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The presence of *Pseudomonas aeruginosa* in the dental unit waterline systems of teaching clinics

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Al-Hiyasat AS, Ma'ayeh SY, Hindiyeh MY, Khader YS. The presence of *Pseudomonas aeruginosa* in the dental unit waterline systems of teaching clinics

Abstract: *Objective:* The objective of this study was to evaluate the extent of *Pseudomonas aeruginosa* contamination of Dental Unit Water (DUW) at a Dental Teaching Center in Jordan. *Methods:* Water samples were collected from 30 dental units, 10 from each of three teaching clinics, namely conservative dentistry, periodontology, and prosthodontics. Samples were collected from the outlet of the air/water syringe, high-speed handpiece and water cup filler, at the beginning of the working day (before use), after 2 min flushing, and at midday. *Results: P. 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 min of flushing and at midday. Conservative dentistry units had the highest counts, followed by periodontology and prosthodontics ($P < 0.05$). Overall, the highest counts ($\log_{10}$ count CFU ml^{-1}) were at the beginning of the working day (1.38 ± 1.05), and the lowest counts after flushing for 2 min (1.10 ± 1.03), and higher numbers were seen again at midday (1.15 ± 1.04) ($P < 0.05$). *Conclusions:* 86.7% of the dental units were contaminated with *P. aeruginosa*, the conservative dentistry units had the highest amount of contamination. Flushing the DUW for 2 min significantly reduced the counts of *P. aeruginosa*.

Key words: dental unit waterline; *Pseudomonas aeruginosa*; teaching clinics

Introduction

The presence of opportunistic and pathogenic bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Legionella*

pneumophila in dental units' water (DUW) has been reported by several previous studies (1–5). One important pathogen, *P. aeruginosa*, is known to be often associated with nosocomial infections (6–8). Despite the fact that *P. aeruginosa* has been isolated from various DUW systems and can reach levels of up to 2×10^5 CFU ml^{-1} (4, 9–13), the source of this contamination could not be due solely to the water source (14–17) as *P. aeruginosa* is present in the oral cavity and can be aspirated back from the patients mouth into the dental unit waterline (DUWL) through a defective check valve (4).

Once a pathogen, such as *P. aeruginosa*, reaches the wall of the tubing of DUWLs, a colonization process begins whereby the bacteria grow and multiply in the biofilm (18). The formation of microcolonies may also lead to an increased level of *P. aeruginosa* in the water that bathes the biofilm. However, the risk of infection lies in the bacteria that are shed from the biofilm and leave the waterline (13). The competitive advantage of *P. aeruginosa* in the colonization of the waterlines is due to the observed capacity of *P. aeruginosa* to inhibit the growth of other bacteria isolated from the waterlines by bacteriocin production (19).

Dental unit water (DUW) may be ingested, inhaled in the form of aerosols or directly contaminate surgical wounds in the mouth (20). *Pseudomonas aeruginosa* derived from DUW has definitely been shown to cause infection (20). Two patients with solid tumours were unwittingly exposed to DUW contaminated with *P. aeruginosa*. Both patients subsequently developed oral abscesses, which pyocine typing confirmed to be caused by the same strain isolated from the DUW (10). *Pseudomonas* species can be transmitted to susceptible patients via direct contact with water (21–24) or after exposure to residual waterborne contamination of inadequately reprocessed medical instruments (25–27).

This current study was conducted is order to evaluate the extent of *P. aeruginosa* contamination in the DUW that both patients and the dental staff are exposed to at the Dental Teaching Center of Jordan University of Science and Technology. In addition, this study aimed to evaluate the level of contamination of DUW in three different specialty teaching clinics.

Materials and methods

Experimental design

Thirty of the most commonly used dental units in the Dental Teaching Center of Jordan University of Science and Technology in Irbid, Jordan, were used for this study. Samples were collected from 10 dental units from each of three groups of teaching clinics including; conservative and pedodontics as a first group and was referred as conservative dentistry, the second group was the periodontology and the third was the prosthodontics. The water samples were collected from the outlet of air/water syringe, high-speed handpiece and water cup filler. Samples were taken at the beginning of the working day (before use of the dental unit), after 2 min of waterline flushing, and at midday when the dental units were being used (after 20 s of DUWLs flushing). In addition, the water source supplying the dental centre was examined (two water tanks and two respective softeners). A 100-ml water sample was taken weekly over the sampling period (3 months) from the water tanks before the entrance of water to the softener filter, and after being softened through an attached faucet. Both temperature and residual chlorine concentration (DPD colorimetric method) were measured for the water tanks, softeners and DUW samples.

Samples collection and plating

Before sample collection the end of each handpiece was disinfected with 70% alcohol to avoid other sources of contamination. A volume of 150 ml of water was collected in sterile containers, containing 0.1 gm/100 ml of sodium thiosulfate ($Na_2S_2O_3{\cdot}5H_2O$) to remove residual chlorine. Water splashing was minimized when filling the container and any contact between the handpiece and the container was avoided. Samples were kept refrigerated at 4–8°C during transfer, and processed directly after arriving to the laboratory in a period of less than 2 h. Water samples (100 ml) were filtered using a 0.45-μm nitro-cellulose membrane filter (Sartorius, Goettingen, Germany). The filters were aseptically removed with sterile forceps and directly plated on cetrimide agar base plates. The plates were incubated for 24–48 h at 37°C; the colonies were enumerated and recorded as CFU of *P. aeruginosa* per ml.

Verification of *P. aeruginosa* colonies

The isolated colonies were subjected to biochemical identification using API 20NE system (Biomerieux, Marcy L'Etoìle, France), in addition to Gram stain, catalase and oxidase reaction, pyocyanin production, casein milk hydrolysis and fluoresceine detection (UV light of 280 nm). *Pseudomonas aeruginosa* (ATCC 27853) was used as a reference in the confirmatory testing.

Statistical analysis

For statistical analyses of the data, the Statistical Package for Social Sciences software (SPSS, version 11.5: SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel 2003 were used. Because 30 dental units were sampled repeatedly, General Linear Model (GLM) repeated measures was used to analyze data. No data were missing. Results of evaluation of assumptions led to logarithmic transformation of *P. aeruginosa* count ($\log_{10}$ count) to improve the normality. The model comprises the explanatory variables sampling time (at the beginning of working day, after 2 min of flushing and at midday), source of sample (water syringe, high-speed handpiece and the water cup filler) and clinic (conservative dentistry, prosthodontics and periodontology). Cells count means for the dependent variable on the original and logarithmic scales over all combinations of time, source and clinic were presented. Bonferroni test was used to perform pairwise comparisons between group means on the log scale. A value of $P < 0.05$ was considered statistically significant.

Results

Pseudomonas aeruginosa was not detected in any 100-ml sample obtained from the first water tank (located on the ground floor) that supplies the prosthodontic and the conservative clinics neither from its softened water samples. However, the second water tank (located on the roof of the centre) that supplies the pedodontics and periodontology clinics harboured *P. aeruginosa* with an average of 9 ± 2 CFU ml^{-1} while an average of 100 ± 9 CFU ml^{-1} was found for its softened water samples. Statistically significant differences were found between the second water tank and its softened water samples (Paired *t*-test, $P < 0.001$). The water temperature was 24 ± 1°C for the tanks and softeners samples, and the residual chlorine concentration was measured to be steady over the sampling period for the first and the second water tank (0.3 and 0.1 mg l^{-1} respectively). However, both softener samples residual chlorine content was zero. On the other hand, the measured temperature of the DUW of conservative, pedodontics and periodontology clinics was 34 ± 1°C, while it was 24 ± 1°C for the prosthodontics clinic. All of the other examined DUW samples had a zero residual chlorine concentration.

Pseudomonas aeruginosa counts obtained from the DUW samples of the air/water syringe, high-speed handpiece and the water cup filler at three sampling times (at the beginning of working day (before use), after 2 min of flushing and at midday) in conservative dentistry (conservative and pedodontics), prosthodontic and periodontology clinics ranged between 0 and 9.4×10^3 CFU ml^{-1}. *Pseudomonas aeruginosa* was detected in 86.7% (26/30) of the dental units at the beginning of working day, in 73.3% (22/30) after 2 min of flushing and 73.3% (22/30) at midday. At the beginning of the working day, all dental units in the conservative dentistry and periodontology clinics were contaminated with this micro-organism. Of the total positive samples at the beginning of working day, 60.9% had *P. aeruginosa* counts <100 CFU ml^{-1} and 39.1% had counts >100 CFU ml^{-1}. In addition, 63.6% and 59.1% of the water samples after 2 min of flushing and at midday, respectively, were contaminated at low levels (<100 CFU ml^{-1}). The two respective sampling periods had 36.4% and 40.9% of the DUW samples heavily contaminated with *P. aeruginosa* (>100 CFU ml^{-1}). Tables 1–3 represent the mean plate counts in categories for the dental units investigated.

Table 1. ***Pseudomonas aeruginosa*** **mean plate counts obtained from the air–water syringe in the conservative dentistry, prosthodontic and periodontology clinics at the three sampling times (before use, after 2 min of flushing and at midday)**

		Pseudomonas aeruginosa mean plate counts* (CFU ml^{-1})				
Clinic	No. of units	0	1–10	11–100	101–1000	>1000
Before use						
Conservative	10	0	0	1	6	3
Prosthodontics	10	6	1	3	0	0
Periodontology	10	1	4	5	0	0
After 2 min of flushing						
Conservative	10	0	0	2	6	2
Prosthodontics	10	6	2	2	0	0
Periodontology	10	2	7	1	0	0
At midday						
Conservative	10	0	0	1	7	2
Prosthodontics	10	6	2	2	0	0
Periodontology	10	2	6	2	0	0

*Values represent the number of units that were contaminated within the range of the column title.

Table 2. ***Pseudomonas aeruginosa*** **mean plate counts obtained from the high-speed handpiece in the conservative dentistry, prosthodontic and periodontology clinics at the three sampling times (before use, after 2 min of flushing and at midday)**

		Pseudomonas aeruginosa mean plate counts* (CFU ml^{-1})				
Clinic	No. of units	0	1–10	11–100	101–1000	>1000
Before use						
Conservative	10	0	0	1	6	3
Prosthodontics	10	3	5	2	0	0
Periodontology	10	1	7	2	0	0
After 2 min of flushing						
Conservative	10	0	0	2	8	0
Prosthodontics	10	4	5	1	0	0
Periodontology	10	5	3	2	0	0
At midday						
Conservative	10	0	0	1	9	0
Prosthodontics	10	4	5	1	0	0
Periodontology	10	5	3	2	0	0

*Values represent the number of units that were contaminated within the range of the column title.

Table 3. ***Pseudomonas aeruginosa*** **mean plate counts obtained from the water cup filler in the conservative dentistry, prosthodontic and periodontology clinics at the three sampling times (before use, after 2 min of flushing and at midday)**

		Pseudomonas aeruginosa mean plate counts* (CFU ml^{-1})				
Clinic	No. of units	0	1–10	11–100	101–1000	>1000
Before use						
Conservative	10	0	0	2	6	2
Prosthodontics	10	4	4	2	0	0
Periodontology	10	0	4	6	0	0
After 2 min of flushing						
Conservative	10	0	0	2	8	0
Prosthodontics	10	6	3	1	0	0
Periodontology	10	3	4	3	0	0
At midday						
Conservative	10	0	0	2	8	0
Prosthodontics	10	5	3	2	0	0
Periodontology	10	3	3	4	0	0

*Values represent the number of units that were contaminated within the range of the column title.

The averages of bacterial counts in DUW samples (original and log scales) according to the type of clinic, source of the sample and sampling time are depicted in Table 4. *Pseudomonas aeruginosa* was unevenly distributed among the three different clinics at the Dental Teaching Center, with the conservative dentistry having the highest counts on the log scale at the three sampling times, followed by periodontology and prosthodontic clinics. Moreover, the air/water syringe demonstrated the highest overall mean counts on the log scale at the three sampling times for all the clinics (1.24 ± 0.49 CFU ml^{-1}) followed by the high-speed handpiece (1.20 ± 0.48 CFU ml^{-1}) and the water cup filler (1.19 ± 0.48 CFU ml^{-1}). Overall, the highest *P. aeruginosa* counts were obtained at the beginning of the working day ($\log_{10}$ count 1.38 ± 1.05 CFU ml^{-1}), followed by reduced counts in response to flushing for 2 min ($\log_{10}$ count 1.10 ± 1.03 CFU ml^{-1}), and then regaining higher numbers at midday ($\log_{10}$ count 1.15 ± 1.04 CFU ml^{-1}) that remained lower than those obtained at the beginning of the working day.

Figure 1 shows the profiles for the three sampling times over the three clinics. For all clinics, the log count was the highest at the beginning of the day (before use). Purging the lines for 2 min reduced the $\log_{10}$ count from 2.64 to 2.41 CFU ml^{-1} in conservative dentistry, from 0.55 to 0.38 CFU ml^{-1} in prosthodontics, and from 0.94 to 0.52 CFU ml^{-1} in periodontology. At midday, the log counts increased but remained lower than that obtained at the beginning of working day for all clinics.

The results of GLM repeated measures analysis showed a statistically significant clinic by sampling time interaction ($P < 0.001$). Although time and clinic main effects also were

Table 4. **The averages of bacterial counts (CFU ml^{-1}) in dental unit waterline samples on the original and log scales according to the type of clinic, source of the sample and sampling time**

		Sampling time					
		At the beginning of working day (before use)		After flushing for 2 min		At midday	
Clinic	Source of sample	Original scale, Mean	Log_{10} scale, Mean ± SD	Original scale, Mean	Log_{10} scale, Mean ± SD	Original scale, Mean	Log_{10} scale, Mean ± SD
Conservative	Air/water syringe	1850.80	2.73 ± 0.69	460.40	2.48 ± 0.43	505.90	2.51 ± 0.43
	High-speed handpiece	1484.50	2.68 ± 0.65	357.30	2.42 ± 0.38	414.10	2.48 ± 0.39
	Water cup filler	609.70	2.50 ± 0.51	306.50	2.32 ± 0.41	351.50	2.37 ± 0.42
	Total	1315.00	2.64 ± 0.61	374.73	2.41 ± 0.40	423.83	2.45 ± 0.40
Prosthodontics	Air/water syringe	5.70	0.47 ± 0.61	3.60	0.39 ± 0.51	4.00	0.41 ± 0.53
	High-speed handpiece	6.20	0.65 ± 0.50	3.60	0.47 ± 0.45	4.20	0.50 ± 0.48
	Water cup filler	5.20	0.53 ± 0.53	2.30	0.29 ± 0.43	2.90	0.34 ± 0.45
	Total	5.70	0.55 ± 0.53	3.17	0.38 ± 0.46	3.70	0.42 ± 0.48
Periodontology	Air/water syringe	11.60	0.98 ± 0.42	3.90	0.56 ± 0.38	5.10	0.64 ± 0.40
	High-speed handpiece	8.30	0.80 ± 0.44	4.10	0.38 ± 0.51	4.50	0.41 ± 0.53
	Water cup filler	15.40	1.06 ± 0.44	7.50	0.63 ± 0.56	8.80	0.70 ± 0.58
	Total	11.77	0.94 ± 0.43	5.17	0.52 ± 0.49	6.13	0.59 ± 0.51

Fig 1. The profiles of *Pseudomonas aeruginosa* for the three sampling times over the three clinics.

statistically significant, they were not interpreted in the presence of strong interaction. All other 2- and 3-way interactions were not statistically significant ($P > 0.05$). Furthermore, there was no significant difference in the average log counts between the three types of water source of the dental units ($P = 0.915$).

Pairwise comparisons using Bonferroni test showed a significant difference in the means of the log counts between the three different sampling times for all clinics. Bonferroni test showed statistically significant differences in the log counts at the beginning of the day between the three clinics with the highest significant differences between conservative dentistry and prosthodontics. However, the log counts after flushing for 2 min and at midday was significantly different between conservative dentistry and the other two clinics but not between prosthodontic and periodontology clinics.

Discussion

The main source of water used in the Dental Teaching Centre is coming from the municipal authority in Irbid Province which uses chlorination as a method for water disinfection. This water is treated in a softening system mounted at the main water pipeline supplying the centre to remove particles that may damage and corrode the dental units and water pipelines, then distributed to the clinics in the centre. The concentration of residual chlorine in the main water source lied within the international standards for drinking water (0.2–0.5 mg l^{-1}) (28). However, a slight reduction in the resid-

ual chlorine concentration was noticed in the second water tank samples of the dental centre which can be ascribed to the instability of chlorine particles as they usually dissipate in 15 min after its addition to the distribution systems during the purging process (29). Moreover, the chlorine may have sedimented during water flow inside the pipelines or ascending to high buildings as the roof of the Dental Teaching Center (29). The absence of residual chlorine in both softeners can also be ascribed to chlorine dissipation, and the possible removal of calcium carbonate ($CaCO_3$) and many other salts that can combine with the chlorine leading to its loss in the softener salt discarding system. None of the DUWLs samples contained chlorine as it is already dissipated within the softening system and the water tanks. Higher temperatures were found for the DUWLs samples of conservative dentistry and periodontology clinics (34 ± 1°C) in comparison with the water source, the softened water samples, and those of the prosthodontic clinic (24 ± 1°C). Such elevation in DUW samples temperature is due to the fact that the Sirona dental units (Sirona C6, in used since 2001) that are used in the conservative, pedodontics and periodontology clinics posses a heating system to warm water in the dental units. Such heating systems can comprise a favourable condition for microbial amplification inside the tubes of dental units. In comparison, the dental units that were used in the prosthodontic clinic were from the A-dec and the Castellini type (in used since 1995) that lack or not functioning water heating systems. Stampi *et al.* (30) reported that the residual chlorine tends to diminish within the apparatus (dental unit) and they recommended the addition of chlorine in high doses to the water systems using the softening and heating to circumvent problems associated with chlorine dissipation and contamination within the softening system it self that can affect the supplied dental units (5).

Pseudomonas aeruginosa was not detected in first water tank neither in its softened water samples which can be linked to the high quality of the water source. Similarly, Barbeau *et al.* (4) could not have detected *P. aeruginosa* in the tap water samples (control) in his study. However, the presence of *P. aeruginosa* in second water tank can be ascribed to the decrease in the residual chlorine concentration which when present in sufficient amounts has an inhibitory effect to *P. aeruginosa* growth (31) combined with the ability of *P. aeruginosa* to thrive even in low nutrient environments, such as distilled water (20). The increase in *P. aeruginosa* counts from 9 ± 2 to 100 ± 9 CFU ml^{-1} in the second softener can be justified by the fact that the softener filter may constitute an environment suitable for amplifying *P. aeruginosa* when this micro-organism uses the accumulated nutrients and minerals from water to grow.

Furthermore, it should also be mentioned that due to the similarity of the types of treatment performed in both the conservative and pedodontic clinics, the data for both clinics (5 units each) were pooled and analyzed under one group (conservative dentistry). Indeed, our preliminary analysis of the data for *P. aeruginosa* counts in samples collected from the outlet of air/water syringe, high-speed handpiece and water cup filler showed no significant differences ($P > 0.05$) between the conservative and pedodontic clinics at the three sampling times.

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that has been isolated from various DUW systems (4, 10–12). In this study, *P. aeruginosa* was detected in 86.7% of the dental units at the beginning of the working day, and in 73.3% after 2 min of flushing and at midday. Barbeau *et al.* (4) reported that *P. aeruginosa* was isolated from 24% of the all the tested dental units and from 62% of the removable prosthodontic units. The high percentage of contamination of the dental unit tested in this study can be related mainly to the level of efficiency of the anti-retraction valves, and also to the heating system in the dental units (conservative and periodontology) in addition to the presence of softener that may act as source of contamination when the water passes through, that act collectively in raising the counts to higher rates than those reported in previous studies (4, 5) Indeed, Stampi *et al.* (5) found a notable growth of *P. aeruginosa* in softened and heated waters of the dental units.

Pseudomonas aeruginosa counts obtained from the dental units in the present study were less than those reported by Barbeau (2×10^5 CFU ml^{-1}) (13). The conservative dentistry clinics had higher log mean counts in comparison with the periodontology and prosthodontic clinics which can be ascribed to the more invasive treatments performed in the former clinic which can introduce organic fluids (saliva, blood, pus, tissue, etc.) into the DUWLs that promote bacterial amplification within the dental unit tubing system (32–34). Studies of high-speed handpieces using dye expulsion have confirmed the potential for retracting oral fluids into internal compartments of the device (35–39). This finding suggests that retained patient material may be expelled intraorally during subsequent uses. In turn, the mean counts in the periodontology clinic was higher than that in the prosthodontic clinic where less work is done for the preparation of the occlusal rest in the chrome-cobalt work in comparison with teeth polishing, root planning and periosurgery performed in the periodontology clinic. Moreover, the increase in water temperature (10 ± 1°C) of the conservative dentistry and periodontology dental unit may play a role in increasing *P. aeruginosa* counts in these two clinics as reported in a previous study (5).

The air/water syringe showed the highest overall mean counts followed by the high-speed handpiece and the water cup filler, although the difference was not statistically significant. The higher contamination rate for the air/water syringe can be ruled out due to its higher utilization rate in the conservative and periodontology clinics. Moreover, the bore size of the air/water syringe is larger than the high-speed handpiece. Consequently, it can be proposed that *P. aeruginosa* which can be recovered from the oral cavity of 4% of healthy individuals (4) can be flown back into the DUWLs and in higher counts to the air/water syringe through the opening of the nozzle due to the negative pressure that may form at the end of flushing (40). Moreover, it has been reported that about 1 ml of microbe laden oral fluids might be aspirated back from the patient's mouth, when each time the air syringe or turbine is stopped while it is fitted into the patient's mouth (40). This fluid may contain an average more than 54 000 micro-organisms per ml, including both facultative and obligate anaerobic bacteria from medium to high virulence (40). Robert *et al.* (41) reported that the air/water syringe was more contaminated than the high-speed drill, which was ascribed to low pressure and water flow leading to the accumulation of bacteria in the tubes. However, Barbeau *et al.* (4) reported that the high-speed drill had higher mean counts for *P. aeruginosa* than the air/water syringe proposing that less often a waterline is used, the greater the chance it has to be colonized by *P. aeruginosa*. The difference in the results obtained in this study and those of Barbeau *et al.* (4) could be ascribed also to the frequency and type of treatments performed by the dental students in our clinics. The air/water syringe was frequently in use on a day-to-day basis, and it was used for each patient throughout the treatment procedure in the conservative dentistry clinics, in particular to wash or dry the operating field, which may increase the risk of contamination. While in the previous study (4) the dental units investigated were perhaps used to perform general dental treatments, which mean that the frequency and the nature of use of the air/water syringe and the high-speed handpiece may have differed.

Flushing the DUWLs for 2 min was found to be significantly effective in reducing *P. aeruginosa* counts in this study. However, this process can be proportional and dependent upon the bore size of the handpieces and the water cup filler, which consequently determines the amount of water that can flush in a certain period of time. The Center for Disease Control and Prevention (42) and the British Dental Association (43) recommended that simple flushing for 1 or 2 min can be the first line to decontaminate the DUWLs and for 20–30 s between the patients' treatments. Moreover, many reports dealing with DUWL contamination with heterotrophic bacteria have shown that draining the waterlines for several minutes reduces the heterotrophic plate counts (HPC) significantly (14, 16, 41, 44). Barbeau *et al.* (4) reported that purging the DUWLs for several minutes can reduce the HPC and reported that the first 2 min of flushing produced a decrease of over 96% (from 5.53 to 4.11 CFU ml^{-1} on log scale). Similarly, flushing can be carried out in the dental units to reduce *P. aeruginosa* counts.

The reason for the higher colonization rate (bacterial counts) of the DUWL with *P. aeruginosa* at the beginning of the working day can be justified by the fact that the *P. aeruginosa* was amplified over the night period aided by the accumulated organic particles found in water and water stagnation inside the waterlines leading to an increase in the number of colonies that batches the biofilm (18, 45). Flushing will pose a pressure on the biofilm to detach the weakly attached bacteria and therefore to decrease *P. aeruginosa* counts. However, the use of the waterlines has raised the count slightly which may be supported by the fact that new *P. aeruginosa* cells have been introduced from the patients mouth into the waterlines through a defective anti-retraction valve, back siphonage of oral fluids into the water and biofilm phase (46), and/or more biofilm sloughed bacteria were shed into the water as a result of continuous use of the waterlines when high numbers of patients were being treated. Therefore, the rate of contamination of the water sample obtained from the dental units of different clinics is overall related to the flow and quantity of the water used and the capacity for washing and removal of biofilm within the waterline (30).

The interpretations of the data and the statistical analysis demonstrate the significant effect of the time of sampling and the clinical procedures performed at each clinic. The significant differences in the time of sampling can be ascribed to a correspondence with flushing. *Pseudomonas aeruginosa* counts obtained at the beginning of working day were significantly higher than those obtained at each of the 2 min flushing period and midday in the three clinics investigated. While the significant clinical effect can be ascribed to the different procedures and the degree of invasiveness of the treatment performed in each clinic, and consequently the possible back flow of *P. aeruginosa* from the patients' mouth to the DUWLs. Thus, *P. aeruginosa* contamination rate was significantly higher in the conservative dentistry clinics in comparison with each of the prosthodontic and periodontology clinics at the three sampling times. The presence of significant interaction between time and clinic suggested that *P. aeruginosa* counts obtained at each sampling time depend on the clinic type from which the water samples were obtained.

The infective dose of *P. aeruginosa* for healthy individuals ranges between 10^6 and 10^{10} CFU ml^{-1} (47). This dosage may become much less in the case of immunocompromised individuals. The presence of a pathogen, such as *P. aeruginosa*, in water used for intraoral or invasive treatments indicates that the used water is of low quality and may pose a threat to both patients and the dental team. Heating water must be reconsidered by dental units' manufacturers and dental professionals to overcome problems associated with temperature-enhanced microbial amplification. Moreover, water softeners must be checked and maintained. Anti-retraction valves must be maintained regularly to overcome problems associated with the flow back of bacteria from patient mouth into the DUWLs. In addition, a disinfection method must be applied to reduce or eliminate biofilms harbouring *P. aeruginosa* organisms (48–53). The use of microbial filters fitted into the handpieces may be a good solution to prevent microbial access into the open wounds and oral lesions of the patients' mouth.

Conclusions

Based on the results and the conditions of the present study the following could be concluded:

1 At the beginning of the working day and before use, 86.7% of the dental units investigated were found contaminated with *P. aeruginosa*.

2 The dental unit at the conservative dentistry clinics revealed significantly higher counts of *P. aeruginosa* than the periodontology and prosthodontic clinics, and this could be related to the invasive treatments performed at the conservative clinics.

3 Flushing the DUWL system for 2 min significantly reduced the counts of *P. aeruginosa*.

4 The results and the interpretation of this study may indicate that the source of contamination with *P. aeruginosa* could be from the patient mouth. Thus, the dental units anti-retraction valves may have failed to prevent fully the back flow of bacteria and oral fluids and therefore must be maintained or replaced regularly.

5 Disinfection methods of DUWL system should be considered and applied to eliminate biofilms harbouring *P. aeruginosa* organisms, and to prevent or reduce the exposure of the patients and the dental staff to these opportunistic and pathogenic bacteria.

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