# Evaluation of the Bacteriological Quality of Dental Unit's Waterlines, Tehran, Iran

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## ABSTRACT
Oral and dentistry (O&D) services processes may lead to exposing of personnel and patients with several microorganisms and arising of health problems. This cross-sectional study was investigated the bacteriological quality of dental unit waterlines (DUWLs) in one of the O&D center in Tehran, Iran. One hundred ninety two samples were collected and examined based on standard microbiological procedures for determining and enumeration of heterotrophic plate count (HPC), *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Data were analyzed by t-test, analysis of variance (ANOVA), Kruskal-Wallis and LSD tests with SPSS software (Ver.16). The results revealed that 70% of water samples (126 samples) had a high density of contaminations that higher than recommended values for DUWLs quality. The mean of bacterial density on Saturday was more than Wednesday and was 1838 CFU/ml, 739 CFU/ml and 11 CFU/ml for HPC, *P. aeruginosa*, and *S.aureuse* respectively. The LSD test implied that the mean of bacterial density of inlet and outlet waters had significant statistical difference in various wards of the O&D center ($p < 0.05$). In addition, the results demonstrated that bacteriological quality in discharging water of various wards was higher than the recommended values. This research revealed that microbial water quality assessment in O&D services centers should be considered for providing of an appropriate disinfection procedure from point of infection control in dental operation services.

**Key words:** Dental Center, Water Bacteriological Quality, Dental Unit Waterlines, Heterotrophic Plate Count, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

## INTRODUCTION
Station and none appropriate disinfection of water in dental units waterlines (DUWLs) can lead to biofilm development and depletion of microbiological water quality; hence, exposure of service provider personnel and patients with various types of pathogen and opportunistic bacteria including *Legionella pneumophila, F species, Klebsiella* species and *Pseudomonas aeruginosa* is a crucial health problems, especially in immunosuppressive people.[1–7]. DUWL's biofilm are formed and developed by water aging in the weekend, provided the condition for growth and reproduction of a variety of bacteria available in water [8–9]. Many of dental therapeutic operations lead to producing of bioaerosols, which have infection potential [10–11]. All dental services, which produce bioaerosols, can create disease risk for the exposed population, including employees, immunodeficiency patients, people with chronic diseases and those dealing with corticosteroid drugs and body immune amplifier [12]. The bioaerosols are contain pathogenic microorganisms may be originated from drain water of turbine duct, saliva and blood of patients' mouth [13–15]. Also, the bioaerosols may be contagious diseases of respiratory tract and allergies [16], several research showed the amount of microbial contamination in tap water and water system of dental units was 51200 CFU/ml and 872000CFU/ml, respectively and *Pseudomonas aeruginosa* was found in 2.38% of tap water samples and in 20.06% of DUWS samples; *Legionella spp.* was found in 29.96% of tap water samples and 15.82% of DUWS samples respectively [17].

Szymańska *et al.* reported the amount of *Pseudomonas aeruginosa* contamination in the water system of dental units 20 CFU/ml [18]. Anderson *et al.* showed heterotrophic plate count (HPC) of the top of turbine soaked in patient saliva was 5×105 CFU/ml [19]. Messano *et al.* showed 87.7% of units' using low quality of water [20]; so monitoring and determining of microbial contamination in DUWLs is required for the provision of a suitable health index in O&D services centers [21]. Therefore, this study was

investigated bacteriological quality of DUWLs in one of the largest dentistry centers in Tehran, Iran.

## MATERIALS AND METHODS

This cross-sectional study was performed in a large dentistry center in Tehran in 2013.

### *Water sampling*

One hundred ninety two samples were collected via grab sampling procedure and examined based on standard microbiological procedures for determining and enumeration of heterotrophic plate count (HPC), *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Water samples (50 ml) from DUWLs for microbial tests were collected onto sterile glass bottles that washed and rinsed with distilled water; dechlorination was done by 3% sodium thiosulfate. The water temperature and residual chlorine of samples were measured by a mobile thermometer and a standard kit at sampling points [22].

Sampling was done from two dentistry units in four selected wards, including prosthodontics, restoration and periodontal surgery and endodontics on Saturdays and Wednesdays. Random selection of units was done from above wards. Sampling were taken from four different parts of the unit, including water before entering the unit, glass filling, turbine air/water syringe and duct. Sampling was repeated in three weeks and total samples were 192. The method for selecting 192 samples is as below:

(2*4*2*4)*3= 192

(Two day * four selected wards * two unit * four part of unit) * three week sampling = 192

Samples were kept at a temperature below 4oC and transported to the laboratory in a cold box for bacteriological test which performed as soon as possible.

### *Sample processing*

After preparation of equipment and required test conditions, bacteriological quality of DUWL was done with enumeration and identity of index bacteria, based on standard microbiological procedures. All experiments were conducted and samples taken according to the standard methods for water and wastewater examinations of the American Public Health Association [23]. All of microbial tests were done by using the culture medium manufactured by Merck Co, Germany.

1. Spread plate method was used for HPC experiments with using plate count agar culture medium (tryptone glucose yeast agar)

2. In order to count *Staphylococcus aureus*, first mannitol salt agar culture medium (MSA) was prepared (9213D) and used in experiments. In order to ensure that grown colonies are *S. aureus* the required controls such as observing yellow color resulted from mannitol fermentation on culture medium, gram staining and observing cluster gram-positive cocci, coagulase, catalase and DNAase tests and oxidase test was done [23].

To count *Pseudomonas aeruginosa*, p-agar culture medium (Pseudomonas Agar), was prepared (9213F). In order to ensure that grown colonies on the surface of mentioned culture medium are *Pseudomonas aeruginosa*, the required controls such as gram staining and observing gram-negative bacilli, oxidase and catalase tests and examining pigment on the culture medium were performed [23].

3. Bacteriological tests were done to count HPC, *staphylococcus aureus* and *pseudomonas aeruginosa*, with 1ml culture of the sample on each culture medium for plate count agar, mannitol salt agar and p-agar, respectively. Then above culture mediums were put under heat at $37^{\circ C}$ for 24-48h. After that, the number of growing colonies on the plates was counted definitively and the results were reported as CFU/ml. In order to compare and classify bacteriological quality of water samples, American Dental Association (ADA) recommendation was used that determined HPC as CFU/ml<200 [24].

### *Dental units' operation and maintenance*

Dental units in operation and maintenance conditions were determined by a reliable and valid checklist with 46 questions. Each question had a score between zero and 2 and the value and importance of each question was determined considering appropriate weighting coefficient of one to five and 283 as maximum score for the whole of the checklist. Then overall status of unit operation and maintenance management was evaluated based on the obtained score at three levels of desirable (> 190 score), medium (142-189 score) and undesirable (< 142 score). Operation time (h/day) and working life of the unit (year) was also determined.

### *Statistical analyses*

Results were analyzed by SPSS16 software, using t-test, analysis of one- way variance (ANOVA), Kruskal-Wallis and LSD. Significant differences are reported at $p < 0.05$.

## RESULTS AND DISCUSSION

The number and diversity of microorganisms in DUWLs are considering as the most important index for contamination status of this equipment's. For this reason, control of bacteriological quality of DUWLs is necessary. Bacteriological quality of 69.8% of the water samples were more than ADA recommendation (<200 ml /CFU) and considered as unfavorable. Maximum, minimum and average total number of indicator bacteria in consuming water of units were

4560, 0 and 1500CFU/ml, respectively. Average HPC of all water samples of units on Saturday and Wednesday were 1838CFU/ml and 1164CFU/ml, respectively (Fig. 1) and this difference was statistically significant ($p < 0.05$). Average number of two other bacteria on Saturday and Wednesday are presented in Fig. 1. The results of this study revealed that dental units water is infected more than ADA recommendation and requires special care of health officials in units utilization protocols' implementation.

On the other hand, significant difference of the mean of HPC between sampling on Saturdays and Wednesdays in this study is justified by the fact that in Iran Saturdays is the first working day of the week. After at least one-day, stop of units' activity and water station in DUWL and consumption of water with low residual chlorine biofilm growth was increased and thus more contamination load of water in Saturdays was occurred. Low average of residual chlorine of 0.21 mg/l in the exit of units' different wards and even its zero value in many samples of Saturday can be a plausible reason for the significant difference in the number of bacteria between sampling days of Saturdays and Wednesdays. Therefore, chlorination as one of water disinfection methods can have an effective role in reducing bacterial contamination load of DUWL. Masoumbeigi *et al.* also in their study of a review on control methods for bacteriological water quality and biofilm in DUWL emphasized that chlorination method is one of the most commonly used methods [25]. In addition, in another study (Masoumbeigi *et al.*) on the relation of bacteriological water and air quality in dentistry center similar results was reported [26]. In the study of Memarian *et al.* in the School of Dentistry, Tehran University of Medical Sciences, also Saturday water contamination was more than midweek [27]. Therefore, most studies reported a result of consistency with the present study and higher levels of used water contamination on Saturdays and it is necessary to pay more attention in order to control the water contamination in Saturdays. All effective measures including super chlorination, release of first water to drain and water treatment at the point of use could prevent and reduce microbial load in DUWLs [28].

The mean and standard deviation of temperature and residual chlorine concentration of the dental unit water was obtained 17.4 ± 0.71 and 0.21 ± 0.18, respectively (Table 1). In Table 2, Pearson correlation coefficient was used to assess the relationship between the temperature and residual chlorine concentration with HPC, *pseudomonas aeruginosa* and *staphylococcus aureus* in the DUWL.

One of the most important reasons of high contamination of DUWL is low residual chlorine of units entering water (Tables 1 and 2), Further, more contamination of DUWL can be explained by higher temperature of the water which accelerate biofilm growth especially in Saturdays. Another reason for the lack of water residual chlorine in hand pieces exit is water stagnation in DUWL which resulted in excessive growth of biofilm on the wall of DUWL. Under these conditions, incomplete disinfection and lack of sufficient residual chlorine cause biofilms growth on the wall of the units with tubes. Also, improper utilization and lack of adequate and on time disinfection of different parts of units are also should be added to above problems and cause intensify undesirable quality of output water from different parts of units. Messano *et al.* reported 87.7% of used water quality of studied dental center wards was under poor condition, that the number of their samples with high infection was more than this study [20].

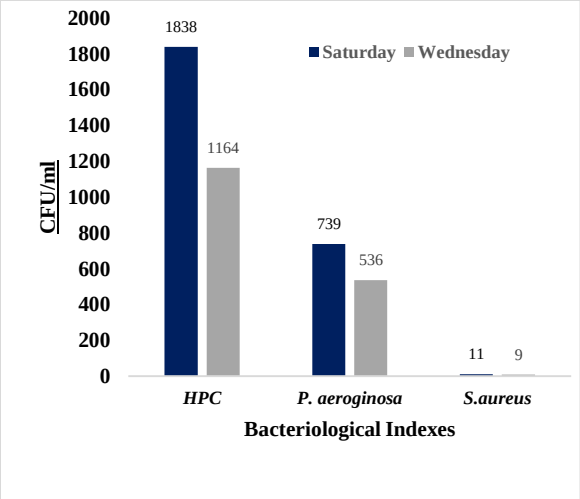


**Fig. 1. T**he average number of index bacteria's in DUWL

**Table 1:** Results of temperature and residual chlorine in DUWL

| Variable | uU | Sample number | Max | Min | X±SD |
|---|---|---|---|---|---|
| **Temperature** | °C | 192 | 19 | 16 | 17.4±0.71 |
| **Residual chlorine** | mg/l | 192 | 0.6 | 0 | 0.21±0.18 |

**Table 2:** Results of temperature and residual chlorine relation with HPC, *P. aeruginosa* and *S. aureus* in DUWL

| Temperature and residual chlorine / Bacteriological index | Residual chlorine (mg/l) | | Temperature (°C) | |
|---|---|---|---|---|
| | r | p-value | r | p-value |
| HPC | -0.71 | 0.0001 | 0.63 | 0.0001 |
| *P. aeruginosa* | -0.72 | 0.0001 | 0.48 | 0.0001 |
| *S. aureus* | -0.1 | 0.02 | 0.24 | 0.014 |

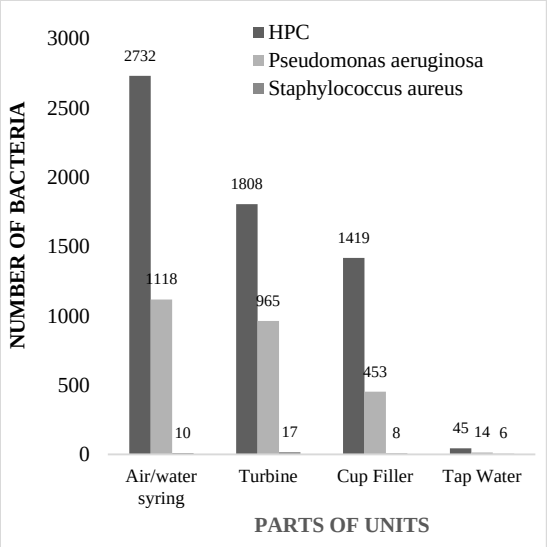


**Fig. 2.** Average number of index bacteria's in water samples from different parts of dental unit

Average number of bacterial index in water samples from different wards of the dental unit was given in Fig. 2. The comparison of the number of isolated bacteria from the water samples of different parts of the dental units showed that the highest number of the bacteria was from air/water syringe part of the units, as follows: HPC, *Pseudomonas aeruginosa* and *Staphylococcus aureus* of 2732, 1118 and 10 CFU/ml, respectively. The lowest number of the bacteria was found in the samples of tap water before entering the unit as HPC (45CFU/ml), *P. aeruginosa* (14CFU/ml), and *S. aureus* (6CFU/ml). A significant difference in number of the bacteria between different parts of units was seen ($p < 0.05$).

The comparison of average HPC of water samples from different parts of units in sections of prosthodontics, restorations and periodontal surgery showed that the air/water syringe segment of unit with 3067, 2258 and 3227CFU/ml, respectively, had the highest rate of contamination compared to other parts of unit. The degree of contamination after the air/water syringe were followed by turbine head duct, cup filler and raw water before entering the unit (tap water), respectively (Fig. 3).

Fig. 4 shows the comparison of the average number of bacteria in water samples collected from different sectors of dentistry equipment. Number of HPC and *P.aeroginosa* in endodentistary sector with 1914CFU/ml and 933CFU/ml respectively and *S. aureus* in the restoration sector with 22CFU/ml is higher than the other wards.

The above mentioned results showed the highest average of bacterial counts was related to air/water syringe and the lowest average of bacterial counts was related to before water entering to the unit and HPC of water samples of air/water syringe in all sectors, especially in endo sector with more contamination (Figs. 2, 3 and 4) and in terms of *Pseudomonas aeruginosa* also endo sector was the most contaminated one (Figure 3).

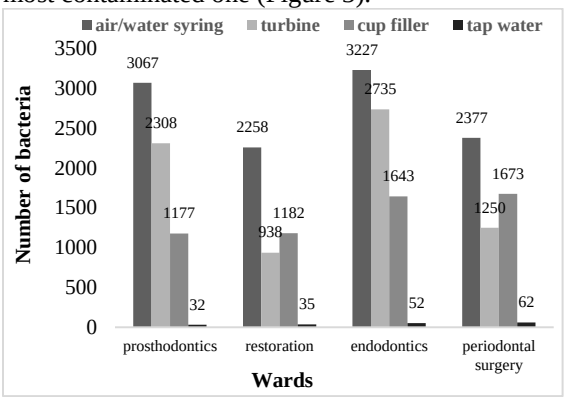


**Fig. 3.** The average number of HPC in water samples of DUWL in different wards

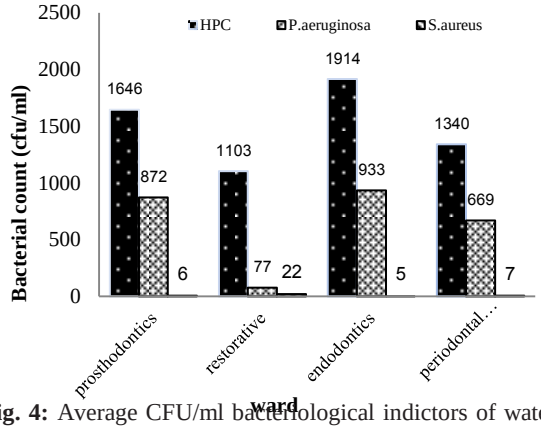


**Fig. 4:** Average CFU/ml bacteriological indictors of water samples from various sections of dentistry

Determining of the residual chlorine indicates that there were not sufficient differences in tap water, the lack of effective disinfection of DUWL and improper utilization and maintenance of units lead to biofilm growth in DUWL wall. The difference of contamination rate in units of different parts also can be due to the amount of their use, the difference of exit velocity of water flow and flushing rate in each unit part. Generally, in cases where the type and size of unit water tubes are the same, water flow rate and frequency of water use per day can affect unit components' contamination. The amount of air/water syringe high infection can also be due to the type and amount of services at the center and it's less useful than other parts of the unit and high stagnation of water and resulting from biofilm formation in the inner wall of its tube. Alipour *et al.* by examining microbial quality of dental units' water of private offices in Bandar Abbas reported that microbial quality of 100% of water samples of high speed hand pieces and air/water syringe exposure was more than

standard and the most infection of air/water syringe exposure was related to HPC, *Pseudomonas aeruginosa* and *Legionella pneumophila.* The average number of HPC and *Pseudomonas aeruginosa* in air/water syringe exposure was reported 8667CFU/ml and 7704 CFU/ml, which are more than air/water syringe exposure infection that in the present study had the highest infection rate [29]. In many studies, the main reason of high infection in DUWL is usually bacterial biofilm overgrowth and bacteria release from biofilm to use water. According to the results of this study and other studies, the type and conditions of utilization and maintenance of units, sampling time, unit water management system and water residual chlorine are also important factors affecting high contamination load in units used water [30]. Smith *et al.* showed that turbine contamination with high speed was more than water exposure and glass filler and the two parts' infection would be higher than reservoir infection [31], which is not consistent with the results of this study. High levels of infection in air/water syringe exposure can be caused as it mentioned the above.

From Table 3, comparing the values of 3 bacteriological indices of used water of studied parts of units revealed that mean difference of the number of bacteria in tap water (before entering the unit) is significantly different with other parts except *Pseudomonas aeruginosa* bacteria in air/water syringe part ($p < 0.05$, LSD test).

**Table 3.** Results of comparison of the mean number of bacteriological indicators in output water of Handpieces

| Bacteriological index | Hand piece | Hand piece | P.value |
|---|---|---|---|
| **HPC** | Air/water syringe | turbine | 0.001 |
| | | Cup filler | 0.0001 |
| | | Tap water | 0.0001 |
| | Turbine | air/water syringe | 0.001 |
| | | Cup filler | 0.147 |
| | | Tap water | 0.0001 |
| | Cup filler | air/water syringe | 0.0001 |
| | | turbine | 0.147 |
| | | Tap water | 0.0001 |
| | Tap water | air/water syringe | 0.0001 |
| | | turbine | 0.0001 |
| | | Cup filler | 0.0001 |
| ***S. aureus*** | Air/water syringe | turbine | 0.547 |
| | | Cup filler | 0.01 |
| | | Tap water | 0.0001 |
| | Turbine | air/water syringe | 0.547 |
| | | Cup filler | 0.047 |
| | | Tap water | 0.0001 |
| | Cup filler | air/water syringe | 0.01 |
| | | turbine | 0.047 |
| | | Tap water | 0.087 |
| | Tap water | air/water syringe | 0.0001 |
| | | turbine | 0.0001 |
| | | Cup filler | 0.047 |
| ***P. aeruginosa*** | Air/water syringe | turbine | 0.361 |
| | | Cup filler | 0.775 |
| | | Tap water | 0.607 |
| | Turbine | air/water syringe | 0.361 |
| | | Cup filler | 0.232 |
| | | Tap water | 0.031 |
| | Cup filler | air/water syringe | 0.775 |
| | | turbine | 0.232 |
| | | Tap water | 0.045 |
| | Tap water | air/water syringe | 0.607 |
| | | turbine | 0.031 |
| | | Cup filler | 0.045 |

Moreover, Table 4 revealed that there was a significant difference considering the amount of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in units' used water in each ward ($p < 0.05$).

Based on various studies, long life and high function of units can be one of the important factors in increasing the thickness of the biofilm layer and result increasing the amount of water contamination. The correlation between the amount of HPC, *Pseudomonas aeruginosa*, *Staphylococcus aureus* in using water and unit's life was significant and showed with more units' life, more used water contamination (Table 5). The results of this study showed that the relationship between the amount of HPC, *Pseudomonas aeruginosa* and Staphylococcus aureus in used water was significant that increasing of units life and more function of the unit led to; more water contamination (Table 5). Montobugno e*t al.* reported using water of newly installed units has less contamination than old one [32]. Also Barbeau *et al.* considered more function of units as one of the factors of increasing the thickness of the biofilm layer and as a result increasing the amount of contamination [30].

The result of one- way variance analysis test (ANOVA) showed that only the relationship between the amount of *Pseudomonas aeruginosa* and *Staphylococcus aureus* and units' utilization, maintenance and management is significant ($p < 0.05$) (Table 6). In other words, if units' utilization, maintenance and management were under good conditions, their bacterial load was lower.

**Table 4:** Results of selective wards relation with HPC, *p. aeruginosa* and *S. aureus* in unite water

| One-way ANOVA / Bacteriological index | ward | X±SE* | Sig (p-value) |
|---|---|---|---|
| **HPC** | Prosthodontics | 1648.8±292.2 | 0.22 |
| | Restoration | 1103.3±228.2 | |
| | endodontics, | 1914.1±298.2 | |
| | Periodontal surgery | 1340.4±247.7 | |
| ***S. aureus*** | Prosthodontics | 6.25±1.9 | 0.0065 |
| | Restoration | 22.5±8.9 | |
| | endodontics, | 5±2.6 | |
| | Periodontal surgery | 7±2.9 | |
| ***P. aeruginosa*** | Prosthodontics | 872±2.8 | 0.007 |
| | Restoration | 76.6±36.1 | |
| | endodontics, | 932.9v237.3 | |
| | Periodontal surgery | 668.7±192 | |

***Mean with error of standard deviation is reported**

**Table 5:** Results of HPC, *P.aeruginosa* and *S. aureus* relation in unite water with Units life

| Units life / Bacteriological index | Units life | |
|---|---|---|
| | **r** | **Sig (**p-value**)** |
| **HPC** | 0.21 | 0.036 |
| ***S. aureus*** | 0.32 | 0.001 |
| ***P. aeruginosa*** | 0.22 | 0.03 |

**Table 6:** Relation of HPC, *P. aeruginosa* and *S. aureus* in used water of units with Units life

| One-way NOVA / Bacteriological index | Units Management | X±SD | Sig. (p-value) |
|---|---|---|---|
| HPC | undesirable | 1627.2±196.3 | 0.35 |
| | mediocre | 1374.5±187.6 | |
| *S. aureus* | undesirable | 14.3±2.4 | 0.028 |
| | mediocre | 6±1.9 | |
| *P. aeruginosa* | undesirable | 800.8±152.2 | 0.035 |
| | mediocre | 474.3±123.7 | |

## CONCLUSION

The purpose of the current study was to assess the bacteriological quality of DUWL in a dentistry center in Tehran, Iran. This study implies that high several parts of dentistry services equipment have a high density of microbial contamination, which can be lead to high risk of service providers and customers. Thus, it is necessary to regularly monitoring, control bacteriological quality of water as one of the priorities of these centers, and notify in the form of unit's utilization protocols or as a health instruction. The second major results of this study implied that financial problems and limitations could be noted as the main obstacle for regularly control of used water bacteriological quality in dental centers. Therefore, supervision and regularly control of bacteriological quality of used water in all dental centers is strongly recommended.

## ETHICAL ISSUES
The authors have observed ethical issues such as plagiarism.

## CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

## AUTHORS' CONTRIBUTION
All authors collaborated equally.

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