

Assessment of the Heterotrophic Bacterial Contamination in Waterlines of Dental Units as a Potential Health Risk Factor in Occupational and Patient's Exposures

Ali Shahryari¹, PhD;
Amineh Zahed¹, BSc;
Abdolhalim Rajabi², PhD;
Maryam Shafipour¹, PhD;
Niloufar Emrani³, PhD; Hadi
Rahimzadeh¹, PhD; Hamidreza
Heidari¹, PhD

¹Environmental Health Research Center, Department of Environmental Health Engineering, Faculty of Health, Golestan University of Medical Sciences, Gorgan, Iran

²Environmental Health Research Center, Department of Biostatistics and Epidemiology, Faculty of Health, Golestan University of Medical Sciences, Gorgan, Iran.

³Department of Pediatric Dentistry, Faculty of Dentistry, Golestan University of Medical Sciences, Gorgan, Iran

Correspondence:

Hamidreza Heidari, PhD;
Environmental Health Research Center, Department of Environmental Health Engineering, Faculty of Health, Golestan University of Medical Sciences, Gorgan, Iran

Tel: +98 17 32457304

Email: Hr.Heidari.tums@gmail.com

Received: 20 October 2024

Revised: 14 November 2024

Accepted: 04 December 2024

Abstract

Background: Waterlines in dental units can harbor various types of bacteria and microorganisms, including heterotrophic bacteria, which can pose a health risk to patients and dental personnel. This study aimed to investigate the extent of heterotrophic bacterial contamination in the waterlines of dental units and evaluate its potential as a health risk factor for occupational and patient exposure.

Methods: This cross-sectional study selected four active departments at the Faculty of Dentistry, Golestan University of Medical Sciences (Gorgan, Iran). Bacterial contamination in the waterlines, dental handpieces, and other water-related components of dental units was investigated and analyzed in terms of Colony-Forming Units per milliliter (CFU/ml). The data were analyzed using the Kruskal-Wallis and Wilcoxon signed-rank tests using SPSS version 23.

Results: The mean and standard deviation of bacterial contamination across all studied departments were significantly higher than the recommended limit of 500 CFU/ml (ranging from 10,250.00±8,924.28 CFU/ml for the inlet to the water tank in the pediatric department to 275,625.00±41,520.86 CFU/ml for the air-water syringe before flushing in the surgery department). No significant differences were observed between departments ($P>0.05$). Furthermore, there were no significant differences in bacterial contamination between different days and hours of sampling ($P>0.05$), although bacterial levels were higher on Saturdays and mornings. Flushing the waterlines for 30 seconds reduced bacterial contamination ($P<0.05$).

Conclusion: Stagnation of water in dental unit waterlines, particularly over weekends or after working hours, using reserve water during dental procedures, and insufficient flushing of dental handpieces resulted in consistently high bacterial contamination levels. Therefore, using fresh water and performing proper flushing in dental handpieces is strongly recommended to mitigate the risks associated with bacterial contamination.

Please cite this article as: Shahryari A, Zahed A, Rajabi A, Shafipour M, Emrani N, Rahimzadeh H, Heidari HR. Assessment of the Heterotrophic Bacterial Contamination in Waterlines of Dental Units as a Potential Health Risk Factor in Occupational and Patient's Exposures. J Health Sci Surveillance Sys. 2025;13(1):100-106.

Keywords: Bacteria, Contamination, Dental unit, Heterotrophic, Waterline

Introduction

The water used in dental units can be supplied either through open water systems, such as municipal water or via water reservoir,¹ which may include small plastic bottles or larger water tanks, particularly in cases where water use restrictions apply. Research has shown that the water used in dental units often harbors numerous microorganisms.^{2,3} Various types of bacteria and microorganisms are typically found in these waterlines, among which heterotrophic bacteria are a significant concern.⁴ These bacteria encompass a broad range of microbial species. Since both patients and dental personnel are at risk of exposure to bacteria-contaminated water—either through direct contact, splatter, or aerosolized water emitted by unit handpieces, including rotating and ultrasonic instruments—the microbial quality of water used in dental units becomes a critical safety concern.⁵

The most important health risk factors transmitted through water from dental units include the presence of opportunistic microorganisms and bacterial endotoxins associated with Gram-negative bacteria.^{5,6} Due to their nutritional characteristics, these bacteria can grow in favorable conditions within water supply systems. As a result, there is a high probability of these microorganisms and pathogens in dental units supplied by municipal water.⁷

One method for assessing water quality and safety is the measurement of Colony-Forming Units (CFU), which indicates pollution. The number of bacteria in the water of dental units should be less than 200 CFU/ml. However, even small numbers of Gram-negative bacteria or respiratory pathogens can pose a health risk. Studies indicate that the water entering the dental unit typically has low levels of contamination (10 to 100 CFU/ml), whereas the water exiting dental equipment such as handpieces, turbines, and scaling devices can be highly contaminated, with more than 100,000 CFU/ml, containing various microorganisms such as *Legionella*, *Pseudomonas*, and *Escherichia coli*. This suggests that water in different parts of the unit can become contaminated.^{6,8-10}

Therefore, ensuring the provision of healthy, non-contaminated water in dental units is crucial, particularly for vulnerable individuals, such as children and older people, as well as those with weakened immune systems.^{11,12} This study evaluated the contamination of heterotrophic bacteria in the water of dental units at the Gorgan Faculty of Dentistry (in northern Iran).

Methods

Sample Size and Setting

In this analytical cross-sectional study, the research community consisted of water samples from four active

departments at the Faculty of Dentistry of Golestan University of Medical Sciences (Gorgan, Iran). The departments were: (1) Surgery, (2) Restorative, (3) Pediatric, and (4) Endodontics. One dental unit was randomly selected from each department. Water samples were taken twice a week (on Saturday and Wednesday, representing the beginning and end of the working week in Iran, respectively) and twice a day (at 08:00 AM and 12:00 PM, representing the start and mid-point of the working day, respectively).

Additionally, water samples were taken from seven locations or conditions within each dental unit, including the air-water syringe before (1) and after (2) flushing; the dental drill or turbine before (3) and after (4) flushing; the water supply nozzle attached to the spittoon (5); the inlet (6); and the outlet (7) of the water tank. The flushing process was defined as the release of water from handpieces for 30 seconds. In total, 112 water samples (4 departments×1 dental unit×2 days per week×2 times per day × 7 locations) were collected for this study. Inactive units and those lacking safe water flow were excluded from the study, and only active dental units were included. This study was approved by the ethics committee with approval number IR.GOUMS.REC.1401.197.

Sampling Protocol

Sampling was performed using sterile 50 ml Falcon tubes as sampling bottles. Each water sample was prepared with a volume of 50 ml. The bottle cap was immediately sealed after sampling to prevent secondary contamination and avoid direct contact between the head of the dental handpieces and the Falcon tube. In the microbiological laboratory, the samples were shaken to create a homogeneous solution. Sample preparation involved dilutions at 1:10 and 1:100 ratios.

Under controlled conditions, 1 milliliter of each sample, along with its corresponding serial dilutions, was added to the surface of two plates containing R2A Agar medium (0.5 ml of each sample on the surface of each plate). A sterile curved glass rod (L-shaped) was then used to distribute the water sample evenly on the surface of the plate in a superficial manner. After ensuring that the water was absorbed into the surface and allowing the plates to dry in laboratory air for approximately 15 minutes, the plates were inverted and placed in an incubator at 37°C for 48 hours.

After incubation, bacterial contamination was counted separately on each plate using a counting device. The average count of both plates was reported as the result of a single water sample, expressed in terms of Colony-Forming Units (CFU/ml).

Finally, the data were analyzed using the Kruskal-Wallis and Wilcoxon signed-rank tests with SPSS software version 23.

Results

Table 1 presents the results of the average bacterial counts in different departments of the dental school and various points of the dental units. The surgical department did not use a turbine during sampling, so data for this section are unavailable. The table shows that bacterial contamination's mean and standard deviation were significantly higher than the recommended limit of less than 200 CFU/ml. No significant differences were observed between the departments.

The results of Table 2 illustrate the effect of the working day on bacterial contamination at different sampling points. The mean and standard deviation (CFU/ml) of heterotrophic bacteria on different days showed no significant differences. Saturday was selected as the representative of the first working day (when dental units had been inactive for 48 hours), and

Wednesday was chosen as the representative of the end working day (when dental units had been active for 4 days).

Similarly, the effect of different times of the day (morning and noon) on bacterial counts in the water was investigated to determine the potential impact of water usage duration on bacterial contamination. As shown in Table 3, no significant differences between the morning and noon times of the day were observed regarding bacterial contamination.

The effect of flushing on the quantity of heterotrophic bacteria (regardless of the dental operational sections, day, and time of the sampling) was investigated. The results presented in Table 4 illustrate the significant reduction in bacterial contamination before and after 30 seconds of flushing of the air-water syringe ($P < 0.05$).

Table 1: The mean and standard deviation (CFU/ml) of the heterotrophic bacteria at different points of the dental units and across departments

Sampling points	Condition	Dental departments				P value**
		Surgery	Restorative	Pediatric	Endodontics	
Air-water-syringe	Before flushing	275625.00±41520.86	272375.00±56870.62	174625.00±138318.40	195250.00±121839.17	0.340
	After flushing	175125.0000±96508.23	173625.00±111628.12	116375.00±127035.35	133875.00±129306.32	0.414
Dental Drill	Before flushing	-	207250.00±106052.21	166625.00±142747.46	219750.00±120841.98	0.758
	After flushing	-	185625.00±120146.26	158125.00±151690.88	142750.00±132265.69	0.755
Water supply nozzle	-	161375.0000±116732.82	200625.00±138216.12	184125.00±129498.41	216625.00±116121.78	0.864
Water tank	Inlet to tank	19000.00±9071.14	16000.00±11414.27	10250.00±8924.28	74750.00±86326.20	0.335
	Outlet to unit	33500.00±23742.66	82500.00±70793.46	94000.00±128394.48	40500.00±60325.30	0.387

*Colony-forming unit/milliliter; **Kruskal-Wallis test

Table 2: The average counts of the heterotrophic bacteria on different days of the week

Sampling points	Condition	Colony-forming units (CFU/ml)*, M±SD**		P value***
		Saturday	Wednesday	
Air-water-syringe	Before flushing	238312.50±113580.93	220625.00±97205.53	0.271
	After flushing	160937.50±127372.14	138562.50±101884.88	0.819
Dental Drill	Before flushing	187416.66±136829.32	208333.33±107602.24	0.578
	After flushing	183583.33±139941.19	140750.00±122733.35	0.764
Water supply nozzle	-	183625.00±123593.89	197750.00±122080.02	0.775
Water tank	Inlet to tank	47375.00±65767.13	12625.00±9478.92	0.131
	Outlet to unit	86875.00±101263.27	38375.00±43972.52	0.096

*Colony-forming unit/milliliter; **Mean±Standard deviation; ***Mann-Whitney U

Table 3: The average counts of the heterotrophic bacteria at different times of day

Sampling points	Condition	Colony-forming units (CFU/ml)*, M±SD**		P value***
		Morning	Noon	
Air-water-syringe	Before flushing	217125.00±109669.73	241812.50±100816.14	0.330
	After flushing	134500.00±109699.58	165000.00±119754.19	0.517
Dental Drill	Before flushing	226000.00±103712.01	169750.00±134376.76	0.253
	After flushing	179916.66±130797.87	144416.66±133612.36	0.569
Water supply nozzle	-	204062.50±115708.52	177312.50±128511.85	0.462
Water tank	Inlet to tank	34625.00±64607.40	25375.00±28973.26	0.326
	Outlet to unit	52000.00±44754.14	73250.00±105823.43	0.597

*Colony-forming unit/milliliter; **Mean±Standard deviation; ***Wilcoxon W

Table 4: The differences in the number of heterotrophic bacteria before and after 30 seconds of flushing (regardless of the dental operational sections, day, and time of the sampling).

Sampling points	Condition	Colony-forming units (CFU/ml)*	
		M±SD**	P value***
Air-water-syringe	Before flushing	229468.75±104379.26	0.001
	After flushing	149750.00±114027.16	
Dental Drill	Before flushing	197875.00±120853.95	0.287
	After flushing	162166.66±130571.54	

*Colony-forming unit/milliliter; **Mean±Standard deviation; ***Paired Samples T-Test

Discussion

The results of this study indicate that the mean concentration of heterotrophic bacteria in all monitored sections exceeded the permissible limit of 500 colonies per milliliter, a threshold recommended by the American Dental Association as the bacteriological limit for water in dental units.¹³ This finding aligns with other similar studies that reported concentrations higher than the recommended limit.^{3,9,14} One potential source of water contamination in dental units is the lack of a non-retraction water system in handpieces, which can occur due to the malfunction of water return valves in dental devices.

Water lines and wastewater drainage systems are known to be prime locations for biofilm formation, which can harbor microorganisms. Most microorganisms found in the water used in dental units are waterborne and often opportunistic pathogens. These microorganisms pose a significant risk to patients, especially those with compromised immune systems, potentially leading to severe infections.^{15,16}

Moreover, the low velocity of water within the internal parts of water lines can promote bacterial colonization and biofilm formation. While this biofilm tends to remain adhered to the interior surfaces of the water lines, microorganisms released from the biofilm can enter the water stream. These microorganisms can then be introduced into a patient's mouth or aerosolized into the dental clinic's respirable air when handpieces are used. As a result, if bleeding occurs during dental procedures, pathogenic agents may penetrate the mouth's tissues and lead to infections.¹⁷ The risk of infection, however, depends on the individual's immune system, with the risk being higher for those with weakened immune systems or individuals who have undergone organ transplants.¹⁸

According to Table 1, comparing the mean and standard deviation of heterotrophic bacteria in water samples before flushing in different departments revealed that the highest levels of microbial contamination were observed in the surgical, restorative, endodontics, and pediatric departments, respectively. However, this difference was not statistically significant ($P>0.05$).

Based on the results in Table 2, the mean and standard deviation of heterotrophic bacteria were

higher on Saturday than on Wednesday in most sampling points and conditions, particularly in the inlet and outlet of the water tank. Although these differences were not statistically significant ($P>0.05$), the data highlighted the effect of stagnant water on the increase in bacterial contamination. This increase occurred due to the closure of dental departments for nearly 60 hours (from Wednesday to Saturday), which has been observed in other studies as well.^{9,19,20}

The effect of different times of the day was also investigated, and the results indicated no consistent or defined changes in bacterial contamination between morning and noon. Although these differences were not statistically significant ($P>0.05$), noticeable changes were observed in the bacterial contamination at the water tank outlet to the dental unit. In the morning, bacterial contamination was significantly higher than at noon due to the stagnant water that had remained in the tank for at least 14 hours (from 6:00 pm to 8:00 am). This could be attributed to the longer stagnation period. In contrast, another study reported that while there were no meaningful differences in contamination between morning and noon for dental handpieces, their contamination levels were consistently higher compared to the water source.²¹

A comparison of the colony-forming units (CFU/ml) of heterotrophic bacteria in the air-water syringe before and after flushing revealed that bacterial contamination before flushing (229,468.75±104,379.26 CFU/ml) was significantly higher than after flushing (149,750.00±114,027.16 CFU/ml) ($P<0.05$). This finding suggests that the flushing process, which involves discharging water at a high pressure for approximately 30 seconds, effectively reduces bacterial contamination by expelling some bacteria from the water lines.

In situations where the water flow rate is low in piping systems, there is no water flow for extended periods (e.g., at night), or when water pressure is insufficient, biofilm formation is common. The reduced water speed within the water lines allows bacteria to adhere and colonize. Water stagnation in dental units, especially when the unit is turned off for long periods, further promotes biofilm development.²² In the faculty clinics, units are typically shut down for over 15 hours, creating conditions favorable for biofilm formation. Biofilm is a complex, heterogeneous

microbial mass that develops on surfaces in contact with liquid. Therefore, it is recommended that appropriate disinfection protocols be implemented to minimize microbial concentrations.²³

The results of a study by Aghakochekezadeh et al. also indicated that bacterial contamination levels in water leaving the unit are high, and flushing significantly reduces this contamination.²⁴ Similarly, Khorakian et al. found that *Pseudomonas* bacteria decreased from 83.3% to 4.44% after flushing.²⁵ Furthermore, another study suggested that flushing, combined with chemical disinfection, is an effective physical technique to reduce microbial load in dental units.²⁶

Our study showed that the abundance of heterotrophic bacteria in water samples from the air-water syringe before flushing was highest in the endodontics, pediatric, restorative, and surgical departments, respectively. In general, the number of heterotrophic bacteria in water samples taken from the air-water syringe was significantly higher before flushing compared to after flushing.

Similar results were observed in the turbine water samples, where the average number of heterotrophic bacteria before flushing was higher than in the water samples after flushing. Several studies have demonstrated the effectiveness of flushing in reducing the microbial load in dental units.^{21, 27} Additionally, various studies have shown that extending the flushing time can further decrease the contamination levels in dental unit waterlines.^{28, 29}

Microbial analysis of the water samples entering the dental clinic tanks revealed that the concentration of heterotrophic bacteria was significantly higher than the 500 CFU/ml limit set for urban tap water by the World Health Organization. Studies indicate that while the water entering the dental unit typically has contamination levels of less than 100 CFU/ml, water emerging from dental equipment such as handpieces, turbines, and scaling devices can be heavily contaminated, with levels exceeding 100,000 CFU/ml, containing a variety of microorganisms.^{30, 31}

Comparing the bacterial counts before and after the water enters the tanker showed that bacterial contamination before the tanker was almost half that of the contamination levels observed after the tanker (Table 3). Additionally, the number of heterotrophic bacteria in the water supply nozzle was found to be significantly higher than the limit set for dental unit water.

Barbot et al.'s study showed that the water entering the dental unit becomes contaminated inside the unit, with pollution originating from biofilm attached to the inner surfaces of the waterlines.³²

This study was conducted in a faculty of dentistry, where water samples were taken from all parts of the

inlets, outlets, and storage containers related to the dental units. This comprehensive sampling can be considered a strength of the study, as it examined the microbial load of water at various points in the dental unit. However, a limitation of this study was the inability to investigate the effect of flushing duration on microbial contamination in dental handpieces due to the working conditions at the faculty. It is suggested that future studies consider this factor and explore the impact of varying flushing times on microbial contamination levels in dental handpieces.

Conclusion

The findings of this study revealed that the concentration of heterotrophic bacteria in both urban water samples (entering the faculty) and all the studied departments within the dental units exceeded the defined permissible limits. This poses a potential health risk, particularly for vulnerable populations, such as patients with compromised immune systems, pregnant women, children, and the elderly. To mitigate these risks, control measures such as disinfection of waterlines with a chlorine solution (100 to 500 mg/liter), daily draining of the water tank, the use of activated carbon filters, on-site water purification systems, and flushing waterlines for several minutes before starting work are recommended. Future studies should also focus on evaluating disinfectant solutions for the dental unit piping system and their effectiveness in reducing bacterial load.

Authors' Contribution

HH and NE conceptualized the study. AZ and NE collected the data. HH, HR, AS, MS, and AR performed data analysis. HH, AS, and NE reviewed the analysis, wrote the manuscript, and edited it. All authors read and approved the final manuscript.

Acknowledgment

This research was sponsored by Golestan University of Medical Sciences (Grant No. 112891). The authors would like to express their sincere appreciation for the university's cooperation, especially the faculty of dentistry, in providing the necessary data.

Funding

This research was sponsored by Golestan University of Medical Sciences (Grant No. 112891).

Ethics Approval

This research was approved by the Ethics Committee (the approval ethics number: IR.GOUMS.REC.1401.197).

Conflict of Interest: None declared.

References

- 1 Szymańska J, Sitkowska J. Bacterial contamination of dental unit waterlines. *Environ Monit Assess.* 2013;185:3603-11. doi: 10.1007/s10661-012-2812-9. PMID: 22899458. PMCID: PMC3613572.
- 2 Błaszczyk B, Pajęzkowska M, Nowicka J, Szymonowicz M, Zakrzewski W, Luboński A, et al. Microbiological Evaluation of Water Used in Dental Units. *Water.* 2022;14(6):915. doi: 10.3390/w14060915.
- 3 Ji X-Y, Fei C-N, Zhang Y, Liu J, Liu H, Song J. Three key factors influencing the bacterial contamination of dental unit waterlines: a 6-year survey from 2012 to 2017. *Int. Dent. J.* 2019;69(3):192-9. PMID: 30565215. PMCID: PMC9379084. doi: 10.1111/idj.12456.
- 4 Some S, Mondal R, Mitra D, Jain D, Verma D, Das S. Microbial pollution of water with special reference to coliform bacteria and their nexus with environment. *Energy Nexus.* 2021;1:100008. doi: 10.1016/j.nexus.2021.100008.
- 5 Castellano Realpe OJ, Gutiérrez JC, Sierra DA, Pazmino Martinez LA, Prado Palacios YY, Echeverría G, et al. Dental Unit Waterlines in Quito and Caracas contaminated with Nontuberculous Mycobacteria: A potential health risk in dental practice. *Int J Environ Res Public Health.* 2020; 17(7):2348. PMID: 32244267. PMCID: PMC7177417. doi: 10.3390/ijerph17072348.
- 6 Hikal W, Kačaniová M, Hussein DE, Ghit A, Smaoui S, Aleem M, et al. Dental unit waterlines and health risks of pathogenic microbial contamination: An update review. *J. Biol. Studies.* 2023;6(4):282–98. doi: 10.62400/jbs.v6i4.8729.
- 7 Igboke H, Bhattacharyya S, Gradus S, Khubbar M, Griswold D, Navidad J, et al. Preponderance of toxigenic *Escherichia coli* in stool pathogens correlates with toxin detection in accessible drinking-water sources. *Epidemiol & Infect.* 2015;143(3):494-504. PMID: 24787554. PMCID: PMC9507053. doi: 10.1017/S0950268814001046.
- 8 Miller CH, Palenik CJ. Infection Control and Management of Hazardous Materials for the Dental Team-E-Book: Elsevier Health Sciences; 7TH Edition, 2016. eBook ISBN: 9780323764933.
- 9 Hajisadeghi S, Aghaali M, Aghighi Hatamipour S, Khalifeh-Gholi M. Evaluation of Bacterial Contamination Level of Waterlines in Dental Units in Dentistry School of Qom University of Medical Sciences in 2018. *Qom Uni Med Sci J.* 2020;14(8):30-8. doi: 10.29252/qums.14.8.30.
- 10 Honarmand M, Shahraki S, Farhad-Mollashahi L, Gholipour R, Ghaedi M. Evaluation of bacterial contamination of water supply in dental unit water lines at Zahedan dental school 2008. *Zahedan J Res Med Sci.* 2010;11(4). doi: 10.1234/knh.v11i1.1132.
- 11 Malakootian M, Nowroozi H. Gram-positive cocci contamination in dental unit water lines at Kerman dental school during 2015. *Kaums J (FEYZ).* 2017;21(3):280-5. URL: <http://feyz.kaums.ac.ir/article-1-3024-en.html>.
- 12 Mazloomi S, Nourmoradi H, Abbasi M, Eslami A, Kazembeigi F, Nikzad N, et al. Survey on the Water Quality of Dentistry Units in Ilam City by Heterotrophic Plate Count (HPC). *Journal of Occupational and Environmental Health.* 2017;3(2):112-9. URL: <http://newresearch.medilam.ac.ir/article-1-252-en.html>.
- 13 Allen MJ, Edberg SC. Health concerns of heterotrophic plate count (HPC) bacteria in dental equipment water lines. *Am J Dent.* 2016;29(3):137-8. PMID: 27505988.
- 14 Bayani M, Raisolvaezin K, Almasi-Hashiani A, Mirhoseini SH. Bacterial biofilm prevalence in dental unit waterlines: a systematic review and meta-analysis. *BMC Oral Health.* 2023;23(1):1-14. PMID: 36934281. PMCID: PMC10024400. doi: 10.1186/s12903-023-02885-4.
- 15 Amirabadi F, Dehghan HJ, Bazrafshan E, Forghani F. Evaluation of Bacterial Contamination in Dental Unit Water Lines in Zahedan in 2016-2017. *Jmsthums* 2019; 7 (2) :44-52. URL: <http://jms.thums.ac.ir/article-1-658-en.html>.
- 16 Hoseini MZ, Naghmachi M, Zirakfard F, Raiganshirazi A, Rezaei S, Yosefi S, et al. A study on microbial quality of water used in the dentistry units. *Armaghanj.* 2014; 19 (8) :707-716 URL: <http://armaghanj.yums.ac.ir/article-1-134-en.html>.
- 17 Olewi R. Bacterial contamination of dental unit water lines (DUWL) in Baghdad city. *IOSR J Dent Med Sci.* 2017;16:47-50. doi: 10.9790/0853-1608034750.
- 18 Uzel A, Cogulu D, Oncag O. Microbiological evaluation and antibiotic susceptibility of dental unit water systems in general dental practice. *Int J Dent Hyg.* 2008;6(1):43-7. PMID: 18205653. doi: 10.1111/j.1601-5037.2007.00269.x.
- 19 Malakootian M, Molazadeh P, Eskandarizadeh A. Microbial and Fungal Contamination of Air at Dental Clinics and Dental Offices in Kerman. *TB.* 2015;13(5). URL: <http://tbj.ssu.ac.ir/article-1-1607-en.html>.
- 20 Memarian M, Fazeli M, Jamalifar H, Karami S. Microbial evaluation of dental units waterlines at the department of operative dentistry, Tehran university of medical sciences in the year 2006. *Jdm.* 2008;21(1):65-71. URL: <http://jdm.tums.ac.ir/article-1-197-en.html>.
- 21 Szymanska J. Control methods of the microbial water quality in dental unit waterlines. *Ann Agric Environ Med.* 2003;10(1):1-4. PMID: 12852726.
- 22 O'Donnell MJ, Boyle MA, Russell RJ, Coleman DC. Management of dental unit waterline biofilms in the 21st century. *Future microbiol.* 2011; 6(10):1209-26. PMID: 22004039. doi: 10.2217/fmb.11.104.
- 23 Pepper I, Gerba CP, Gentry T, Maier RM. Environmental microbiology: Academic press; 2nd Edition, 2008. ISBN-13 : 978-0123705198.

- 24 Aghakouchakzadeh A, Mohamadzadeh M, Hamian M, Rahim Zadeh M, Esmail Shrazi S. Evaluating Bacterial Contamination of Water Supply in Dental Units at Alborz University of Medical Science Using Microbial Culture and Colony Count in 2019. *Aumj* 2020;9(4):31-8. URL: <http://aums.abzums.ac.ir/article-1-1171-en.html>.
- 25 Khorakian F, Movahhed T, Karbasi S, Nezafat ST, Bahramian L. Frequency evaluation of *Pseudomonas* SPP contamination in the dental unit water line system at Mashhad dental school. *J Res Dent Sci* 2019; 16 (3) :201-209 URL: <http://jrds.ir/article-1-1061-en.html>.
- 26 Baudet A, Lizon J, Martrette J-M, Camelot F, Florentin A, Clément C. Dental unit waterlines: a survey of practices in Eastern France. *Int J Environ Res Public Health*. 2019; 16(21):4242. PMID: 31683783. PMCID: PMC6862618. doi: 10.3390/ijerph16214242.
- 27 Cobb CM, Martel CR, McKnight III SA, Pasley□ Mowry C, Ferguson BL, Williams K. How Does Time□Dependent Dental Unit Waterline Flushing Affect Planktonic Bacteria Levels? *J Dent Educ*. 2002 Apr; 66(4):549-55. PMID: 12017153.
- 28 Teixeira R. Water-quality of Westbrabantse dental units and the effect of flushing. *Ned Tijdschr Tandheelkd*. 2002 Aug;109(8):307-11. Dutch. PMID: 12212457.
- 29 Ghasempour M, Ghobadi Nejad M, Haji Ahmadi M, Shakki H. Microbiological evaluation of dental unit water at dental offices and dental school in the city of Babol. *Jmds*. 2005; 29(1, 2):97-104. doi: 10.22038/jmds.2005.1512.
- 30 Nourmoradi H. Studying Microbial quality of dental unit water in Ilam town with Heterotrophic plate count method. *Tech Res Info System*. 2018;1(2). URL: <http://newresearch.medilam.ac.ir/article-1-252-en.html>.
- 31 Hoogenkamp MA, Brandt BW, Laheij AM, de Soet JJ, Crielaard W. The microbiological load and microbiome of the Dutch dental unit; ‘please, hold your breath’. *Water Res*. 2021; 200:117205. doi: 10.1016/j.watres.2021.117205. PMID: 34058484.
- 32 Barbot V, Robert A, Rodier M-H, Imbert C. Update on infectious risks associated with dental unit waterlines. *FEMS Immunol Med Microbiol*. 2012 Jul;65(2):196-204. doi: 10.1111/j.1574-695X.2012.00971.x. PMID: 22469485.