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Research Article:

Bacterial colonization of the dental unit waterlines in dentistry in Umm Al-Qura University teaching hospital, Makkah, Saudi Arabia

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ABSTRACT

Background:
The aim of the current study was to investigate bacterial colonization of the dental unit waterlines (DUWLs) in Umm Al-Qura University's Dental Teaching Hospital in Makkah, Saudi Arabia.

Methods:
Eight dental units were selected to collect 48 water samples from DUWls, two samples from each water outlet of the dental unit (handpiece, air-water syringe, and cup-filler water). Each sample (300 ml) was treated with sodium thiosulfate (10 mg /100 ml) and then were filtered using 0.2 µm synthetic filter paper. The filter was then placed on 5% blood agar and incubated for 48 hours at 37 oC incubator.

Results:
The average number of heterotrophic plate count (HPC) bacteria in the water samples collected from the output of the 8 DUWLs was 57.79 colony-forming unit (CFU) per 300 ml water sample (0.19 CFU/ml). There were no statistically significant differences among CFU among the air/water syringe, the high-speed handpiece cooling water, or the cup filler water ($p = 0.791$). These findings were -within the acceptable limit according to Egyptian standards for drinking and domestic use of water.

Conclusion:
The current study has shown the level of CFU in DUWLs is acceptable in the dental care unit water system at Umm Al-Qura University's dental teaching hospital.

1. INTRODUCTION

The water quality in dental units is of considerable importance because patients and dental staff are regularly exposed to the water and aerosol generated by the dental unit (Szymanska, 2007). Air scalers, dental handpieces, and ultrasonic scalers are supplied by the unit's water, like most instruments, which also irrigates and cools the tooth surface during dental treatment (Abdouchakour et al., 2015). Additionally, water is provided to the dental unit cup filler outlet patients use for oral washing and the bowl rinse outlet for rinsing the dental unit spittoon. Therefore, dental unit waterlines (DUWLs) comprise a complex organisation of meters of narrow-bore plastic tubing, mostly 2-3 mm inner diameter. In limited-bore tubes, a thin immobile layer of fluid, named the hydrodynamic boundary layer, is located at the interface of the lumen wall and the moving water (Abdouchakour et al., 2015; Fan et al., 2021), a silver coating placed on the luminal surface of commercial waterline tubing failed to prevent biofilm growth, according to research (Lal et al., 2016).

A routine check for microbial contamination in the dental unit's waterlines is performed to avoid enormous microbial infections and recognize pathogenic bacteria's presence. Most microbial species depicted in massive DUWLs infection are aerobic, Gram-negative, heterotrophic environmental species with minimum pathogenicity (Abdouchakour et al., 2015). Back-contamination (the sucking back of biological fluids from patients' oral cavities) has also been identified as a

major source of DUWLs contamination (Costa et al., 2015; Cristina et al., 2008). An anti-retraction device can be installed with the handpiece to avoid backflow from the oral cavity to the waterlines, but some documented evidence proved valve failures (Abdouchakour et al., 2015).

Microorganisms can colonise the surfaces of water supply tubes, including DUW. The number of expected microorganisms and patients' oral microflora contribute significantly to the contamination of DUWLs (Szymańska & Sitkowska, 2013).

Bacterial contamination of the output water from DUWLs is dangerous for patients and healthcare staff. Many different types of bacteria, such as *pseudomonas, leptospira, legionella pneumophila, mycobacterium spp.*, and *staphylococcus spp*., have been found to live and grow in dental equipment, including amoebae species (Barbeau & Buhler, 2001; Lin et al., 2011; Nikaeen et al., 2009; Singh et al., 2003; Walker et al., 2000). Several studies have shown that *P. aeruginosa* is one of the most challenging pathogens to treat, and it is commonly found in DUWLs (De Oliveira et al., 2008; James et al., 2015).

Pseudomonas aeruginosa is the commonest colonizer of DUWLs. Al-Hiyasat et al. (Al-Hiyasat et al., 2007) reported that *pseudomonas aeruginosa* was present in higher counts (86.7%) in dental units at the start of the day, where a reduction of 13.4% was observed after two minutes of flushing and in the middle of the day. *Pseudomonas aeruginosa* can cause a broad range of severe infections and strengthens the issue with its ability to form biofilms. It is difficult to treat infections caused by P. aeruginosa because the organism is naturally resistant to many drugs and can also develop new resistance mechanisms, such as the development of β-lactamases and carbapenemases (Gawish et al., 2019). *P. aeruginosa* has *12*-to *100-fold* lower outer membrane permeability to various compounds than *Escherichia coli*, a feature central to its high intrinsic resistance to antimicrobials (Hancock & Brinkman, 2003).

P. aeruginosa has numerous porin families, including the OmpA family's so-called structural porins, the OmpW family's small porins (8 β-sheets) and the 18 β-sheet larger diffusion porins. However, the TonB-dependent receptors for the absorption of siderophores, heme, and organic sulfur molecules are present in the outer membrane with larger channels with 22 antiparallel β-sheets. The 19 members of the OprD family called Occ (outer membrane carboxylate channel) are divided into two subfamilies. Phylogenetically, OccD is involved in the uptake of essential amino acids, and OccK is involved in the uptake of cyclic molecules that are negatively charged (Chevalier et al., 2017). Regarding the colonising nature of these bacteria, using various types of continuous water disinfection systems proved effective in treating contaminated DUWLs (Offner et al., 2016).

Legionella species can be present in a variety of water systems, including cooling towers, spas, cisterns, and showers (APHA et al., 1998; Bollin et al., 1985). After isolating them from these water systems, standing water in dental units has also been identified as a possible source for this microorganism and its infection (Ma'ayeh et al., 2008). Legionella species are pathogenic microorganisms that can be spread by aerosols and may cause pneumonia and Pontiac fever (APHA et al., 1998). *Legionella pneumophila* is the most common cause of Legionella pneumonia, with serogroups 1-6 most commonly associated with respiratory infections (Edelstein, 1988).

There is no data or record regarding the bacterial colonization of DUWLs in Umm Al-Qura University Dental Teaching Hospital; therefore, we hypothesized that there is no bacterial contamination in DUWLs. The aim of this study is to investigate the bacterial colonization of the dental units in Umm Al-Qura University's dental teaching hospital.

2. MATERIALS AND METHODS

2.1 Sample size determination

The sample size calculation was performed a using software sample size calculator (https://clinical.com/stats/samplesize.aspx). The ample size was calculated based on the mean number of colony-forming unit (CFU) and the mean difference between the number of CFU in drinking water and DUWLs determined by a previous study (Abdouchakour et al., 2015), with a standard error of 5% (p = 0.05) and a power of 90% (0.9). The estimated sample size was 48.

2.2 Sample collection

A total of 48 water samples were collected from 8 randomly selected dental care units of the Dental Teaching Hospital, Umm Al-Qura University. Each dental unit received a dual water supply, where the high-speed handpiece and the air/water syringe waterlines were supplied by a refillable water reservoir attached to the unit. In contrast, the cup filler waterline was supplied by tap water. Six samples (300 ml/sample) per unit were collected; two samples were collected from the water outlets of each high-speed handpiece cooling water, the air/water syringe, and the cup filler water.

2.3 Bacterial isolation

Before filtering the samples, all samples were treated with 10 mg of sodium thiosulfate per 100 ml to neutralize the residual chlorine present in the water. Each sample (300 ml) was filtered using 0.2 µm polycarbonate (PCTE) membrane filters (STERLITECH, USA). The filters were placed on a Borosil® Filtration Assembly with a 1L funnel (Foxx Life Sciences, USA) attached to a vacuum suction unit (GIMA, Italy). The filters were removed with sterile forceps, placed on 5% blood agar plates, and incubated for 48 hours at 37 °C. After 48 hours, the plates were removed from the incubator, and CFU were counted using a stereomicroscope under reflected light (Optika Microscopes). CFU is a unit used in microbiology to estimate the number of viable bacteria in a sample.

2.4 Statistical analysis

The collected data was statistically analyzed using SPSS software version 23, and the data was presented as mean ± SD. A one-way ANOVA test was used for comparison among the total microorganisms in the high-speed handpiece cooling water, the air/water syringe, and the cup filler water, p value less than 0.05 was considered statistically significant.

3. RESULTS

Heterotrophic plate count (*HPC*) is widely used to measure the heterotrophic microorganisms' population in drinking water. Due to the absence of specific standards regarding DUWLs in Umm Al-Qura University, the acceptable limit for HPC bacteria in potable drinking water (<50 CFU/mL) according to the Egyptian standards for drinking and domestic use water, has been used as a guideline in this study (EWQS, 2007).

The average number of HPC bacteria in the water samples collected from the output of the 8 DUWLs was 57.79 CFU per 300 ml water sample (0.19 CFU/ml). The mean CFU in cup filler water was 55.88 ± 28.95, the air/water syringe was 54.31 ± 35.03, and the high-speed handpiece cooling water was 63.19 ± 50.03 (Table 1).

Table 1: The average CFU of different water samples

Sample (No)	CFU Mean ± SD	p
Cup filler water (16)	55.88 ± 28.95	0.791
Air/water syringe (16)	54.31 ± 35.03	
High-speed handpiece cooling water (16)	63.19 ± 50.03	
Total (48)	57.79 ± 38.38	

CFU = Colony Forming Units, p= p value calculated by one-way ANOVA test.

There were no statistically significant differences between CFU of the air/water syringe, the high-speed handpiece cooling water and cup filler water (p = 0.791). CFU on 0.2 µm filter paper placed on blood agar from different water samples were illustrated in figure 1.

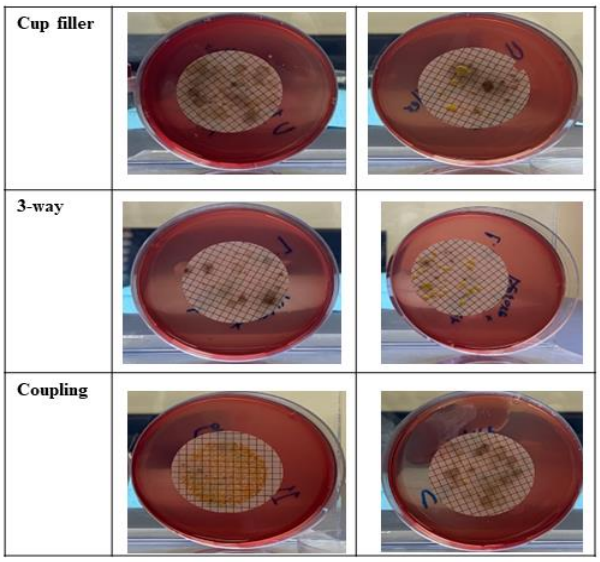

Figure 1: CFU on 0.2 µm filter paper placed on blood agar. Water samples were collected from the cup filler, 3-way, and coupling at the beginning of the session.

4. DISCUSSION

Water contaminated with bacteria may pose a danger to dentists and patients because of their exposure to water and aerosols released from dental units. The present study has shown that the average number of HPC bacteria in the water samples collected from the output of the 8 DUWLs was 57.79 CFU per 300 ml water sample (0.19 CFU/ml). These findings were found to be at the acceptable limit according to Egyptian standards for drinking water and domestic uses (EWQS, 2007), the American Dental Association (ADA), and the Center for Disease Control (Gerberding et al., 2003).

Egyptian standards for drinking and domestic use water stated that the acceptable limit for HPC bacteria in potable drinking water is less than 50 CFU/mL (EWQS, 2007). However, the American Dental Association recommends that water from dental units should not have more than 200 CFU/ml of bacteria, whereas the Center for Disease Control (CDC) stated that the maximum contamination of dentally treated water should be 500 CFU/ml (Gerberding et al., 2003).

Studies done in Göteborg, Sweden, and St. Gallen, Switzerland, have shown that most DUWLs did not have acceptable water quality (<100 CFU/ml and >300 CFU/ml, respectively) (Barben et al., 2009; Dahlén et al., 2009). Nikaeen et al. (Nikaeen et al., 2009) reported that HPC levels significantly exceeded the acceptable levels for DUWL water quality in both the air/water syringe and the high-speed handpiece. A recent study in Saudi Arabia (Alkhulaifi et al., 2020) reported significant high levels of bacterial contamination in DUWLs that were not disinfected, whereas the DUWLs that were disinfected with citric acid disinfectant showed no bacterial contamination.

Biofilms in DUWLs are one of the leading causes of increased bacterial load (Franco et al., 2005). It has been demonstrated that disinfectant exposure and flushing reduce the viable count by 9.1% and biofilm saturation by 0.5% (Agarwal et al., 2023; Fotedar & Ganju, 2015). There is strong evidence to suggest that flushing is not enough to enhance the water quality in dental treatment (Ling et al., n.d.; Rice et al., 2006) and chemical disinfectants are recommended for removing biofilms from DUWLs (Salam et al., 2017).

The DUWLs could be contaminated with microorganisms from different sources, including water that is shared by both domestic users and the dental professionals, patients' saliva sucked back into dental water units due to retraction valve failure, the skin microflora of dental staff, and free-living protozoan vectors such as amoebae (Spagnolo et al., 2020). The growth of microorganisms and the formation of biofilm on the inner surface of the pipes of DUWLs may be facilitated by the environment inside the pipes of the dental unit.

Tall et al. (Tall et al., 1995) have demonstrated multiple layers of dimorphic microorganisms developed in the lumen of dental air/water syringes over six months. Many dental professionals are in danger of occupational exposure to *Legionella* (Petti & Vitali, 2017). *Legionella* antibodies in serological examinations have been discovered to be more prevalent in dental workers than in the general population, suggesting that dental aerosols are a source of *Legionella* exposure (Fotos et al., 1985; Reinthaler et al., 1988).

It has been reported that the longer the period of dental practice, the higher the risk of such infections (Estrich et al., 2017). Thus, a combination of preventative measures is necessary to reduce the risk of infection in dental care settings. It is equally crucial to keep track of DUWL's water quality and perform regular maintenance on dental equipment. The effectiveness of these preventative measures has been proven by several scientific investigations, which have been cited by multiple international organizations (Alkhulaifi et al., 2020; Artini et al., 2008; Baudet et al., 2020; Costa et al., 2017).

Precautions should be used in daily clinical practice to reduce aerosol formation, such as high-velocity air evacuation and air conditioning systems. Dental healthcare professionals should try to keep their DUWLs at least as clean as the ADA standard of 200 CFU/ml of aerobic heterotrophs, or even superior. It has been hard to meet this goal consistently, not only because there aren't any standards or laws, but also because dental unit manufacturers haven't been prompted to address these concerns with engineering and design changes and with technical instructions on DUWL disinfection. The current study was a single-center study, and therefore the water quality estimated in the present study cannot be generalized to other dental units in different centers in Saudi Arabia.

5. CONCLUSION AND RECOMMENDATION

The current study has shown the level of CFU in DUWLs is acceptable in the dental care unit water system at Umm Al-Qura University's dental teaching hospital. We recommend monitoring the microbiological quality of the water in DUWL and disinfecting it regularly.

AUTHOR CONTRIBUTION

Faisal K. Alotaibi, Mustafa F. Almutawwif, Ehab A. Alsayed, Mohammed R. Tamboosi and Khaled R. Althebeti: sample collection, performing the experiments and writing the manuscript. Wahdan M. A. Elkwatehy: performed statistical analysis. Abdel-Rahman Youssef: designing the study, writing, and editing the manuscript.

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CONFLICT OF INTEREST

No conflict of interest

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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*, *81*(21), 7509. https://doi.org/10.1128/AEM.01279-15

Agarwal, D., Sunitha, S., & Reddy, C. V. K. (2023). 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*(11), 46. https://journals.lww.com/aphd/pages/default.aspx/article.asp?issn=2319-5932;year=2008;volume=6;issue=11;spage=46;epage=52;aulast=Agarwal;type=0

Al-Hiyasat, A. S., Ma'ayeh, S. Y., Hindiyeh, M. Y., & Khader, Y. S. (2007). The presence of Pseudomonas aeruginosa in the dental unit waterline systems of teaching clinics. *International Journal of Dental Hygiene*, *5*(1), 36–44. https://doi.org/10.1111/J.1601-5037.2007.00221.X

Alkhulaifi, M. M., Alotaibi, D. H., Alajlan, H., & Binshoail, T. (2020). Assessment of nosocomial bacterial contamination in dental unit waterlines: Impact of flushing. *The Saudi Dental Journal*, *32*(2), 68–73. https://doi.org/10.1016/J.SDENTJ.2019.07.003

APHA, AWWA, & WEF. (1998). Standard method 6232B. Trihalomethanes and chlorinated organic solvents: liquidliquid extraction gas chromatographic method. *Standard Methods for the Examination of Water and Wastewater*, *6000*, 40, 46. https://www.worldcat.org/title/40733179

Artini, M., Scoarughi, G. L., Papa, R., Dolci, G., De Luca, M., Orsini, G., Pappalardo, S., Costerton, J. W., & Selan, L. (2008). Specific anti cross-infection measures may help to prevent viral contamination of dental unit waterlines: A pilot study. *Infection*, *36*(5), 467–471. https://doi.org/10.1007/S15010-008-7246-5/METRICS

Barbeau, J., & Buhler, T. (2001). Biofilms augment the number of free-living amoebae in dental unit waterlines. *Research in Microbiology*, *152*(8), 753–760. https://doi.org/10.1016/S0923-2508(01)01256-6

Barben, J., Kuehni, C. E., Schmid, J., Barben, J., & Monatsschr, S. (2009). Water Quality in Dental Chair Units A Random Sample in the Canton of St. Gallen. *Schweiz Monatsschr Zahnmed*, *119*.

Baudet, A., Lizon, J., Martrette, J. M., Camelot, F., Florentin, A., & Clément, C. (2020). Efficacy of BRS® and Alpron®/Bilpron® Disinfectants for Dental Unit Waterlines: A Six-Year Study. *International Journal of Environmental Research and Public Health*, *17*(8). https://doi.org/10.3390/IJERPH17082634

Bollin, G. E., Plouffe, J. F., Para, M. F., & Hackman, B. (1985). Aerosols containing Legionella pneumophila generated by shower heads and hot-water faucets. *Applied and Environmental Microbiology*, *50*(5), 1128. https://doi.org/10.1128/AEM.50.5.1128-1131.1985

Chevalier, S., Bouffartigues, E., Bodilis, J., Maillot, O., Lesouhaitier, O., Feuilloley, M. G. J., Orange, N., Dufour, A., & Cornelis, P. (2017). Structure, function and regulation of Pseudomonas aeruginosa porins. *FEMS Microbiology Reviews*, *41*(5), 698–722. https://doi.org/10.1093/FEMSRE/FUX020

Costa, D., Bossard, V., Brunet, K., Fradin, B., & Imbert, C. (2017). Planktonic free-living amoebae susceptibility to dental unit waterlines disinfectants. *Pathogens and Disease*, *75*(8), 99. https://doi.org/10.1093/FEMSPD/FTX099

Costa, D., Mercier, A., Gravouil, K., Lesobre, J., Delafont, V., Bousseau, A., Verdon, J., & Imbert, C. (2015). Pyrosequencing analysis of bacterial diversity in dental unit waterlines. *Water Research*, *81*, 223–231. https://doi.org/10.1016/J.WATRES.2015.05.065

Cristina, M. L., Spagnolo, A. M., Sartini, M., Dallera, M., Ottria, G., Lombardi, R., & Perdelli, F. (2008). Evaluation of the risk of infection through exposure to aerosols and spatters in dentistry. *American Journal of Infection Control*, *36*(4), 304–307. https://doi.org/10.1016/J.AJIC.2007.07.019

EWQS (Egyptian Drinking Water Quality Standards) (2007). Ministry of Health, Population Decision Number458. - References - Scientific Research Publishing. (n.d.-b). https://scirp.org/reference/referencespapers.aspx?referenceid=2528915

Dahlén, G., Alenäs-Jarl, E., & Hjort, G. (2009). Water quality in water lines of dental units in the public dental health service in Göteborg, Sweden. *Swedish Dental Journal*, *33*(4), 161–172. https://pubmed.ncbi.nlm.nih.gov/20162927/

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 : [Publication of the Brazilian Society for Microbiology]*, *39*(3), 579–584. https://doi.org/10.1590/S1517-838220080003000032

Edelstein, P. H. (1988). Nosocomial Legionnaires' disease: a global perspective. *Journal of Hospital Infection*, *11*(SUPPL. A), 182–188. https://doi.org/10.1016/0195-6701(88)90185-5

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. *The Journal of the American Dental Association*, *148*(3), 164–171. https://doi.org/10.1016/J.ADAJ.2016.11.03

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. https://doi.org/10.3389/FCIMB.2021.670211

Fotedar, S., & Ganju, S. (2015). Microbial contamination of dental unit water lines in H.P. Government Dental College, Shimla. *The Saudi Journal for Dental Research*, *6*, 129–132. https://doi.org/10.1016/j.sjdr.2014.11.002

Fotos, P. G., Snyder, I. S., Miller, R. W., Mutchler, B. M., & Westfall, H. N. (1985). Prevalence of Legionella-specific IgG and IgM Antibody in a Dental Clinic Population. *Http://Dx.Doi.Org/10.1177/00220345850640121101*, *64*(12), 1382–1385. https://doi.org/10.1177/00220345850640121101

Franco, F. F. S., Spratt, D., Leao, J. C., & Porter, S. R. (2005). Biofilm formation and control in dental unit waterlines. *Biofilms*, *2*(1), 9–17. https://doi.org/10.1017/S1479050504001450

Gawish, S., Abbass, A., & Abaza, A. (2019). Occurrence and biofilm forming ability of Pseudomonas aeruginosa in the water output of dental unit waterlines in a dental center in Alexandria, Egypt. *Germs*, *9*(2), 71. https://doi.org/10.18683/GERMS.2019.1160

Gerberding, J. L., Director Dixie Snider, M. E., Chu, S. Y., Thacker, S. B., Ward, J. W., Hewitt, S. M., Kay Smith-Akin, C., Douglas Weatherwax, Me. W., Bright, K. L., Quang Doan, M. M., & Erica Shaver, M. R. (2003). Dental Health-Care Settings-2003. *MMWR*, *52*(17). http://www.ada.org.

Guidelines for Infection Control in Dental Health-Care Settings --- 2003. (n.d.). Retrieved June 5, 2023, from https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5217a1.htm

Hancock, R. E. W., & Brinkman, F. S. L. (2003). Function of Pseudomonas Porins in Uptake and Efflux. *Https://Doi.Org/10.1146/Annurev.Micro.56.012302.160310*, *56*, 17–38. https://doi.org/10.1146/ANNUREV.MICRO.56.012302.160310

James, A., Shetty, A., Hegde, M. N., & Bhandary, S. (2015). Detection & Quantification of Microorganisms in Dental Unit Waterlines. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN*, *14*(2), 88–91. https://doi.org/10.9790/0853-14228891

Lal, S., Pearce, M., Achilles-Day, U. E. M., Day, J. G., Morton, L. H. G., Crean, S. J., & Singhrao, S. K. (2016). Developing an ecologically relevant heterogeneous biofilm model for dental-unit waterlines. *Http://Dx.Doi.Org/10.1080/08927014.2016.1260710*, *33*(1), 75–87. https://doi.org/10.1080/08927014.2016.1260710

Lin, S. M., Svoboda, K. K. H., Giletto, A., Seibert, J., & Puttaiah, R. (2011). Effects of Hydrogen Peroxide on Dental Unit Biofilms and Treatment Water Contamination. *European Journal of Dentistry*, *5*(1), 47. https://doi.org/10.1055/s-0039-1698858

Ling, M. L., Ching, P., Cheng, J., Lang, L., Liberali, S., Poon, P., Shin, Y., & Sim, C. (n.d.). *GUIDELINE Open Access Antimicrobial Resistance and Infection Control APSIC dental infection prevention and control (IPC) guidelines*. https://doi.org/10.1186/s13756-023-01252-w

Ma'ayeh, S. Y., Al-Hiyasat, A. S., Hindiyeh, M. Y., & Khader, Y. S. (2008). Legionella pneumophila

contamination of a dental unit water line system in a dental teaching centre. *International Journal of Dental Hygiene*, *6*(1), 48–55. https://doi.org/10.1111/J.1601-5037.2007.00280.X

Nikaeen, M., Hatamzadeh, M., Sabzevari, Z., & Zareh, O. (2009). Microbial quality of water in dental unit waterlines. *Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences*, *14*(5), 297. /pmc/articles/PMC3129099/

Offner, D., Fioretti, F., & Musset, A.-M. (2016). Contamination of dental unit waterlines: assessment of three continuous water disinfection systems. *BDJ Open 2016 2:1*, *2*(1), 1–6. https://doi.org/10.1038/bdjopen.2016.7

Petti, S., & Vitali, M. (2017). Occupational risk for Legionella infection among dental healthcare workers: meta-analysis in occupational epidemiology. *BMJ Open*, *7*(7), e015374. https://doi.org/10.1136/BMJOPEN-2016-015374

Reinthaler, F. F., Mascher, F., & Stünzner, D. (1988). Serological Examinations for Antibodies against Legionella Species in Dental Personnel. *Http://Dx.Doi.Org/10.1177/00220345880670061001*, *67*(6), 942–943. https://doi.org/10.1177/00220345880670061001

Rice, E. W., Rich, W. K., Johnson, C. H., & Lye, D. J. (2006). The Role of Flushing Dental Water Lines for the Removal of Microbial Contaminants. *Public Health Reports*, *121*(3), 270. https://doi.org/10.1177/003335490612100308

Salam, N., Mulamoottil, M. ., & George, B. (2017). *Assessment of Microbial Contamination in Dental-Unit Water Lines: An Analytical Study*. *March*, 97–101. https://doi.org/10.4103/2319-5932.201925

Singh, R., Stine, O. C., Smith, D. L., Spitznagel, J. K., Labib, M. E., & Williams, H. N. (2003). Microbial diversity of biofilms in dental unit water systems. *Applied and Environmental Microbiology*, *69*(6), 3412–3420. https://doi.org/10.1128/AEM.69.6.3412-3420.2003

Spagnolo, A. M., Sartini, M., & Cristina, M. L. (2020). Microbial Contamination of Dental Unit Waterlines and Potential Risk of Infection: A Narrative Review. *Pathogens 2020, Vol. 9, Page 651*, *9*(8), 651. https://doi.org/10.3390/PATHOGENS9080651

Szymanska, J. (2007). Dental bioaerosol as an occupational hazard in a dentist's workplace. *Annals of Agricultural and Environmental Medicine*, *14*(2), 203–207. https://www.aaem.pl/Dental-bioaerosol-as-an-occupational-hazard-in-a-dentist-s-workplace-,72974,0,2.html

Szymańska, J., & Sitkowska, J. (2013). Bacterial contamination of dental unit waterlines. *Environmental Monitoring and Assessment*, *185*(5), 3603. https://doi.org/10.1007/S10661-012-2812-9

Tall, B. D., Williams, H. N., George, K. S., Gray, R. T., & Walch, M. (1995). Bacterial succession within a biofilm in water supply lines of dental air-water syringes. *Canadian Journal of Microbiology*, *41*(7), 647–654. https://doi.org/10.1139/M95-088

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*, *66*(8), 3363–3367. https://doi.org/10.1128/AEM.66.8.3363-3367.2000