E Watanabe
AM Agostinho
W Matsumoto
IY Ito

Dental unit water: bacterial decontamination of old and new dental units by flushing water

Authors' affiliations:
Evandro Watanabe, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
Alessandra Marçal Agostinho, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Wilson Matsumoto, Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
Izabel Yoko Ito, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

Correspondence to:
Evandro Watanabe
Rua Barão do Amazonas
1167, apto 123, CEP: 14010-120
Ribeirão Preto – SP
Brazil
Tel.: +55 16 3602 4217
Fax: +55 16 3635 8241
E-mail: evandrowatanabe@gmail.com

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Abstract: *Objective:* To evaluate by means of Petrifilm™ system (3M, St Paul, MN, USA) the level of bacterial contamination in water from old and new dental units (air–water syringes and high-speed turbines) before and after flushing water through waterlines. The old dental units had been used for 13 years and the new dental units for 1 year. A fast method named Petrifilm™ AC (3M) was employed to evaluate the level of water contamination with total aerobic bacteria and Petrifilm™ EC for *Escherichia coli* and coliforms. *Methods:* Dental unit water were collected before and after flushing of 4 (air–water syringes) and 2 min (high-speed turbines) from 24 old and new dental units. Thereafter, samples were diluted, inoculated onto Petrifilm™ plates and incubated. *Results:* The filtered tap water that filled up dental unit reservoirs showed a low level of bacterial contamination (4 and 15 CFU ml^{-1}). However, all water samples from old and new dental units were highly contaminated. The flushing of dental unit waterlines reduced the bacterial count in all dental unit water, but the reduction was better in water from new dental units than from old dental units. *E. coli* and coliforms were not detected in any water samples analysed. *Conclusion:* Flushing water is a simple measure that should do part of dental routine, because it was able to reduce the level of total aerobic bacteria in water from old and new dental units.

Key words: bacterial count; dental unit waterline; water

Introduction

The goal of infection control in dentistry is to reduce or eliminate exposures of patients and the dental team to microorganisms (1).

Several authors have related microbial contamination from dental unit water (2–6).

By the early 1960s, dental researchers had already observed that microorganisms seemed to flourish in the dental unit water.

Blake (7), a dentist in the UK, was the first to report high levels of bacteria in dental unit water. Moreover, Kelstrup *et al.* (8) were the first to demonstrate microbial colonies attached to dental unit waterlines, currently, known as biofilm.

In Japan (9), the standard for the microbial quality of drinking water (called potable water) is no more than a total of 100 CFU ml^{-1} (colony forming units per millilitre of water). However, the Environmental Protection Agency (10), the American Public Health Association (11), the American Water Works Association (11) and the Brazilian Ministry of Health (12) have set a maximum limit for heterotrophic mesophilic bacteria in drinking water at 500 CFU ml^{-1}.

The biofilm in dental unit waterlines is the cause of a high number of microorganisms in water caused by a low level of microbial contamination in public water distribution systems (13).

The dental unit waterline is a flexible tube with diameter approximately 0.5–1.0 mm (14, 15). This system is a solid surface in contact with water that is a home to remarkable microscopic communities, the biofilms (16).

According to Donlan & Costerton (17), the new definition of a biofilm is a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription. Moreover, according to Costerton *et al.* (18), biofilm is not a compact mass, but a mass of microorganisms developed into columns and floors with primitive channels where flowing liquids with nutrients, biocides, by-products and gases.

The CDC – Centers for Disease Control and Prevention recommends that dentists should use sterile saline or sterile water as a coolant/irrigator when surgical procedures involving the cutting of bone are performed (19).

In 1996, the ADA – American Dental Association published a statement on dental unit waterlines that challenged dental equipment manufacturers to produce systems that could reduce the level of bacteria in water used for non-surgical dental treatment to 200 CFU ml^{-1} or fewer by the year 2000 (20). In November 1999, the ADA reaffirmed this goal and reported on scientific and technological developments that had occurred since the panel first convened (21).

Several authors have been reported flushing water through waterlines to reduce the level of microbial contamination of dental unit water (22–27).

The microbial quality of water may be evaluated by several methods, among them there is a fast method denominated Petrifilm (3M, St Paul, MN, USA) that has shown a great value because of facility and fast execution (2, 4).

The aim of this study was to evaluate by means of Petrifilm^{TM} system (3M, St Paul, MN, USA) the level of bacterial contamination in water from old and new dental units (air–water syringes and high-speed turbines), before and after flushing water through waterlines; besides, the level of bacteria in water from dental unit reservoirs and a tap.

Materials and methods

The water samples were collected from 24 dental units of two clinics at the Dental Association of Ribeirão Preto, São Paulo, Brazil.

A clinic with 12 dental units and in use for 13 years, it was considered as old. Ten dental units (Delta B) were from Dabi Atlante (Ribeirão Preto, SP, Brazil) with individual reservoirs as polyethylene terephthalate (PET) bottles (500.0 ml) and two dental units were from Gnatus (Ribeirão Preto, SP, Brazil) with individual fixed reservoirs (2000.0 ml).

Another clinic with 12 dental units in use for 1 year, it was considered as new. All dental units (Croma Millennium) were from Dabi Atlante (Ribeirão Preto, SP, Brazil) with individual reservoirs as PET bottles.

Collection of water samples

Approximately 10.0 ml of each water sample before and after flushing water through waterlines was collected from air–water syringes and high-speed turbines in sterile test tubes (25×125 mm).

The first water samples from high-speed turbines and air–water syringes were collected when the dental units were turned on and the second water samples after flushing for 2 and 4 min, respectively.

Furthermore, 24 water samples from reservoirs of old and new dental units and four samples of filtered tap water supplied to the reservoirs of these dental units were collected in different days.

All samples were treated with 0.05 ml of 2.0% sodium thiosulfate (*Reagen*, Rio de Janeiro, RJ, Brazil) to neutralize the residual chlorine present in water.

The water samples were transported to the Microbiology Laboratory at Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo in a cool box (4–8°C) being the congest time 2 h after the actual collection.

Plating onto Petrifilm™ plates (3M)

According to 3M™, Petrifilm™ Aerobic Count (AC) Plate is a ready-made culture-medium system that contains Standard Methods nutrients, a cold-water-soluble gelling agent, and a tretazolium indicator that facilitates colony enumeration of total aerobic bacteria.

Petrifilm™ EC – *Escherichia coli* and *Coliform Count* Plate contains violet red bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide (BCIG), and a tetrazolium indicator that facilitates colony enumeration. The top film traps gas produced by the lactose fermenting.

The top film of Petrifilm™ plate was lifted and 1.0 ml of sample or diluted sample was dispensed onto the centre of bottom film. Slowly and preventing air bubbles from being trapped, the top film was dropped down onto the sample. Thereafter, a plastic spreader was placed and pushed gently downward onto the centre of the plate to distribute the water sample. The plastic spreader was removed and plates were left undisturbed for at least 1 min to permit the gel solidification. These plates were incubated in a horizontal position, with the clear side up in stacks of up to 20 plates, in a wet chamber at 37°C for 48 h.

After incubation, all red regardless of size or intensity CFU were enumerated by a stereomicroscopic (Nikon, Tokyo, Japan) under reflected light.

Statistical methods

The Wilcoxon and Mann–Whitney U are non-parametric tests that were used to compare the value of bacterial contamination (28).

Statistical significance was assumed at a P value <0.05.

The comparisons were for:

1. Air–water syringes of old and new dental units:
   (i) Before × after flushing.
2. High-speed turbines of old and new dental units:
   (i) Before × after flushing.
3. Air–water syringes of old × new dental units.
4. High-speed turbines of old × new dental units.
5. Air–water syringes × high-speed turbines of old dental units:
   (i) Before flushing;
   (ii) After flushing.
6. Air–water syringes × high-speed turbines of new dental units:
   (i) Before flushing;
   (ii) After flushing.
7. Reservoirs:
   (i) Old × new dental units.

Results

The results are shown in Tables 1 and 2 and Fig. 1.

According to the statistical analysis (Wilcoxon test), flushing water showed a statistically significant reduction in total aerobic bacteria presented in the water of both air–water syringes and high-speed turbines from old and new dental units.

However, according to Mann–Whitney U test, flushing water showed a better bacterial reduction in the water from new dental units than from old dental units (air–water syringes and high-speed turbines).

The filtered tap water that filled up dental unit reservoirs had a low level of bacterial contamination (4 and 15 CFU ml^{-1}). Nevertheless, the reservoirs water of old dental unit was highly contaminated with exception of the old dental unit reservoir #10 (12 CFU ml^{-1}). On the other hand, only the reservoir water of the new dental unit #9 was highly

Table 1. **Statistical comparison between the level of bacterial contamination in water from old and new dental units, before and after flushing water**

	P-value	Significance
Old × new AWS	>0.0500	NS
Old × new HST	>0.0500	NS
Old AWS × HST (before flushing)	0.3981	NS
New AWS × HST (before flushing)	0.0023	**
Old AWS × HST (after flushing)	0.0378	*
New AWS × HST (after flushing)	0.8014	NS

AWS, air–water syringes; HST, high-speed turbines; NS, not significant.
*Significant, **more significant.

Table 2. **Averages of total aerobic bacteria (CFU ml^{-1}) in water from old and new dental units, before and after flushing water**

	Air–water syringes*		High-speed turbines*	
Dental units	Before	After	Before	After
Old	8 270 509	53 357	56 711 918	8465
New	322 358	204	1 628 833	374

*CFU ml^{-1}, colony forming units per millilitre of water.

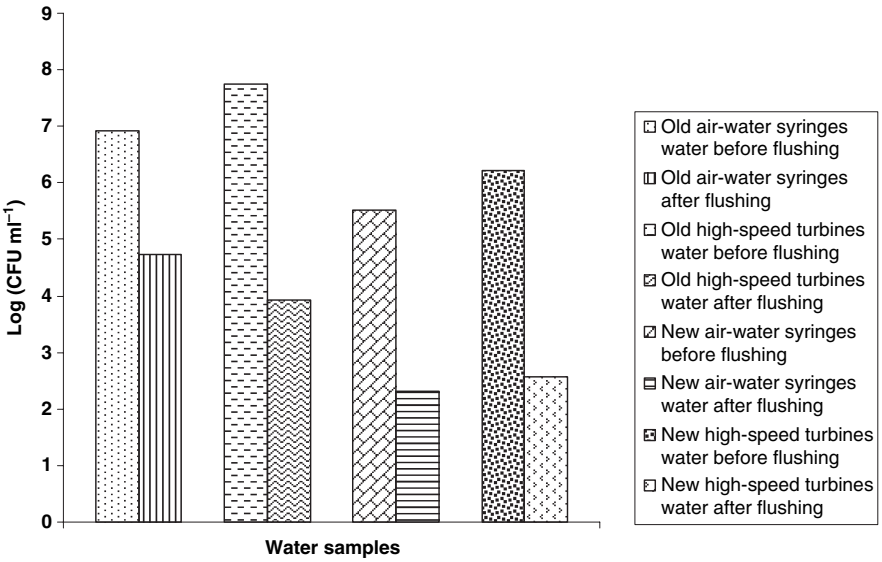

Fig. 1. Averages of total aerobic bacteria log (CFU ml^{-1}) in water from old and new dental units, before and after flushing water.

contaminated (32 650 CFU ml^{-1}). Besides, it was observed that the levels of total aerobic bacteria in reservoirs water from old dental units (average: 137 909 CFU ml^{-1}) were statistically significantly higher than from new dental units (average: 2 726 CFU ml^{-1}) – Mann–Whitney U test.

Petrifilm™ EC plates

Escherichia coli and coliforms were not detected in any water samples analysed.

Discussion

According to American Dental Association by the year 2000, the level of bacterial contamination from dental unit water should be to 200 CFU ml^{-1} or fewer (20).

Authors have related bacterial contamination in dental unit water to 400 000 CFU ml^{-1} (29), 100 000CFU ml^{-1} (30), 39 400 000 CFU ml^{-1} (4) and even 300 000 000 CFU ml^{-1} (3). In this study, the highest levels of total aerobic bacteria were found in water from old dental units: a high-speed turbine (610 000 000 CFU ml^{-1}) and an air–water syringe (81 500 000 CFU ml^{-1}).

According to Noce *et al.* (24), the addition of sodium thiosulfate to neutralize the residual chlorine in water samples collected from dental units is an important procedure, because the level of bacterial contamination before chlorine neutralization was reported to 140 000 CFU ml^{-1} and after to 220.000 CFU ml^{-1}. Therefore, these results demonstrated an increase of bacteria recovery of 57.0%.

In this study, the final concentration of sodium thiosulfate in water samples was 100 mg l^{-1} (4, 6).

Authors have reported the use of dental units for 1 year (31), 5 years (32), 10 years (33), 15 years (34) and even 20 years (31). This study was realized with dental units in use for 13 years (old) and 1 year (new).

The municipal water distribution systems may be connected directly to dental units (23, 25, 27, 35). Nevertheless, water may supply independent reservoirs made of metal, plastic or glass. Therefore, independent reservoirs may be located on floor or attached directly to dental units (7, 29).

Nowadays, water reservoirs (bottles) made of PET and with capacity of 5000 ml are the most common type attached directly to dental units (9, 31, 32, 35, 36).

According to Depaola *et al.* (37), independent reservoirs isolate dental units from municipal water distribution system permitting to utilize water of good microbiological quality. The user may introduce cleaners and germicides to control or eliminate biofilm formation within the water delivery system. Besides, the PET reservoirs have more advantages than other types of metal, or plastic, or located on floor, because they are easy to clean because of the small size and transparency. Moreover, the small amount of water stagnates in bottle reservoirs decrease the development of microorganisms as well as biofilm formation.

In this study, two old dental units had individuals water reservoirs located on floor and with capacity of 2000.0 ml. In addition, 22 water reservoirs (PET bottles with capacity of 500.0 ml) were attached directly to dental units (10 old and 12 new).

The water from municipal distribution system was used to fill up all dental unit reservoirs and came from a filter connected a tap that located at old dental clinic.

Several authors have reported tap water that fill up dental unit reservoirs with bacterial contamination fewer than 200 CFU ml^{-1} (4, 9, 23, 25, 29–31, 36, 38). On the other hand, other authors have related bacterial contamination in tap water above 500 CFU ml^{-1} (35, 39).

Water from dental unit reservoirs has shown bacterial averages of 0 (38), 660 (9), 22 000 (35), 118 667 (7), 167 500 (7) and 223 399 CFU ml^{-1} (3).

In this study, the filtered tap water that supplied the reservoirs of dental units was collected and analysed in four different days. Although two water samples showed the presence of bacteria, the levels of contamination were well below 500 CFU ml^{-1} (4 and 15 CFU ml^{-1}). These results indicate that the water from municipal distribution system was with excellent bacterial quality.

The CDC (19) and the ADA (20) have recommended the microbial contamination control of dental unit waterlines by means of flushing water through waterlines at the beginning and end of the working day as well as between patients as recommended for a minimum of 20–30 s. Although flushing does not remove biofilms on dental unit waterlines, it may reduce the microbial count in water temporarily and help to clean the waterlines of materials and oral microorganisms that may have entered via suck-back from the patient's mouth.

We agree with Pankhurst *et al.* (14) that flushing may be instituted as a simple and expedient measure, immediately as a stop-gap procedure in all dental surgeries. In addition, it may be applied in whatever dental units (age or type) without the need to purchase additional equipment.

Nevertheless, according to Santiago *et al.* (40), flushing has a transient effect and the initial levels of microbial contamination come back after 30 min.

The flushing times have been reported from 1 to 20 min (9, 22–27, 35). All authors observed a reduction of bacterial contamination in dental unit water. However, with flushing times from 4.5 min (22, 23, 26) were found levels of bacterial contamination below 200 CFU ml^{-1} as recommended by ADA (20, 21).

In this study, the water samples were collected before and after flushing procedure. Air–water syringes water was flushed for 4 min and high-speed turbines water during 2 min.

We agree with Prevost *et al.* (9) and Santiago *et al.* (40) that flushing is a very important procedure to reduce the bacterial count from dental unit water.

Dental unit water has shown a high level of microbial contamination caused by biofilm formation on walls of waterlines (1, 4, 13).

Intermittent stagnation of the water inside the waterlines commonly occurs between patients, overnight and over the weekends. Consequently, the dark, damp and warm interior of dental unit waterlines may serve as an ideal incubator for microbial proliferation (1, 15, 16, 36).

Thus, we agree with Dolci & Montebugnoli (13), who consider the dental unit waterline as an 'amplifier system' from the low number of microorganisms present in public system water.

We did not find *E. coli* and coliforms in dental unit water as reported in the study of Walker *et al.* (5) and Abel *et al.* (38).

Various methods and culture mediums have been applied to evaluate the level of bacterial contamination in water from dental unit waterlines. The Plate Count Agar medium has been used to count total aerobic bacteria in water by pour plate technique (3, 24). Moreover, the employment of plating onto surface of R_2A Agar and Trypticase Soy Agar, as well as the use of membrane filtration technique is reported in the literature (24, 25, 27, 36, 39).

In this study, Petrifilm^{TM} AC (total aerobic bacteria) and EC (*E. coli* and coliforms) was used to evaluate the level of bacteria in dental unit water and filtered tap water.

The Petrifilm^{TM} system has shown several advantages when compared with other conventional methods. Firstly, it is convenient because it is ready-to-use and eliminates steps of medium culture preparation and necessity of glasses. Secondly, the plates occupy less space (in stacks of up to 20 plates) in incubator chambers, refrigerators and cupboards. Thirdly, the plates do not break and they are easily discarded or stored in refrigerator.

It is a fact that the biofilm formed on dental unit waterlines is a real problem in dentistry by causing microbial contamination in water. The flushing through waterlines of old dental units showed to be less efficient to reduce the bacterial count in water than in new dental units. Possibly, this happens because of the formation of a biofilm more mature and well-established (stronger) in dental unit waterlines that have been used for a long time.

In our opinion, this problem should be seriously confronted in the dental routine with periodic flushing water and additional chemical treatment (cleaner/disinfectant) for safety of patients and dental team. Moreover, water reservoirs should be usually cleaned with mechanical and chemical methods (brushing and soap) to remove the biofilm.

Periodically, a control of microbiological quality in dental unit water (air–water syringes, high-speed turbines and reservoirs) is fundamental to check the effectiveness of flushing and chemical treatment. Therefore, the Petrifilm^{TM} is a fast method that may be used for this purpose because of low cost, facility and fast execution when compared with conventional methods.

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