

Detection and Quantification of Bacterial Flora in Dental Unit Water Systems and the Effect of Flushing on its Reduction

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ABSTRACT

Statement of Problem: The origin of Dental Unit Water Lines (DUWL) contamination is specially related to the formation of biofilm which is composed of microorganisms within water and is located on the tubing lines.

Purpose: In this descriptive study, we evaluated the degree of contamination with Gram positive and Gram negative bacteria in DUWLs of the dental school and also determined the efficacy of flushing on reducing its microbial count.

Materials and Methods: Thirty dental units from all the departments of dental school in Tehran University of Medical Sciences were selected for this study. Sampling consisted of a two step procedure before and after one minute of flushing. The samples were taken from air/water line of each selected dental unit separately. Air/water syringe of each unit was completely disinfected with Deconex before sampling.

Results: The range of the contamination varied from 190 to 23×10^5 CFU/ml. The bacterial contamination included anaerobic Gram negative *bacilli*, non-fermenting Gram negative bacteria, Gram positive *cocci* and Gram positive *bacilli*. In all the samples taken from water taps, contamination was noted, varying from 25 to 1700 CFU/ml. This was significantly lower than the contamination of air/water syringe of the dental units.

Conclusion: Applying the right principles for infection control such as using disinfectants or sterile water in dental settings and daily flushing before visiting patients can be of great significance.

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Introduction

Several different types of microorganisms have been identified and separated from Dental Unit Water Lines (DUWL) in recent years. The origin of this contamination is specially related to the format-

ion of biofilm which is composed of microorganisms within water and is located on the tubing lines [1]. Most of these microorganisms are not pathogenic in healthy individuals but may be of great importance in patients with systemic diseases and may

cause morbidity in the immune compromised patients. Although the results of some epidemiologic studies show that contamination of DUWL can be dangerous in patients with immune-deficiency or other immune system problems [2], it can be true for pregnant women, elderly, graft recipients or even smokers. Contamination of DUWL can be of great importance since the patients and dental personnel are in intimate contact with water and aerosols produced in the environment [3].

Several microorganisms including Gram positive and Gram negative bacteria have been detected in different researches. The biofilm plays the main role in this contamination [4]. Biofilm is indeed the microbial colony attached to the tubing surfaces which are more resistant to antiseptics and antimicrobials than microorganisms within water. So, the bacteria living in the biofilm have a more chance to survive than floating microorganisms.

Martin detected *Pseudomonas Aeruginosa* (P.A) in the dental unit water system. It had resulted in Pseudomonal abscess in the oral cavity of two immunocompromised patients [5]. Disinfecting the dental unit water system with chlorine was evaluated by Fiehn in 1998. It was stated that besides the carcinogenic characteristics of chlorinated water for laboratory animals, it could also result in corrosion of the dental equipment [6].

Ozcan showed that both Alpron and Bio 2000 appear to be an effective disinfectant for use in eliminating the cfu (Colony-Forming Units) in DUWL totally at the end of 2 weeks [7]. Walker assessed the microbiology of DUWS and biofilms in general dental practices across seven European countries including the United Kingdom, Ireland, Greece, Spain, Germany, Denmark, and the Netherlands. Water supplied by 51% of 237 dental unit water lines exceeded the current American Dental units.

The current Center for Disease Control and Prevention (CDC) guidelines for infection control in dental healthcare settings recommend that dental

unit output water should amount to 500 CFU/ml of the aerobic heterotrophic bacteria. The American Dental Association has set a standard for dental unit output water which is equal to 200 CFU/ml of aerobic heterotrophic bacteria [1, 2]. The study emphasizes the need for effective mechanisms to reduce the microbial burden within DUWS, and highlights the risk of occupational exposure and cross-infection in general dental practice [8]. A study was carried out by Zanetti (to evaluate the decontaminating efficiency of an alternative product, hydrogen peroxide, on dental unit water supply). The results of this study indicated that the disinfectant is able to keep contamination under control, as long as the treatment is repeated daily, before starting work, and especially after long interruptions [9]. Al-Hiyasat evaluated the extent of *Pseudomonas aeruginosa* contamination of DUW at a Dental Teaching Center in Jordan. *Pseudomonas aeruginosa* was detected in 86.7% (26/30) of the dental units at the beginning of the working day, and in 73.3% (22/30) after 2 minutes of flushing and at midday; flushing the DUW for 2 minutes significantly reduced the counts of P.A [10].

A study was carried by Ketabi in 2010 to evaluate the effect of Chlorine-dioxide on reduction of bacterial contamination of Dental Unit Water Systems. This study indicated that the amount of bacterial contamination in DUWS of Khorasgan dental school was higher than the accepted level. After using chlorine dioxide, the amount of bacterial colonies was significantly reduced ($p < 0.05$). The major group of bacteria observed included Gram negative bacilli (*pseudomonas*), Gram positive cocci and a few Gram positive bacilli [11].

This study evaluated the degree of contamination with Gram positive and Gram negative bacteria in DUWLs of the dental school in Tehran University of Medical Sciences for the first time. We also determined the efficacy of flushing in reducing the microbial count of dental unit water system.

The extent of contamination with *Pseudomonas aeruginosa* was also determined.

Materials and Methods

Thirty dental units from all the departments of the dental school in Tehran University of Medical Sciences were selected for this study. More dental units were selected from departments with greater numbers of dental units and more patients visiting daily. Sampling consisted of a two step procedure before flushing and after one minute of flushing. A control sample was also taken from tap water of each ward. The samples were taken from air/water line of each selected dental unit separately. Water samples were taken approximately mid-morning around 10 o'clock on Mondays. CDC has developed special recommendations for use in dental offices. For example, air/water syringes should be operated for a minimum of 20 to 30 seconds after each patient to flush out the retracted material. Between patients, the coverings must be changed and the underlying surface cleaned. Between clinical sessions, all surfaces including those apparently uncontaminated (out-side the zoned area) should be thoroughly cleaned and decontaminated with detergent and a suitable veridical disinfectant. Fresh solutions of disinfectant should be made up and used according to the manufacturer's instructions [1-2]. Thus, we considered all recommendations in our study. Air/water syringe of each unit was completely disinfected with Deconex before sampling.

Sampling Method

Ten CC of water from each unit water line was collected into a sterile water bottle using aseptic techniques. Another sample was taken after 1 minute of flushing and collected into a separate sterile bottle. In this study, water sample collection was done thrice and then an average was taken for the results. The head of the water syringe did not contact with the bottle so that no other contamination was added to the collected sample. The bottles were then label-

ed and returned to the laboratory in 2 hours.

Culturing media

The following media were used for detection of bacterial growth in the laboratory:

Cetrimid Agar (for detection of *Pseudomonas*. A, Merk, Germany), Mac conkey Agar (for Gram negative bacteria, Himedia, India), Blood Agar (Himedia, India), R2A media (for colony count, Gibco, UK) MHA (Muller Hinton Agar, Himedia, India) Oxidative/Fermentative medium, Merck, Germany), KIA media (Kliger Iron Agar, Himedia, India) and Water samples were completely mixed for 15 seconds and decimal dilutions were prepared by adding 1^{cc} of the sample to 9^{cc} of sterile water. Dilutions of 1/100 and 1/400 were prepared for each tube and plated on Cetrimid, BA, R2A and Mac Agar. After all, 16 plates were cultured for each sample (before and after flushing). After 48 hours, the total colony count of each plate was enumerated. For confirmation of presumptive *Pseudomonas* and non-fermentative bacteria, a slide was prepared from the present colonies and was stained by Gram technique. Bacillus and Gram negative *coco bacillus* bacteria were selected and purified for MHA media. For purifying, the bacteria were sampled from the related colonies and incubated for 24 hours in MHA plates. The purified bacteria were placed on Oxidative Fermentative medium and Kliger iron Agar and incubated for 18-24 hours in the incubator. After the growth of bacteria in this media, non-fermentative bacteria were identified. These bacteria were placed on ERIC kits. Bacterial suspension was prepared (3×10^8 CFU/ml) and placed on ERIC kits in sterile condition. It was incubated for 4 hours and the type of bacteria was identified using ERIC software according to the present template. Data were analyzed by SPSS software (version 11.5) and statistical tests including Wilcoxon and descriptive analysis.

Results

In all the 30 samples taken from air/water syringes,

Table 1 The extent of contamination of DUWS in different departments before flushing

Dept.	Before flushing (CFU/ml)	N	Std. deviation	Std. error mean
Orthodontics	2300000	1	0	0
Pediatrics	440960.0	5	400300.3	179019.7
Restorative	277645.6	7	550685.8	208130.7
Periodontics	209000.0	4	156411.9	78205.9
Partial prosth	175733.3	3	241437.8	139394.2
Endodontics	69000.0	5	100040	44739.2
Fixed prosth	60600.0	5	49561.1	22164.4
Total	281983.98	30	506041.2	92390.1

bacterial contamination was noted. The range of this contamination varied from 190 to 23×10^5 CFU/ml. The bacterial contamination included anaerobic Gram negative *bacilli*, non-fermenting Gram negative bacteria (N.F.B), Gram positive *cocci* and Gram positive *bacilli*. In all the samples taken from tap water, contamination was noted which varied from 25 to 1700 CFU/ml. This was significantly lower than the contamination of air/water syringe of dental units (Tables 1, 2). Of the most important findings of this research was that in all the samples cultured from water syringes contamination with N.F.B was seen while in none of the tap water samples contamination with N.F.B was noted. In all the sources, the total count of Gram negative bacteria was more than Gram positive bacteria (70% Gram negative and 30% Gram positive).

Isolating and detecting *Pseudomonas aeruginosa* from dental unit water supply has been one of the main aims of this study. This was done by using special media and in this regard *Brev. Diminuta* previously known as *Burkholderia cepacia* which can lead to meliodosis infection was detected. Also, *B. Cepacia* which is an opportunistic bacteria and can cause severe problems in patients with cystic fibrosis, *P.pseudoalcali* genes which is commonly found in hospital environment and can lead to meningitis, *Flavo Bacterium meningosepticum* which can be isolated from soil, water, food and can be found in hospital environment were observed. It can

Table 2 The extent of contamination of DUWS in different departments after flushing

Dept.	After flushing (CFU/ml)	N	Std. deviation	Std. error mean
Orthodontics	26000.00	1	0	0
Pediatrics	13540.00	5	22238.78	9945.48
Restorative	62597.14	7	163780.33	61903.15
Periodontics	29550.00	4	29065.62	14532.81
Partial prosth	2633.33	3	1289.70	744.61
Endodontics	446.00	5	869.184	388.71
Fixed prosth	471.40	5	293.043	131.05
Total	22085.6	30	79517.38	14517.82

cause meningitis and septicemia in susceptible patients. Moreover, *Morax lacunata* which was previously known as *Moraxella liqefaciens* and can be isolated from respiratory and eye infections was seen. It grows on the agar media. All of the mentioned bacteria are opportunists and can somehow cause different infections and diseases in susceptible patients.

Discussion

Contamination of DUWS has been known as long as 30 years ago. However, the fact still persists in spite of making many problems for immune compromised and other at risk patients [13]. There are two main sources for this contamination. The first one is oral microbial flora of the patients which is flowed back to water system of dental units by means of high speed or suction and the second is biofilm which can act as the potential source of contamination. According to the findings of this study, all the 30 dental units had different counts of bacterial contamination which in some cases has been unusually higher than standard amounts. Similar to the study in Khorasgan dental school, the amount of bacterial contamination in DUWS was higher than the accepted level [11]. The results also showed that the degree of contamination of air/water syringes has been higher than tap water and that Gram negative bacteria have been more prevalent than Gram positive ones. In most of the similar studies,

Gram negative bacteria have been more prevalent than Gram positive ones in dental unit water systems [11-13]. The most important data found was the higher number of non-fermenting Gram negative bacteria in samples taken from dental units compared to those found in samples from tap water which is due to higher tendency of these bacteria for living in open aquatic environments and also due to the bacteria's simple growth requirements enabling such bacteria to live and even proliferate in water [3, 8]. One of the important issues regarding the bacterial infection of water supply of dental units is the fact that nearly all of these units are very old and worn out and because most of them are not supplied with perfect and modern water system, biofilm is readily formed and bacterial colonization is facilitated in their water system. Another important issue is that most of these bacteria are opportunistic pathogens which can readily cause severe and life threatening diseases in some immune compromised and other susceptible patients. The presence of bacteria can be of great risk for health care workers too.

Due to the importance of these microorganisms in the pathogenicity and infectivity, applying the right principles for infection control such as using disinfectants or sterile water in dental settings and daily flushing before visiting patients can be of great help. One minute of flushing before visiting each patient compared to other ways of infection control such as using disinfectants and sterile water needs no special equipment and has no cost.

In a study by A.J. Smith, it was shown that the extent of infection in air/water syringes of dental units was far greater than tap water. The study was conducted in some dental clinics in the west of Scotland and the results are consistent with our findings [14].

In another study in the southwest of England by J.T.Walker, 55 dental units were selected and bacterial infection in different parts were evaluated and compared. The samples were taken from water

syringe and head of high speed. Turbin although the extent of bacterial infection was lesser than what we have found in our study showed, in both studies Gram negative bacteria to be more prevalent than Gram positives. In this study, 68 percent of the units were contaminated while 100 percent of our samples have been shown to be contaminated [15].

In a study by Meiller, the mean bacterial contamination of the dental units was reported to be 10^5 CFU/ml. This is significantly lower than the contamination of the dental units in our study [16]. Walker conducted a research on 20 dental units to assess their extent of contamination. The contamination in their study was largely related to Gram negatives which are similar to our findings [17]. Teixeira compared the effect of 20 seconds of flushing with 2 minutes of flushing and concluded that 2 minutes of flushing can be more efficacious in eliminating and reducing the bacteria in dental unit water lines [18]. According to the findings of Watanabe, ten minutes of flushing is highly helpful in reducing the bacterial counts of DUWS [19]. Whitehouse states that 20 minutes of flushing can reduce the count of bacteria to zero but after 24 hours the count will increase gradually [13]. Although long flushing can lead to greater reductions in bacterial counts, this is not practical in most dental clinics. It is suggested that 1 to 2 minutes of flushing is suitable in most situations because it can lead to considerable reduction in bacterial contamination. In this research, we were able to detect and isolate some different types of opportunistic bacteria including *P.aeruginosa*. Watanabe and Leggat detected *E.coli* in dental unit water lines [19-20].

Barbeau isolated *P.Aeruginosa* from water lines of more than 25% of the dental units under the study which had a concentration of more than 10 CFU/ml [21]. Also, Ma'ayeh could prove the presence of *P.Aeruginosa* in dental unit waterlines [22]. *Legionella* has been known as the main cause of respiratory infection and Legionellosis [22]. Isolating this

bacterium requires special media, takes a long time to complete and is a very complex procedure. Detecting *Legionella* was not considered in this study.

Conclusion

The presence of biofilm in dental unit water lines is a much known fact and its quantity depends on so many factors such as oldness and quality of dental unit and how strictly the infection control guidelines are observed. Considering these guidelines according to the present conditions and facilities such as flushing before visiting each patient can reduce the count of bacteria and lead to a safer environment for both the practitioner and the patient.

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