Impact of cleaning regimes on dental water unit contamination

Soad A. Abdallah and Ahmed I. Khalil

ABSTRACT

Microorganisms that have been identified in dental unit waterlines (DUWLs) are of concern because they can cause infections, especially in immunocompromised patients. This study aimed to assess the incidence of microbial contamination in DUWLs before and after intervention to reduce contamination, and to investigate the presence of coliforms, *Escherichia coli* and *Pseudomonas aeruginosa*. Water samples were collected aseptically from the waterlines. The high-speed hand-piece and dental chair units were served by one distillation apparatus, which was fed by the potable tap water of four dental clinics. Different interventions were used: chlorination, flushing before clinics and between patients, draining at the end of the day, and freshly distilled water on a daily basis. There was a significant difference between the level of contamination in the high-speed hand-piece (1.5–2.7 log CFU/ml) and dental chair unit water (2.0–3.5 log CFU/ml). Coliforms (0.9%) *E. coli* (0.9%) and *Pseudomonas* (1.8%) were detected during 2008. This study indicates the need to monitor water quality regularly and prevent stagnation in DUWLs to reduce the number of viable bacteria to <100 CFU/ml. We recommend flushing the DUWL for 2 min before the first patient and for 10–20 s between patients, flushing the dental unit at the end of the day and draining it overnight to reduce the development of biofilms, and chlorination of the DUWLs.

Key words | bacterial contamination, chlorination, dental unit waterline, flushing

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INTRODUCTION

Dental unit waterlines (DUWLs) are an essential part of dental surgery equipment, and provide water for the tubes that connect the high-speed hand-piece, air/water syringe and ultrasonic scalers. Cross-contamination usually occurs from contaminated instruments. The water supplies in dental units can become contaminated as a result of a transient retraction of oral fluid into the waterline of a dental hand-piece if it is shut off while still in the mouth (Berlutti *et al*. 2003); DUWLs are a potential problem in the control of infection in dental practice because they can be easily contaminated by patients and water impurities (Sacchetti *et al*. 2006). Patients and dental staff might inhale fine spray from DUWLs as it splashes off the surface of the patient's mouth (Samaranayake 2003).

Dental units are considered to be a source of infection with a highly variable clinical spectrum; such infections can cause overwhelming acute diseases, especially in immunocompromised patients (Walker & Marsh 2007). Microbial contamination of DUWLs and their water supplies is frequent, either with oral bacteria or with environmental bacteria, especially human opportunistic pathogens (Merne *et al*. 2000). Fungi and protozoa have also been shown to contaminate DUWLs (Simões *el al.* 2008). These microorganisms colonize the interior surfaces of the waterline tubing and replicate, which inevitably results in adherent heterogeneous microbial accumulations that are termed biofilms. This bacterial growth depends on factors including concentrations of disinfectant, water temperature and waterline substrate. The characteristics of the material composing waterlines may greatly influence the densities of bacteria (Momba & Makala 2004).

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The biofilm is an optimum habitat for a great variety of bacteria, and it protects the bacteria from disinfectants by providing a suitable matrix of polysaccharides and glycoproteins, which holds the bacteria in place (Walker *et al.* 2003). The attached bacteria flourish, multiply and build up more matrix material, which in turn, hold more species of bacteria, until the biofilm is visible to the naked eye and nearly obstructs the lumen of the water line (Walker & Marsh 2007). At low flow rates, fluid at the center of any lumen travels fastest. Closer to the the tubing walls, its rate of flow is slowed increasingly by biofilm roughness (Herd *et al.* 2007). Water at the tubing walls is virtually stagnant, which permits bacteria to adhere and colonize the internal surfaces (Kettering *et al.* 2002).

Species found in the biofilm on DUWLs include coliforms and *Escherichia coli*, in addition to true human pathogens, such as *Moraxella* spp., *Flavobacterium* spp., *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Mycobacterium* spp., *Staphylococcus* spp. and *Klebsiella* spp. (Coleman *et al.* 2007).

To reduce DUWL contamination, water that is used for restorative procedures should be of the same quality as drinking water (Depaola *et al.* 2002). The American Dental Association (ADA) has proposed that dental water should contain <200 CFU/ml (Walker *et al.* 2000). The European Union has drinking water standards set at 100 CFU/ml; however, dental unit water is not considered as drinking water.

The presence of extended narrow bore tubing and long periods of stagnation means that dental water units (DWUs) can be prone to relatively high levels of microbial contamination, including the biofilm formation and the presence of opportunistic pathogens, irrespective of the source and quality of the inflowing water. The present study was concerned with establishing why microbial contamination is so prevalent in DWUs, as well as interventions that reduce DWU contamination and achieve microbial water quality levels recommended by the ADA.

METHODS

Study design

The study consisted of two phases. The first (cross-sectional) phase was carried out to investigate the clinics in the Hawally Province Centers of Kuwait (Sabah El-Salem, Rumathyia, Salmiya, Salwa and Western Hawally Health Centers) during 2007–2008. Two hundred samples were collected and examined during this period. The second phase (cohort study) was carried out to evaluate the impact of the interventions at all Western Hawally Health Center clinics, and the possible ways of treating dental chair unit water, and to contribute to the growing body of information on DWU contamination. Two hundred and five samples were collected during the second part of the study (99 in 2007; 106 samples in 2008). Different interventions were applied during this study.

Interventions

Water in the main storage container, which received water from the main public water network, and was the source of water to the distillation apparatus was chlorinated using Actichlor™ disinfectant tablets (5.0 g tablets/250 ml water) to give a 1% concentration, according to the Antichlor manufacturer's instructions. Flushing of the hand-piece, the three-in-one syringe and other instruments by operating them continuously might be expected to decrease aspirated oral flora and other organisms in the distal part of the system. This was achieved by flushing water through the DUWLs for 2 min at the beginning of each day, and for 20–30 s after the treatment of each patient. All the water that was left in the water lines was drained off at the end of the day. Finally, we used freshly distilled water on a daily basis, directly from the distillation apparatus (stored distilled water was not used). Combining these strategies was necessary to control biofilm formation and to achieve the desired level of water quality. Western Hawally Health Center clinics were selected for these interventions because these clinics showed the highest level of contamination.

Water sampling

Dental chair units and hand-pieces were connected to bottled distilled water that had been in daily use. Dental unit waterlines samples were collected from two sites in four clinics: the chair scaler and the hand-piece in addition to a distillation apparatus source (as control) that feed all dental clinics in this center.

Water samples of 250 ml were discharged from the DUWLs in each clinic, just before the start and midway through the morning session. These were collected aseptically in sterile bottles that contained 0.1 g sodium thiosulfate to neutralize any residual chlorine or disinfectant in the water (Martin & Gallagher 2005; Pankhurst *et al.* 2005). Water samples were collected and delivered to the laboratory within 3 h of collection. Ten-fold serial dilutions of each sample were then made in a phosphate buffer solution and the sample was vigorously agitated by vortexing for 15 s. Aliquots of each dilution (0.1 ml) were plated on R2A Agar (Oxoid) in triplicate, for determination of total viable count (TVC), and incubated at 20–22 °C for 7–10 days. Total viable count was evaluated according to the ADA Standard for Dental Water (Anonymous 1996) and the European Drinking Water Standards (European Council Directive 98/83/EC 1998). A number of selective media were also used for the detection of coliforms, *E. coli* and *P. aeruginosa*, as described by Walker *et al.* (2000). Identification to species level was done by growth on selective agar, as described by Walker *et al.* (2003). The BioMerieux system API 20E was used for species identification of coliforms, *E. coli* and *P. aeruginosa*. Appropriate controls for each agar medium were evaluated for sterility by pouring onto agar plates with no samples for each time and temperature used. The colonies on each plate (TVC) were counted immediately after incubation.

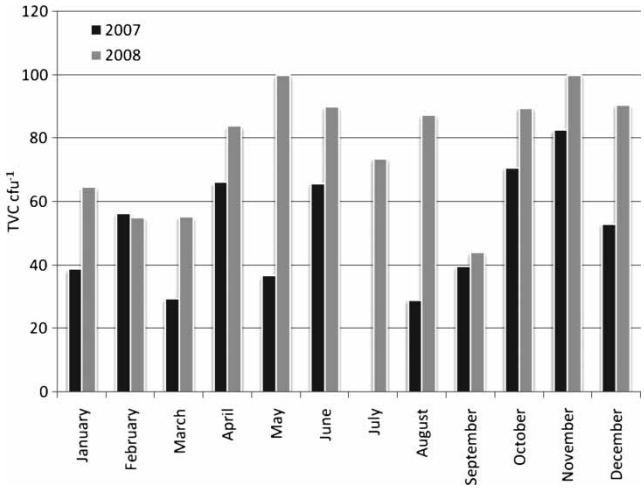

Figure 1 | The comparison between the total viable count (log CFU/ml) during the years 2007 and 2008.

Statistical methods

For statistical analysis, the TVC values were converted into $\log_{10}x$. Data analysis was carried out using SPSS for Windows version14 and Microsoft Office Excel 2003. The *t*-test (paired) was used to compare the bacteriological contamination of the two water lines in the four clinics, and the degree of contamination before and after intervention. The means of the cell counts before and after intervention were presented. A probability of $p < 0.05$ was considered statistically significant.

RESULTS

Two hundred samples were assessed from the centers of Sabah El-Salem, Rumathyia, Salmiya, Salwa and Western Hawally Health Centers during 2007–2008.

Figure 1 presents a comparison between the TVC (log CFU/ml) in 2007 and 2008. During 2007, 22 samples (32%) had a TVC above the permissive level according to the ADA and EU standards, whereas 46 (68%) had TVCs below the permissive level. In contrast, during 2008, 62 samples (46%) had TVCs below the permissive level, whereas 70 (53%) samples had TVCs that exceeded the ADA and EU standards. It is worth noting that 62 of 70 (88%) samples showed a TVC $>10^3$ log CFU/ml; five samples (7%) were contaminated with a mixed culture of *E. coli* (0.9%) and *P. aeruginosa* in addition to having a TVC above the standard level. Finally, three samples (4%) were contaminated with *P. aeruginosa* alone. It is concluded that the level of contamination increased during 2008 in comparison with 2007. This contamination (*E. coli*, coliforms and *P. aeruginosa*) was the reason that we focused on the highly contaminated clinics of Western Hawally Health Center during 2008 and the application of intervention during the year 2009.

One hundred and six water samples were collected from the Western Hawally Health Center during 2008, with the objective of estimating the microbial load during this period, before any intervention. Only 48 samples (45%) showed TVCs below the standards, whereas 58 samples (55%) had TVCs above the permissive level according to ADA and EU standards.

Table 1 | Total viable counts (log CFU ml^{-1}) of source waters

Clinic	No. of samples	% Positive samples	Mean log CFU/ml	Range log CFU/ml
Clinic 1				
Chair	11	73.2	2.7	2.0–3.0
Hand-piece	13	91.3	3.1	2.4–3.7
Clinic 2				
Chair	10	51.1	2.8	2.7–2.8
Hand-piece	12	96.9	2.7	1.5–3.8
Clinic 3				
Chair	13	50.4	2.8	2.7–2.9
Hand-piece	15	90.4	3.2	2.4–3.8
Clinic 4				
Chair	10	75.6	3.1	2.6–3.5
Hand-piece	10	58.6	3.5	3.4–3.5

The data illustrated in Table 1 show that the samples collected from hand-pieces were more contaminated than those from chair scaler water samples. The TVC were in the range 2.4–3.7 log CFU/ml in Clinic 1, 1.5–3.8 log CFU/ml in Clinic 2, 2.4–3.8 log CFU/ml in Clinic 3 and 3.4–3.5 log CFU/ml in Clinic 4. In chair scaler water samples, the lowest TVC was 2.07 log CFU/ml in Clinic 1, and the highest was 3.55 log CFU/ml in Clinic 4. *P. aeruginosa* isolates were detected in hand-piece water samples in Clinics 1 and 2 during October and April 2007 respectively. A paired *t*-test revealed a statistically significant difference between the TVC of the water samples that had been collected from high speed hand-pieces and chair water samples ($p < 0.05$).

Escherichia coli (0.9%) and coliforms (0.9%) were also detected in hand-piece water samples from Clinic 1. In 2008 and before intervention, microbial species of clinical significance were isolated from some waterlines, including total coliforms and *E. coli* that were isolated in October, and *P. aeruginosa* that was isolated in April and October. All of these pathogens were detected in samples that had been collected from hand-pieces.

Table 2 presents the percentage of samples that had TVCs below and above the ADA and EU permissive levels before and after intervention. Intervention took place in January–May 2009. A slight increase in TVC was observed in April and May. A total of 30 samples were collected during this period. Twenty-four samples (80%) showed TVCs below the ADA and EU permissive levels, and were lower than in 2008. Statistically, a *t*-test showed a significant difference before and after intervention ($p < 0.05$).

Table 2 | Percentage of samples having log CFU/ml below and above the permissive level of ADA & EU Standards before and after intervention

Month	Percentage of samples before intervention CFU/ml < 100 (%)	Percentage of samples before intervention CFU/ml > 100 (%)	Percentage of samples after intervention CFU/ml < 100 (%)	Percentage of samples after intervention CFU/ml > 100 (%)
January	50	50	80	20
February	75	25	75	25
March	50	50	100	0
April	25	75	87.5	12.5
May	0	100	66.7	33.3

DISCUSSION

This study investigated a limited number of dental units. The microbiological quality of water in units in general dental practice did not conform to accepted guidelines for potable water, which agrees with previous findings (Smith *et al.* 2002).

Dental unit waterlines and their water effluents were colonized by heterotrophic bacteria from water that was pumped from the public water supply to the waterlines. Human pathogens are returned to the DUWLs during dental treatment processes as a result of insufficient anti-retraction in dental units (Putnins *et al.* 2001). This leads to the proliferation of microorganisms on the inner surface of the DUWLs, and biofilm formation (Whitehouse *et al.* 1991).

The present study confirms that DUWLs are highly contaminated when dental units are in use for several months and receive no decontamination treatment. The amount of DUWL contamination differed between the hand-piece (high TVC) and chair (low TVC), which showed that some of the water samples that were taken had reached CFU/ml values above the limits of ADA

Heterotrophic bacteria and oral pathogens can be responsible for severe enteric diseases such as diarrhea,

and immunocompromised patients are particularly at high risk (Mills 2000). Waterborne pathogens were detected in the present study: *P. aeruginosa* (in hand-pieces), coliforms and *E. coli*. *P. aeruginosa* has been reported in other studies (Barbeau *et al*. 1996), and there has been a documented case of infection with this opportunistic pathogen, which was associated with DUWLs (Pankhurst *et al*. 2005). This was in a medically compromised patient, who might have been more susceptible to infection in this type of setting. Coliforms and *E. coli* were isolated in small number of water samples from hand-pieces that were supplied with water that was stored in the open and in a highly contaminated storage area.

Factors inherent to equipment in the dental unit, such as age and model, frequency of usage, water source, presence or absence of anti-retraction/check valves, and the position of the water bottle, are thought to account for variability in bacterial distribution and numbers (Robert *et al*. 1994). We questioned the effectiveness of flushing and disinfection because high levels of bacteria were isolated from hand-piece water samples that had been regularly flushed and/or disinfected. Flushing temporarily reduces microbial counts in the DUWLs, but might not reduce biofilm formation in the tubing. This might be due to the fact that biofilms often enclose bacteria within carbohydrate-containing material (Xu *et al*. 2003) that protects them from the effect of disinfectants, or lack of physical removal of the biofilm. Others have shown that flushing fails to reduce DUWL microbial contamination by >9% (Walker *et al*. 2003). In addition, bacterial counts can increase; for example, when portions of biofilm detach from the inside layer of the tubes and slough off into the water (Mills 2000). For this reason dental units should be re-designed to minimize the build-up of biofilms (Smith *et al*. 2002). Dental water units cannot be considered microbiologically clean because biofilms might contaminate the water with bacteria and vice versa (Walker *et al*. 2000). One major concern is whether these bacteria are pathogenic. Therefore, dental specialists should make concerted efforts to ensure that water of potable quality emerges from the DWUs. This is due to the fact that once the bacteria succeed in entering the system, and sufficient nutrients from the plastic tubing occur, the bacteria will turnover to support biofilm growth (Colbourne *et al*. 1984).

There have been several efforts to decrease the microbial contamination of DUWLs: flushing of the unit prior to use; autoclaving of hand-pieces; replacement of the hand-piece between patients; anti-contamination devices to prevent oral secretions from entering the DUWL; connection to a separate water supply (e.g. to bottles of distilled water); chemical or UV disinfection; and the use of water filters (Lux 2008a, b). These decontamination practices include best practice that aims to achieve higher standards in infection control through continuous improvements, cleaning methods, washer-disinfectors, ultrasonic cleaning, manual cleaning, inspection, sterilization, instrument storage and decontamination areas. These are in addition to general practice principles such as hand hygiene, personal protective equipment (gloves, disposable plastic apron, face and eye protection, clothing, uniforms, laundry) and surface and equipment decontamination. Flushing the hand-piece with water before use can reduce bacterial counts (Smith *et al*. 2002). However, as demonstrated in the present study, a high level of microbial contamination can persist even after applying the recommended flush times. The most important factor that affects microbial contamination of DUWLs is water stagnation inside the equipment lines, which can allow bacteria to propagate within a biofilm.

Dental chairs and units are considered to be medical devices and must meet the appropriate requirements. This includes cleaning, disinfection or sterilization instructions. It is also clear from the present study that, regardless of the method of decontamination selected, it is important to ensure that it is stringently applied in a busy general practice setting.

CONCLUSIONS

Flushing the water line for two minutes before the first patient and for 10–20 s between patients is recommended so that the number of bacteria in the water line can be decreased. However, this reduction is only temporary because the microorganisms will multiply back in a very short time. Therefore, an alternative strategy could be a flushing of the dental unit at the end of the day and draining it overnight to reduce the development of the biofilm. It is also recommended to daily prepare freshly distilled water in addition to chlorination.

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