

## Investigation of the Bacterial Contamination of Dental Unit Waterlines and the Effectiveness of Flushing on the Contamination Level in Rafsanjan, Southeastern Iran

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### ABSTRACT

**Introduction:** Dental unit water lines (DUWLs) are potential sources of microbial contamination that threaten patients and dental personnel. This study aimed to determine the bacterial quantity and quality of DUWLs in Dental School in Rafsanjan and to determine the effect of flushing on the contamination rate.

**Materials and Methods:** In this cross-sectional study, 124 water samples were collected from 20 active units in five departments (endodontics, periodontics, pediatrics, prosthetics, and restorations) at Rafsanjan Dental School (July 2024). Sampling was carried out using a standard method from the water inlet and the connection point of the turbine and scaler in three stages: before work, after 30-second flushing, and after work. To identify the bacterial contamination load, heterotrophic plate counting (HPC), Gram staining, and standard biochemical tests for each bacterial species were used. Kruskal-Wallis and Mann-Whitney tests were used to analyze the data.

**Results:** The results showed that the contamination level in 53.2% of the samples was above the permissible limit. The highest contamination level was observed in the periodontics department (890 CFU/mL), and the lowest was in the endodontics department (380 CFU/mL). 30-second flushing significantly reduced contamination and the number of bacteria ( $p \leq 0.001$ ), while the difference between contamination levels in different departments and components of the dental units was not significant ( $p > 0.05$ ).

**Conclusion:** Given the contamination of DUWLs, continuous water disinfection, cleaning of water lines, and flushing before and during work in dental units are recommended.

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### Introduction

Dental unit waterlines (DUWLs) are essential components of dental treatment systems that supply the water required to operate several dental

instruments and devices, including turbines, air and water pumps, and ultrasonic scalers <sup>1</sup>. The structure of DUWLs leads to the rapid formation and growth of biofilms <sup>2</sup>. The average water temperature in

DUWLs is in the range of 20 to 30 °C, which is suitable for the growth and formation of biofilms<sup>2</sup>. However, the long-term residence of water in DUWLs causes the formation of biofilms, and the contamination of the water outlet from the dental unit originates from the biofilm attached to the walls of its pipes<sup>3</sup>. In addition, contamination of DUWLs can be caused by saliva reflux from the patient's oral cavity, which occurs when the equipment creates negative pressure<sup>4</sup>.

Contact of patients and staff with contaminated water or aerosols through the digestive tract and the entry of aerosols into the respiratory system through the air can lead to infection, especially in immunocompromised individuals, children, and the elderly<sup>5, 6</sup>. Studies have reported the prevalence of various microbial contaminants, including bacteria, fungi, and protozoa, in DUWLs<sup>1, 3</sup>. However, the most common contaminations are related to various bacterial species. Studies have reported the presence of heterotrophic pathogenic bacteria, such as *Staphylococcus aureus*, *Legionella*, *Pseudomonas*, especially *Pseudomonas aeruginosa*, and *Escherichia coli* in DUWL systems; in many cases, the concentrations of these contaminants were higher than the permissible limit set by the American Dental Association (ADA), which is 500 colony-forming units per milliliter (CFU/mL)<sup>7-9</sup>.

High concentrations of *Pseudomonas aeruginosa* have been implicated in pulmonary infections in patients with cystic fibrosis, and a dentist reportedly died of pneumonia after exposure to contaminated dental unit water<sup>10</sup>. Indeed, bacteria transmitted through aerosols can cause illnesses such as influenza and the common cold, as well as respiratory diseases such as tuberculosis and Legionnaires' disease<sup>6, 11</sup>. Studies have shown that Infections caused by *Mycobacterium abscessus* in 95 children (24 and 71 children) in two pediatric dental clinics in the United States were directly linked to contaminated water from dental units used for pulpotomy<sup>12-14</sup>. Also, two deaths in elderly patients, one in Italy and the other in Sweden, have been reported after exposure to water contaminated with *L. pneumophila* in DUWLs<sup>12, 15, 16</sup>. Studies conducted in Iran have also reported varying

degrees of bacterial contamination in dental units. In the study by Yazdanbakhsh et al., the bacterial contamination rate of water in Shahrood dental units was reported to be 64%<sup>17</sup>, and in the study by Ghaem Maghami et al., the bacterial contamination rate of water in Shahid Beheshti dental units was reported to be 50%<sup>18</sup>.

Epidemiological research in different countries and cities has yielded different results, and it seems necessary to conduct extensive and comprehensive research in this field to determine the causes and factors of contamination and methods of their control and elimination. Therefore, considering that water contamination in dental units can pose risks to the health of patients, personnel, and dentists, and considering the necessity of conducting these studies in all parts of the world with the aim of updating information in this field in order to determine the causes and factors of contamination and methods of their control and elimination, and also with the aim of providing better and more effective treatments, this study was conducted to evaluate the level of bacterial contamination of the DUWLs at the Rafsanjan Dental School and determine the effectiveness of the flushing method in reducing microbial contamination.

## Materials and Methods

### Sampling

This descriptive, cross-sectional study was conducted in July 2024 at the School of Dentistry, Rafsanjan University of Medical Sciences. A total of 124 water samples were collected from 20 active units in five departments: endodontics, periodontics, pediatrics, prosthetics, and restorative dentistry. Samples were collected from three areas of each unit: the air and water pump, turbine connection, and ultrasonic scaler. Sampling was performed in three-time steps: before starting work, after finishing work, and after performing a 30-second flushing. Four municipal water samples were collected for the control group. Sampling was carried out according to the standard for microbial water sampling, and samples were collected aseptically in sterile containers and immediately

transferred to the microbiology laboratory<sup>19</sup>. All samples were transported to the laboratory and tested within two hours of collection.

### Microbial Analysis

Heterotrophic plate counting (HPC) or standard colony counting was used to determine the number of bacterial colonies. Samples were plated on nutrient agar using the pour plate method and incubated for 48 h at 35–37°C. The results are reported as CFU/mL. To identify the bacterial species, the colonies were subjected to Gram staining and biochemical tests, including IMViC, catalase, coagulase, DNase, and oxidase. A catalase test was performed to detect colonies suspected to be gram-positive *cocci*. The catalase-positive and catalase-negative bacteria were *staphylococci* and *streptococci*, respectively. In catalase-positive cases, deoxyribonuclease (DNase) and coagulase tests were used to diagnose *Staphylococcus aureus*. The Novobiocin test was used to distinguish *Staphylococcus epidermidis* from *Staphylococcus saprophyticus*. In the case of gram-negative *bacilli*, the oxidase test is used to differentiate *Pseudomonas* from *Enterobacteriaceae*, which shows a green pigment. The IMViC test was also used to differentiate between different bacteria in the *Enterobacteriaceae* family. The IMViC test is a

set of four different biochemical tests, including the indole, Methyl Red, Voges-Proskauer, and Citrate Utilization tests, which are used to identify and differentiate bacteria, especially members of the *Enterobacteriaceae* family, and the samples were tested according to the standard method<sup>20</sup>.

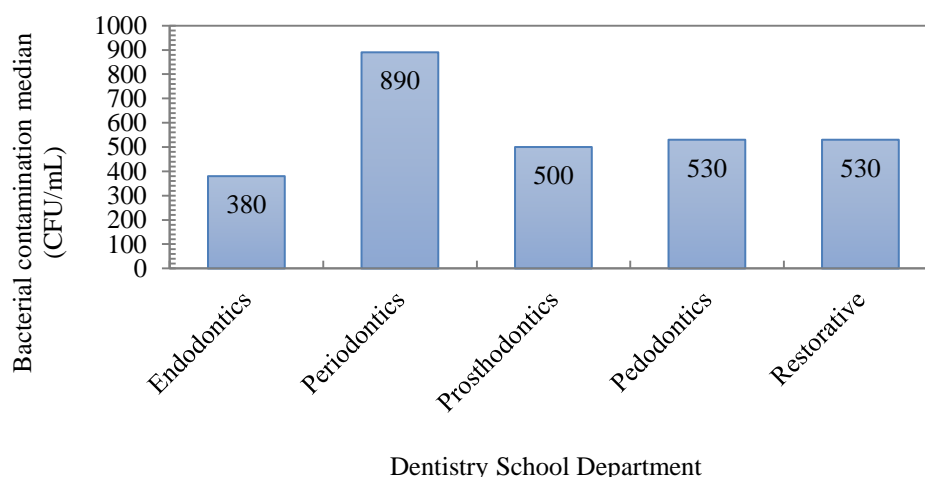
### Statistical analysis

Data were analyzed using SPSS version 22. Descriptive statistics, including median, interquartile range, and frequency, were calculated. Mann-Whitney U and Kruskal-Wallis tests were used to compare the groups. The level of statistical significance was set at  $< 0.05$ .

## Results

### Bacterial concentration in DUWLs

The median bacterial contamination (CFU/mL) in the different departments is shown in Figure 1. The highest contamination was observed in the periodontics department (890 CFU/mL), and the lowest was in the endodontics department (380 CFU/mL). The median values were for the prosthetic, restorative (530 CFU/mL), and pediatric (500 CFU/mL) departments. However, the Kruskal-Wallis test did not show a significant difference between the contamination of the departments ( $p = 0.736$ ).



**Figure 1:** Bacterial contamination load of dental units in different departments of the dentistry school.

The median bacterial contamination (CFU/mL) in different parts of the unit, divided into different departments of the School of Dentistry, is presented in Table 1. Accordingly, the highest level of contamination was related to the scaler in the perioperative department, and the lowest level

of contamination was related to the turbine in the pediatric department. Based on the statistical results of the Kruskal-Wallis test, there was no significant difference between the levels of contamination in different parts of the unit, divided into different departments ( $P > 0.05$ )

**Table 1:** Median and interquartile range of *heterotrophic* bacteria in different parts of the units in dentistry departments (n= 120)

Dentistry departments	Units	Median (CFU/mL)	Interquartile range (CFU/mL)	P-value
Endodontics	Air-water syringe	430	320-1952.5	0.707
	Air turbine handpiece	510	65.5-1975	
Periodontics	Air-water syringe	780	290-1657.5	0.285
	Air turbine handpiece	995	383.75-3950	
Pedodontics	Air-water syringe	895	130-3049	0.401
	Air turbine handpiece	400	222.5-1882.5	
Prosthodontics	Air-water syringe	740	382.5-1050	0.885
	Air turbine handpiece	527.5	410-3982.5	
Restorative	Air-water syringe	417.5	335-830	0.236
	Air turbine handpiece	895	382.5-985	

### Microbial species

Microscopic and biochemical analyses revealed the presence of various bacterial species. The prevalence of various microorganisms in the collected samples and the prevalence of each

microorganism in different sections are presented in Table 2. The highest prevalence was related to gram-positive bacilli (69.2%), and the lowest prevalence was related to *Streptococcus* (5.8%) and *coliforms* (5.8%).

**Table 2:** Percentage prevalence of identified bacterial types in different departments of the School of Dentistry (n=120)

Microorganisms	Number of Contaminated Samples (%)	Faculty departments					P-value
		Restorative	Prosthodontics	Pedodontics	Periodontics	Endodontics	
<i>Gram-positive bacillus</i>	83(69.2)	22.9%	22.9%	19.3%	14.5%	20.5%	0.166
<i>Micrococcus</i>	32(26.7)	12.5%	18.8%	21.9%	25%	21.9%	0.743
<i>Staph aureus</i>	23(19.2)	26.1%	34.8%	13%	13%	13%	0.223
<i>Staph epidermidis</i>	17(14.2)	23.5%	23.5%	11.8%	11.8%	29.4%	0.651
<i>Pseudomonas aeruginosa</i>	12(10)	33.3%	33.3%	0%	16.7%	16.7%	0.269
<i>Streptococcus</i>	7(5.8)	28.6%	14.3%	42.9%	14.3%	0%	0.414
<i>Coliforms</i>	7(5.8)	28.6%	14.3%	42.9%	14.3%	0%	0.414

### Effect of Flushing

The median bacterial contamination (CFU/mL) by the flushing procedure is presented in Table 3. The contamination level decreased after flushing, and according to the Mann-Whitney test, there was a significant difference between the contamination levels of the samples before and after flushing ( $p \leq$

0.001). After 30 s of flushing, a significant decrease in contamination was observed; the median CFU decreased from 905 to 350, which was statistically significant ( $p \leq 0.001$ ). In contrast, the difference between the samples before and after daily clinical work was not significant ( $p = 0.152$ ). The contamination level of the samples

after work was lower than that of the samples before work, but based on the statistical results of the Mann-Whitney test, there was no significant

difference between the contamination level of the samples before and after work ( $p=0.152$ )

**Table 3:** Heterotrophic bacteria prevalence in DUWLs according to flushing procedure

Sampling time	Number of samples	Median (CFU/mL)	Interquartile range (CFU/mL)	p-value
Before work	40	905	612.5-2300	-
After 30 seconds of flushing	40	350	6-530	< 0.001
After work	40	685	395-2000	0.152

Table 4 shows how many samples had contamination higher than the ADA standard and how many had contamination lower than the ADA standard at different sampling times. Based on the results, 77.5% of the initial samples and those before flushing had contamination levels higher than the ADA standard, which decreased to 30% in the samples taken after flushing. In addition,

57.5% of the samples taken after work had contamination levels higher than the standard. Based on the statistical results of the Chi-Square test at different sampling times, there was a significant difference between the number of samples with contamination higher than the ADA standard and those with contamination lower than the ADA standard ( $p \leq 0.001$ ).

**Table 4:** Contamination levels of DUWLs from Dentistry school at different times according to ADA standards

Sampling time		> 500 CFU/mL	≤ 500 CFU/mL	p-value
Before work	Number	31	9	< 0.001
	Percent	77.5%	22.5%	
After 30 seconds of flushing	Number	12	28	
	Percent	30%	70%	
After work	Number	23	17	
	Percent	57.5%	42.5%	

Table 5 compares the contamination levels of unit water samples in different departments of the dental school according to ADA standards. The results showed that overall, 53.2% of the samples

had contamination levels above the permissible limit (500 CFU/mL), most of which were related to the periodontics and prosthetics departments.

**Table 5:** Comparison of contamination levels of unit water samples in different sections according to ADA standards

Dentistry departments		> 500 cfu/mL	≤ 500 cfu/mL	p-value
Endodontics	Number	11	14	0.634
	Percent	44%	56%	
Periodontics	Number	16	9	
	Percent	64%	36%	
Pedodontics	Number	12	13	
	Percent	48%	52%	
Prosthodontics	Number	14	10	
	Percent	58.3%	41.7%	
Restorative	Number	13	12	
	Percent	52%	48%	
Total	Number	66	58	
	Percent	53.2%	48.8%	



## Discussion

### Dental Unit Water Contamination Rate

This study showed that the contamination level in DUWLs at the Rafsanjan dental school was above the permissible limit set by the American Dental Association (500 CFU/mL) in 53.2% of the samples. These findings are consistent with the results of similar studies in Iran and other countries, especially in areas where DUWLs are not properly maintained and disinfected<sup>1,11</sup>. In the study by Buitrago et al., the rate of dental unit water contamination was reported to be 21%<sup>21</sup>, which was lower than that in the present study. Yazdanbakhsh et al. reported bacterial contamination rate of Shahrood dental school to be 64%<sup>17</sup>, which was higher than that in our study. Ghaem Maghami et al., reported the water contamination of Shahid Beheshti dental school to be 50%<sup>18</sup>. Studies have shown that the duration of use of the dental units (years of use) increases the thickness of the biofilm layer and, as a result, increases the level of contamination<sup>22</sup>. Water contamination in dental units can be due to two main reasons: the microbial flora of the patients' mouths, which can enter the unit's water supply system due to the suction effect and return of the patient's saliva (backflow) through suction or the turbine head duct, and the stable microbial environment deposited in the unit's water pipes, or the biofilm, which acts as a potential source of contamination<sup>23</sup>.

The results of this study showed that the highest level of contamination was observed in the periodontal department. In the study by Blaszczyk et al., the highest level of contamination was in the perioral section, which was consistent with our study<sup>24</sup>. In the study by Hajisadeghi et al., the lowest level of contamination was in the perioral section, which was inconsistent with our study and could be related to the level of operation of the units or the condition of the municipal water piping<sup>25</sup>. According to previous studies, the longer the dental unit is inactive and the more water remains in the unit lines or pipes, the higher the contamination level of the outlet water. Therefore, it can be said that in our study, the reason for the

higher contamination level in the periodontal department could be related to the less active units and less use of power in this department, as well as the use of manual scalers by lower-entry students and the unused scalers during that period of time<sup>26,27</sup>. In this study, the lowest contamination level was recorded in the endodontics department, which may be due to the continuous use of units and better adherence to the hygiene protocols.

The results of this study showed that the highest contamination rate was related to the scaler part, and the lowest contamination rate was related to the turbine part of the units. In the study by Abbasi et al., the highest contamination rate was related to the scaler, which was consistent with our study<sup>23</sup>. In the study by Aghakochekezadeh et al., the highest contamination rate was related to the turbine, which was inconsistent with our study and could be related to a different statistical population<sup>28</sup>.

### Bacterial species

The most common bacterial species grown in our study were gram-positive bacilli (diphtheroid, spore-forming), *Micrococcus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Streptococcus*, and coliforms. In the study by Abbasi et al., the most commonly reported species were gram-positive bacilli, which is consistent with our results<sup>23</sup>. In the study by Aghakochekezadeh et al., the species found in order of prevalence were *Escherichia coli*, *Klebsiella*, non-pathogenic *Staphylococcus*, and *Micrococcus*<sup>28</sup>. Moradania et al. reported the presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and coliforms in the unit water<sup>29</sup>. In our study, *Escherichia coli* was not detected in any of the samples, although species in the coliform group were detected, all of which were non-intestinal or non-fecal. These species can also be commonly identified on surfaces, skin, and saliva<sup>29</sup>. In addition, the presence of microorganisms such as *Staphylococcus epidermidis* can indicate contamination due to the return of patient saliva through suction or the turbine head duct into the unit water duct<sup>30</sup>.

In addition, identification of bacterial species indicated the presence of potentially pathogenic microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *coliforms*, which can pose a high health risk to patients with weakened immune systems <sup>26</sup>.

### The effect of flushing

A key finding of this study was the significant reduction in water contamination after a 30-second flush. This result is consistent with the recommendations of the ADA and the Centers for Disease Control (CDC) <sup>31</sup>. Although flushing cannot completely remove biofilms, it plays an important role in reducing the microbial load as a simple, low-cost, and immediate method of intervention. The results of this study also showed that there was no statistically significant difference between samples before and after daily work; this indicates that daily activities alone do not increase contamination, but that there is already a baseline contamination <sup>32</sup>.

This study showed that flushing at the beginning of the day and before starting work is significantly effective in reducing water contamination levels. This emphasizes the implementation of the American Dental Association guidelines, which require flushing before starting the daily work of the unit, between two patients, and after completing the daily work. The studies by Hosseini Mehraban et al., <sup>30</sup>, Aghakouchakzadeh et al., <sup>28</sup>, Hajisadeghi et al., <sup>25</sup>, and Khondian et al. <sup>33</sup> are also consistent with the present study.

In the present study, 77.5% of the initial samples (before flushing) had contamination higher than the ADA standard, and this contamination decreased to 30% in the samples taken after flushing. According to the results obtained in this and other existing studies, flushing seems to be the best and most practical method for contamination control. However, it should be noted that this is not a perfect method because it cannot remove biofilms attached to the walls of the water path, which requires more detailed and complete studies in this field. In this study, bacterial counts in samples taken after work showed that the level of

contamination decreased compared to samples taken before work. The high level of contamination before work could be due to the stagnation of water in the unit pipes at the beginning of the working day, and the reason for the decrease in microbial load after work could be due to the unit being active and the water being circulated <sup>34</sup>. In the study by Abbasi et al., the level of contamination in samples collected at the beginning of the workday was higher than that in samples taken after work <sup>23</sup>, which is consistent with the present study.

According to the ADA guidelines for controlling dental unit water contamination, and the Centers for Disease Control and Prevention (CDC), the bacterial concentration of dental unit water used in nonsurgical procedures should be less than or equal to 500 CFU/mL <sup>31</sup>. Sterile saline or sterile water should be used as a coolant and rinsed during surgical procedures <sup>21</sup>. The ADA recommends using stored water that is not connected to city water, cleaning air and water outlets daily, using chemical compounds to remove microbes from water, using special filters to control dental unit water, and flushing for 30 s before starting work to control and limit contamination <sup>31</sup>. In a study conducted by Pankhurst et al., the results showed that installing a valve that prevents fluid from flowing back from the patient's mouth into the unit's water system reduces contamination <sup>35</sup>. In a study by Berlutti et al. on the effect of an anti-retraction device on preventing microbial contamination of dental unit water lines, they concluded that even installing an anti-retraction device did not prevent fluid from flowing back from the patient's mouth into the unit's water system in 74% of cases when the turbine stopped moving, resulting in cross-infection between patients <sup>36</sup>.

### Conclusion

In this study, the bacterial contamination of the DUWLs in Rafsanjan Dental School exceeded the acceptable standard in 53.2% of the samples. The presence of pathogenic microorganisms, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*,

and *coliforms*, indicates a potential risk of infection transmission in the dental environment. In the present study, the results before and after the flushing procedure showed a significant reduction in the microbial load in all devices, which is also recommended by the ADA as one of the obvious factors in reducing contamination before starting work. Therefore, flushing before work is recommended as a reliable and accessible method for all departments to reduce the microbial load. In addition, flushing alone cannot completely remove biofilms located in pipes. As a result, the use of complementary methods such as the use of non-return valves, periodic disinfection with chemicals, and the installation of continuous disinfection systems is recommended.

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### Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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### Ethical Considerations

This study approved by Rafsanjan University of Medical Sciences with code 4030027 and ethical code IR.RUMS.REC.1403.087.

### Code of Ethics

IR.RUMS.REC.1403.087.

### Authors' Contributions

All authors contributed to the study conception and design. Data collection were performed by Amirreza Isaei, and data analysis were performed by Somayeh Salari-Sedigh, Farimah Sardari, Hadi Eslami and Parvin Khalili. The first draft written and revision of the manuscript were performed by

Amirreza Isaei and Hadi Eslami. All authors read and approved the final manuscript.

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