These small aerosols which contain microorganisms can induce asthma, rhinitis, allergic alveolitis, or other respiratory problems [44]. In previous studies on DU water, *Fusarium*, *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*, and *Scopulariopsis* have been determined [18,22]. *Aspergillus* and *Penicillium* are the most common genera of those found in aquatic environmental studies. There spores are known to cause allergic reactions [45,46]. Moreover, *Cladosporium* and *Alternaria* are known to provoke respiratory and asthmatic symptoms in immunocompromised people. From our study, isolated fungi such as *Aspergillus*, *Penicillium*, and *Cladosporium* spp.could also create these harmful effects.

The fungi isolated from DUs water contained several yeasts, but mostly filamentous fungi [18,19,22,42,47,48]. *Aspergillus* and *Penicillium* are the prevalent genera which have been isolated in DUWLs. It is known that, the spores of these genus members can cause allergic reactions, asthma, and other respiratory problems.

Indeed, copious evidence of dental unit water contamination has accumulated since the 1960s [49]. Many microorganisms (bacteria, viruses, fungi) have been found in water samples from DUWLs: *Streptococcus mitis*, *Streptococcus salivarius*, *Enterococci*, *Staphylococcus cohnii*, *Staphylococcus warneri* A, *Klebsiella* (*Enterobacter*) *aerogenes*, *Bacillus subtilis*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Serratia marcescens*, *Aeromonas* spp., *Acinetobacter* spp., *Flavobacterium* spp., *Moraxella* spp., *Cladosporium* spp., *Pseudomonas* spp., *Legionella* spp., etc [50-54], and the health of both dental staffand patients could be at risk of infection.

The Guidelines on infection control in dental healthcare settings issued by the US Centers for Disease Control and Prevention (CDC) recommend that the level of heterotrophic plate counts (HPCs) in dental unit water should not exceed 500 CFU/mL [55]. Moreover, the American Dental Association(ADA) has set a limit of≤200 CFU/mL on the heterotrophic bacterial load in water from dental unit waterlines [34]. In the EU, however, there is no current guideline regarding DUWLs, though in some countries the drinking water standard is used as a reference [12].

Abdouchakour et al. [56] reported *P. aeruginosa* and *Achromobacter* sp. of microbial contamination of waterlines in a dental healthcare center. Moreover, confirmation of the literature reports was provided by a recent study [57] which found *Legionella* and *P. aeruginosa* contamination of water samples from DUWLs. In addition, the presence of Gram-negative bacteria in DUWLs can lead to the production of endotoxins (LPS) in the water and air of a dental surgery [58]. Fungi have also been found in water from dental units. In a study conducted by Mazari et al. [59], 18 dental waterlines were analyzed for the presence of yeasts on their internal surfaces. Of the 18 DUWLs studied, 10 were contaminated (55.56%). *Candida albicans*, *Candida guilliermondii* and *Candida glabrata* and two species of non-*Candida*, *Rhodotorula* spp. and *Trichosporon* spp., were identified. In addition to bacteria, fungi and viruses, protozoa such as free-living amoebae have been isolated from DUWLs [60,61].

Free-living amoebae can act as a reservoir for microorganisms (e.g., *Legionella* spp. and *Pseudomonas* spp. etc.) or as pathogens in their own right [62-68]. In previous study Spagnolo et al. [69], assessed the level of contamination by bacteria and amoebae in 30 dental units, the mean concentration of HPCs at 22 and 36°C was1168.53 CFU/mL and 827.90 CFU/mL respectively, while the concentration of *P. aeruginosa* proved to be of 25.13 CFU/100 mL. Of the 30 units, 26.67% displayed a concentration of ≥103CFU/L of *L. pneumophila*; about 23% of cases involved *L. pneumophilas*. The study revealed that the water in the DUWLs contained considerably higher concentrations of microorganisms than the input water supply, there by confirming the role of the water system inside the dental unit in increasing microbial contamination. Previous studies have found a wide range in the rate of recovery of *Legionella* contamination of DUWLs, from 0% to 100% of DUWL systems [70,71] including *Legionella pneumophila* sero group1, reaching levels as high as 105 colony-forming units per milliliter [70,72,73]. The presence and concentration of *Legionella* contamination in DUWLs varies according to the features of the water supply system, the design and model of the dental unit, and the methods of disinfection used [70].

3. PROTOZOANS FOUND IN WATER SUPPLIES

Protozoa are unicellular organisms that are characterized by their locomotion. Amoeba move by use of small lobes called pseudopods. Others have long, whip-like tails called flagella like some bacteria. Others have hundreds of short hair-like structures called cilia covering their outer body. They exist in a variety of shapes and can live by themselves or as parasites. They eat organic substances from their environment or a host. Examples of important protozoans in dental waterlines are species of *Microsporidium*, *Giardia* and *Cryptosporidium* [74-76].

Although there are many protozoans found in drinking water and dental waterlines, there are several that have gained certain notoriety over the past 20 years. *Giardia lamblia* is a single-celled microscopic parasite that lives in the intestine of people and animals and causes a diarrheal disease known as giardiasis. *Giardia* has become one of the most common causes of water borne disease in humans in the United States and around the world. Several community-wide outbreaks of giardiasis have been linked to drinking municipal water or recreational water contaminated with *Giardia*. The parasite is found in drinking and recreational waters and is passed in the stool of an infected person or animal. It's protected by an outer shell that allows it to survive outside the body and in the environment for long periods of time. It's extremely difficult to kill with chlorination and other methods due to its ability to entrance cyst state [74-76].

Cryptosporidiosis is a diarrheal disease caused by the microscopic parasite *Cryptosporidium parvum*. It can live in the intestine of humans and animals and is passed in the stool of an infected person or animal. Like *Giardia*, it is too protected by an outer shell that allows it to survive outside the body for long periods of time and makes it very resistant to chlorine disinfection. Persons with weakened immune systems are at risk for more serious disease. Symptoms may be severe and could lead to serious or life threatening illness. Examples of persons with weakened immune systems include those with HIV/AIDS, cancer and transplant patients who are taking certain immunosuppressive drugs, and those with inherited diseases that affect the immune system. The largest outbreak of water borne disease in the United States occurred in Milwaukee, Wisconsin in 1993. Over 400,000 persons were infected by *Cryptosporidium* resulting in the hospitalization of 4,000 and the deaths of at least 50 people [74-76].

Hassan et al. [77] carried out the study to investigate the contamination of the dental irrigation systems with *Cryptosporidium* species in Alexandria, Egypt. They collected forty water samples from all 20 working dental irrigation machines in a dental center in Alexandria. Water samples were taken from the hand pieces of dental irrigation machines in all studied units. Their results are *Cryptosporidium* spp. was found as a contaminant in 27.5% of water samples taken from dental irrigation machines.

Cryptosporidium is an obligatory, intracellular, coccidian parasite that infects the intestinal epithelial cells, causing diarrhea. It is classified as an emerging pathogen by the Centers for Disease Control and Prevention [78], and can cause infection in people, cattle, and other animals [79]. *Cryptosporidium* may be transmitted by direct person-to-person contact, contact with infected animals, or ingestion of contaminated water or food [80]. The parasite can complete its life cycle within one host, and is known to be resistant to antiparasitic drugs. Upon infection with *Cryptosporidium*, immuno-competent individuals may experience a self-limiting, acute, watery diarrhea lasting for about a week; however, the immuno-compromised typically develop chronic or persistent diarrhea, leading to wasting and even death [81]. Further, chemotherapeutic agents have been shown to have low efficacy in those immuno-compromised individuals who are most affected by the disease [82]. The resistance of *Cryptosporidium* spp., to the typical concentrations of chlorine used to disinfect water has made this an important issue [83]. The ability of *Cryptosporidium* oocysts to survive conventional water treatment, and to withstand chlorine disinfection, make control of water-borne infection particularly challenging in developed and developing countries alike [84].

4. GENUS *ACANTHAMOEBA*

Globally, there has been a great interest and much attention among scientists in studying the occurrence and isolation of free-living amoebae from various water sources, because several species of *Acanthamoeba* (e.g. *A. castellanii*, *A. culbertsoni*, *A. hatchetti*, *A. healyi*, *A. polyphaga*, *A. rhysodes*, *A. astronyxis*, and *A. divionensis*) cause the insidious, chronic and mostly fatal disease granulomatous amoebic encephalitis

(GAE), particularly in immune-compromised, patients with HIV/AIDS or who are chronically ill, diabetic, have undergone organ transplantation or are otherwise debilitated with no recent history of exposure to recreational fresh water [85,86] and *Acanthamoeba keratitis* causes a vision-threatening disease [87].

Studies in past years has confirmed that Free-living amoebae (FLA) of the genus *Acanthamoeba* are ubiquitous and in habit a variety of water, air, and soil environments [88,89], and have been isolated from many ecological habitats [90-93], also they have been isolated from fresh, salt water and arid environment soil, sewage dump sites, and household garden and flower pot soil, water taps, humidifiers, and aquaria [94], and more than that from shower heads, ventilators, hydrotherapy baths and heating, ventilation, and air conditioning units in the hospital environment and dental irrigation systems [95].

The risk of *Acanthamoeba* spp. became more dangerous after it has been shown that bacteria and virus survive and grow within *Acanthamoeba* and some of them are potential pathogens for humans, beside its resistance to water treatments, *Acanthamoeba* became important waterborne pathogens [96-98].

The heightened concern of the *Acanthamoeba* on public health around the world has been recognized with the presence of pathogenic *Acanthamoeba* in the hospital water sources, dental unit waterlines during oral healthcare procedures and tap water and its seriousness of public health [99-103].

Dendana et al. [95] reported that water at the entrance of hemodialysis apparatus contained FLA, with many FLA of the genus *Acanthamoeba*. These authors attributed this contamination to the long stagnation of water, which could lead to the development of biofilms, providing favorable conditions for the growth and proliferation of many microbes and FLA [95]. In addition, these amoebae have been found in dental water lines [38,104]. In addition to disease caused by direct exposure to *Acanthamoeba* spp., these amoebae can act as vehicles form any pathogenic microorganisms with high virulence and resistance to antibiotics [105].

Huws et al. [105] reported the enhancement of methicillin-resistant *Staphylococcus aureus* (MRSA) survival, replication and virulence in association with *Acanthamoeba polyphaga* provides evidence for this role of amoebae. Cirillo et al. [106] reported enhanced invasion of *Legionella pneumophila* grown in *Acanthamoeba* relative to those grown under standard laboratory conditions. A high level resistance of *L. pneumophila* released from *A. polyphaga* to disinfectants and antimicrobials has also been reported [107,108].

Many clinical studies have been carried out to assess the pathologic out comes of amoebal infections in humans. The first human infection by *Acanthamoeba*, previously reported as *Hartmannella*, was described after the discovery of amoebae and cysts in brain sections from a patient with brain abscesses in the early1970s [109]. In addition to causing chronic granulomatous amoebic encephalitis, keratitis, lung and skin infections, *Acanthamoeba* spp. also serve as hosts for bacterial pathogens, such as *Legionella* spp. So, for the danger of *Acanthamoeba* spp., have recently received a lot of attention [110]. Many pathogenic microorganisms that replicate inside free living amoebae, such as MRSA, are more virulent and more resistant, complicating the situation when FLA and other pathogens co-exist in health care settings [105].

Fukumoto et al. [111] demonstrated the co-existence of *Parachlamydia acanthamoebae*, a potential pathogen causing hospital-acquired pneumonia, with *Acanthamoeba* in an actual hospital environment. These authors suggested that the significant impact of *Acanthamoeba* on the long-term survival of this pathogen could facilitate the pathogen's spread through a hospital environment [112]. The situation could be dangerous in health care settings visited by immuno-compromised patients, including those on hemodialysis. Patients in hospitals may be exposed to *Acanthamoeba* associated nosocomial infections, as documented in previous reports [112-115].

In Egypt, only a few studies on *Acanthamoeba* have been published. The presence of *Acanthamoeba* spp. in fresh water sources associated with human activities in the Nile Delta region was revealed using morphologic and molecular parameters [116]. In Alexandria, *Acanthamoeba* spp. were isolated from canals and drains [117]. In addition, *A. gruberi* and *A. rhysodes* were isolated from the nasal passages of six healthy children living near the contaminated canals [117].

However, no previously published appraisal of the hydraulic systems of hemodialysis and dental units for the presence of *Acanthamoeba* spp. exist. only study was carried out by Hikal et al. [101]. They isolated *Acanthamoeba castellanii*, *A. griffin*, *A. hatchitti* and *A. lenticulata* from dental unit waterlines (DUWLs). Also, Hassan et al. [77] isolated *Acanthamoeba* spp. from the hydraulic systems of hemodialysis and dental units in Alexandria. and confirmed that these systems were contaminated with *Acanthamoeba* spp.

5. CONCLUSION

A large number of microorganisms were quantified from biofilm accumulated in the dental units' waterlines. The contamination of the dental units' waterlines is a reality, which can develop individual and collective disorders. Infectious risks associated with DUWLs are not currently a major public health problem, but have been identified. Because of both the increasing number of people with immuno-compromised status and recent technological developments regarding water quality, biofilms, etc., we need to do everything possible to better and better management and application of decontamination protocols for waterlines need to be applied.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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