التلوث الجرثومي لأنابيب مياه الوحدة السنية وأجهزة التقليح فوق الصوتي

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الملخص

الهدف: هدفت هذه الدراسة إلى تقييم التلوث الجرثومي لأنابيب مياه وحدات سنية في عيادات مختلفة في مدينة دمشق

الطرائق: تم جمع 114 عينة مياه من 25 وحدة سنية، 12 وحدة سنية موجودة ضمن عيادات مختلفة في كلية طب الأسنان - جامعة دمشق، و13 وحدة سنية موجودة في عيادات خاصة، أخذت عينات المياه من أجهزة التقليح فوق الصوتية، وقبضات سنية توريينية، ومحقنة هواء/ ماء، ومحقنة هواء/ ماء بعد 3 دقائق من إسالة المياه، وكأس المريض. حُضنَت العينات على صفائح من الآغار المدمى، وبعدها جرى تعداد الوحدات المشكلة للمستعمرات cfu/ml النامية على سطح الآغار.

النتائج: كانت 97،4% من عينات هذه الدراسة ملوثة جرثومياً بمقدار تراوح بين 1000-cfu/ml 350000 ، بمتوسط بلغ 25900 cfu/ml، سجلت أعلى معدلات التلوث الجرثومي في عينات مياه مأخوذة من وحدات سنية في قسم جراحة الفكين في كلية طب الأسنان في جامعة دمشق في حين أكثر المآخذ تلوثاً كانت أجهزة التقليح فوق الصوتي ومحقنة هواء/ ماء بلغ التعداد الجرثومي في عينات أجهزة التقليح فوق

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الصوتي مقداراً تراوح بين 4000-cfu/ml 260000 ، بمتوسط 55214 cfu/ml . وجدت مستعمرات Pseudomonas في 32% من الوحدات السنية المدروسة. يؤدي إسالة المياه مدة 3 دقائق إلى تراجع في عدد الجراثيم الموجودة في أنابيب مياه الوحدة السنية.

الاستنتاجات: تثبت هذه الدراسة أنَّ مياه أنابيب الوحدات السنية وأجهزة التقليح فوق الصوتية ملوثة بشدة، مما يؤكد ضرورة اتباع تدابير تطهير مياه الوحدة السنية.

Microbial Contamination of Dental Unit-and Ultrasonic Scaler Waterlines

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Abstract

Objective: This study aimed to evaluate the microbial contamination in dental unit water lines (DUWL) at different clinics in Damascus.

Methods: 114 water samples were collected from 25 dental units, twelve of the dental units were located in different clinics at the Damascus University, faculty of dentistry, and the other 13 were located in private practices. Samples were collected from ultrasonic scalers, dental high-speed handpieces, air/water syringes, air/water syringes after 3 minute flushing, and cup fillers. Samples were incubated on blood agar plates, then the numbers of colony forming units (cfu/ml) grown were counted.

Results: 97, 4% of the collected samples were contaminated in the range of 1000-350000 cfu/ml, with a median count of 25900 cfu/ml. The highest contamination was detected in samples taken from the oral surgery clinics at the faculty of dentistry. While the highest contaminated sources of dental water were the ultrasound scalers and the air/water syringe outlets. Samples from ultrasound scalers were contaminated in the range of 4000 - to 260000 cfu/ml, with a median count of 55214 cfu/ml. The presence of pseudomonas organisms was detected in 32% of the dental units. Flushing for three minutes can reduce the levels of bacteria present in dental waterlines.

Conclusions: samples from dental unit waterlines and ultrasonic scalers were highly contaminated, indicated the necessity of using antimicrobial procedures.

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Many studies demonstrated that dental unit waterlines are often contaminated with high densities of microorganisms $^{(1, 2, 3)}$. Untreated dental unit waterlines (DUWL) host biofilms that serves as a reservoir of bacteria that are dispersed through the water network $^{(1)}$. The age of dental unit does not appear to influence the level of microbial contamination $^{(2)}$.

Microbial quality of dental unit water is a concern to dental practitioners and patients because patients and dental staff are regularly exposed to water and aerosols generated by the unit ⁽⁴⁾.

Among the microorganism involved in environmental contamination, Legionella and Pseudomonas species are considered an important infectious hazard, and they was found in dental waterlines in many samples collected from many cities ^(3, 5, 6, 7).

Legionella was found in 23,9% of water samples collected from 6 Italian cities ⁽⁸⁾. Seventy six Pseudomonas aeruginosa strains were isolated from the dental practice environment and water systems in samples collected in Sao Paulo, Brazil ⁽⁹⁾. In dental literature the reported prevalence of Legionellae in DUWL vary widely from 0 to 68% ⁽¹⁰⁾. Also different species of fungi have been isolated in DUWL ⁽¹¹⁾. Exposure to these microorganisms within the dental practice possesses a potential health risk especially to immunocompromised patients, young children, the elderly, or those with chronic illness such as cancer or diabetes ⁽¹²⁾.

On the biological level the cell walls of Gram-negative bacteria are composed of an outer membrane made mostly of lipopolysaccharides (LPS, endotoxins). LPS stimulates the release of some proinflammatory cytokines (TNF- α , IL-1, IL-6). With high level of PLS, fever and tachycardia are induced ⁽¹³⁾. Dental waterlines contain large numbers of such organisms (LPS). The LPS level was reduced by 70% with 1 minute of flushing, but flushing times of 5 and 10 minutes were not able to reduce LPS levels to zero⁽¹⁾. A statistically significant correlation was demonstrated between bacterial load and endotoxins ⁽¹⁴⁾.

In 1993, the CDC⁽¹⁵⁾ recommended that dental waterlines be flushed at the beginning of the clinic day to reduce the microbial load. However, this procedure does not affect the amount of biofilm in the waterlines. Commercial devices and procedures designed to improve the quality of water used in dental treatment are available. They include chemical disinfectants, self-contained water systems combined with chemical treatment, anti-retraction valves, sterile water delivery systems, in-line microfilters, and combinations of these treatments ^(15, 16, 17, 18, 19, 20, 21). Even chemical treated waterlines need to be monitored regularly ⁽²²⁾.

In 1995, the ADA addressed the dental water concern by asking manufacturers to provide equipment with the ability to deliver treatment water with \leq 200 cfu/ml of unfiltered output from waterlines. It was recommended to use sterile water during dental surgical procedures. The same problems occur in ultrasonic scalers^(15, 23).

The aim of this study is to evaluate:

- 1- The microbial contamination (cfu/ml) in water samples collected from 25 dental units selected from different clinics in Damascus.
- 2- The contamination of ultrasonic scaler waterlines.
- 3- The precence of Pseudomonas in these water samples.
- 4- The effect of 3 minute flushing procedure on microbial contamination.

Methods:

126 water samples were collected to achieve the purpose of this study. 12 tap water samples, 14 ultrasonic scalers, and 100 dental unit water samples were collected aseptically from 25 dental units that used municipal water, 12 of them are located in different clinics of the faculty of dentistry at Damascus university, and the other 13 dental units are in private practices practicing general dentistry in Damascus. From each dental unit 4 samples were collected, dental high-speed handpiece, air/water syringe, air/water syringe after 3 min flushing (to follow the CDC recommendation), and cup filler (table 1, 2).

No.		Clinics	of dental scho	ool		Private	total
	Paediatr.	Perio. *	Conserv.	surgery	total	clinics	totai
Dental units	3	3	3	3	12	13	25
Water samples	12	17	12	12	53	61	114

* including scalers

Table 1: number and location of dental units and water samples.

	Source of	Source of samples					
	Dental unit water						
	Oral rinsing cup	High- speed handpiece	Air/water syringe	syringe after flushing	total	Scaler	total
No. samples	25	25	25	25	100	14	114

Table 2: number of water samples collected from different sources.

The samples were collected at the beginning of the working day (before the dental unit was used). One tap water sample was also collected from each clinic (12 clinics) to represent the water source that goes into these dental units. All dental units had been in service for more than one year and used municipal water as the main outlet source.

Before collecting the dental unit samples, tap water samples from the same dental clinic were tested for bacterial counts. All Water samples from tap water were in normal range (in this study < 1000 cfu/ml), except one sample which had a number of 7000 cfu/ml. This water is not directly supplied from municipal waterlines, instead, it was supplied by a central reservoir which serves different sites in the clinic, and the samples from this clinic are not included in this study.

Water samples were collected in sterile and evacuated tubes and placed on ice in a sealed container for no more than 4 hours. Samples were incubated for 48h

at 37C on blood agar plates. After the incubation period, the numbers of colony forming units (cfu/ml) grown were counted.

A pilot study of 5 samples was completed prior to the initiatiation of the study. It showed that the colony counts were very high, so a dilution was made to let one colony represent 1000 cfu/ml. The sample was deemed not contaminated if no colonies grew on the agar plate. The presence of Pseudomonas was examined.

Statistical analysis was done with SPSS version-13. T-test, ANOVA test, and Bonferroni-test were applied to study the differences in microbial contamination.

Results:

The results of this study are presented in table 3.

Table 3: cfu/ml in all samples collected from all dental units and ultrasou							
scalers.							
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	No.							
No. sample	sample unit dental unit	Scaler (m,r)†	Air- water syringe	syringe after flushing	High- speed handpiece	Cup filler	P *	
1-4	1	Paediatr. c.		10000	2000	18000	6000	
5-8	2			75000	14000	28000	3000	
9-12	3			34000	9000	21000	4000	
13-16	4	Conserv. c.		4000	2000	6000	4000	

17-20	5			11000	5000	13000	4000	+
21-24	6			7000	3000	9000	3000	
25-28	7	Surgery c.		4000	3000	65000	12000	
29-32	8			350000	150000	15000	230000	+
33-36	9			80000	5000	35000	27000	+
37-41	10	Perio. c	7000 (m)	29000	5000	3000	3000	
42-46	11		210000 (m)	115000	12000	8000	7000	
47-51	12		118000 (m)	65000	8000	6000	7000	+
52			38000 (m)					
53			260000 (m)					
54-58	13	private	4000 (r)	2000	<1000	1000	5000	
59-63	14	Private	7000 (r)	10000	3000	2000	17000	+
64-67	15	Private		4000	< 1000	12000	2000	
68-71	16	Private		3000	2000	3000	1000	
72-75	17	Private		4000	2000	20000	3000	+
76-80	18	Private	15000 (m)	13000	6000	9000	7000	
81-85	19	Private	24000 (m)	76000	8000	33000	18000	
86-89	20	private		9000	5000	16000	6000	
90-94	21	Private	39000 (m)	9000	<1000	16000	6000	+
95-99	22	Private	6000 (r)	25000	6000	7000	7000	
100-104	22	private	32000 (n)	51000	1000	24000	14000	+
105-109	24	private	9000 (m)	11000	4000	36000	6000	
110-114	25	private	4000 (m)	38000	27000	31000	20000	

† scaler m : scaler used municipal water

scaler r : scaler used reservoir distilled water

* P : pseudomonas

1- The microbial contamination (cfu/ml) of collected water samples.

- 111 collected samples from dental units and ultrasound scalers were contaminated (97, 4%) to different extents (1000-350000 cfu/ml), with a median count of 25900 cfu/ml (table 4).

Table 4: degree of dental waterlines microbial contamination.

Water samples	Contaminated	Median count	SD	Largest No.
Total	Samples %	cfu/ml		cfu/ml
114	97,4	25900	52520	350000

- The mean bacterial count of water samples collected from dental units in different clinics at the dental school was 32060 cfu/ml, and from dental units in private clinics was 12340 cfu/ml, the difference (t-test) was significant (P< 0,05) (table 5).

Table 5: the difference of bacterial contamination (cfu/ml) between samples From dental units in dental school clinics, and private clinics.

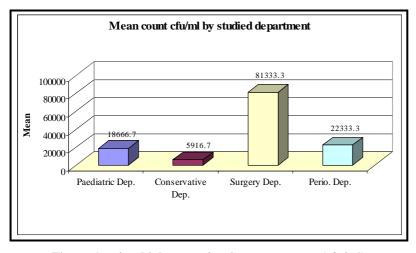
Source of water	No. Samples	Mean	SD	P-value	Sig.
Dental school	48	32060	63520	0,04	S
Private	52	12340	14440	3,31	5

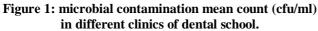
- ANOVA test was applied to study the differences in microbial contamination mean count (cfu/ml) between different clinics of dental school (table 6, figure 1). cfu/ml of the oral surgery clinics was the highest and was significantly greater than the clinic of conservative dentistry (Bonferroni-test).

 Table 6: differences in microbial contamination mean count (cfu/ml)

 between different clinics of dental school.

clinic	No. Samples	Mean cfu/ml	SD	F	P- value	Sig.
Pediatric	12	18666.7	20428.7			
Conservative	12	5916.7	3449.9	2 000	0.012	S
Oral surgery	12	81333.3	109343.8	3.999	0.013	
Periodontics	12	22333.3	34051.7			





- The differences between different water sources are shown in table 7 and figure 2. The ANOVA analysis showed that the highest cfu/ml count was in ultrasound scalers, then air/water syringes, while the lowest contamination was in cup filler outlets, but these differences were not statically significant.

Table 7: difference between different water sources . (cfu/ml)

Source of water	No. Samples	Mean Cfu/ml	SD	F	P- value	Sig.
Cup filler	25	16880.00	44866.8			
High-speed handpiece	25	17480.00	14580.6	2 200	0.094	NS
Air/water syringe	25	41560.00	71337.3	2.200		
Ultrasound scaler	14	55214.29	82266.2			

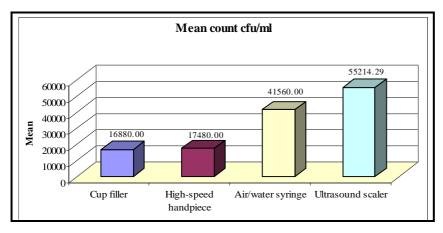


Figure 2: microbial contamination mean count (cfu/ml) in different water sources.

2- The microbial contamination (cfu/ml) in collected water samples from ultrasound scalers.

14 ultrasound scalres were tested, all of them were contaminated, with a median count of 55214 cfu/ml (table 8) .

		Ultrasound scalers		
No. of scaler	rs tested	14		
Lowest No.	(cfu/ml)	4000		
Largest No.	(cfu/ml)	260000		
Median count	(cfu/ml)	55214		
contaminated samples (%)		100		

Table 8: microbial contamination of 14 ultrasound scalers.

Ultrasound scalers that used reservoir with distilled water had significantly less count of cfu/ml (table 9).

 Table 9: differences between ultrasound scalers using reservoir with distilled water and ultrasound scalers using municipal water.

Type of scaler	No. of scalers	Mean	SD	P-value	Sig.
Reservoir scalers	4	5250	1500	0.027	c
Municipal scalers	10	75200	90670	0,037	3

3- presence of Pseudomonas in these water samples(dental units).

On average 32% of tested dental units contained pseudomonas colonies, no significant differences were found between dental school clinics and private clinics (table 10).

Table 10: F seudoli	nonds presence	in actival	acei mi	001		
	Dental school clinics	Private clinics	total	Kay2	P- value	Sig.
No. of dental units	12	13	25			
Pseudomonas + (No./unit)	4	4	8	0,019	0,891	NS
Pseudomonas + (% / unit)	33,3	30,8	32,0			

Table 10: Pseudomonas presence in dental waterlines.

4- The effect of 3 minute flushing on microbial contamination.

Flushing reduced the microbial contamination of dental waterlines significantly (table 11, figure 3).

Table 11: the effect of 3 min. flushing air/water syringe on dental water microbial contamination (cfu/ml)

Flushing procedure	Largest No. cfu/ml	contaminated samples %	Mean cfu/ml	t-test	P- value	Sig.
Before flushing	350000	100	41560	-3.331	0.003	S
After 3 min. flushing	150000	88	11303			

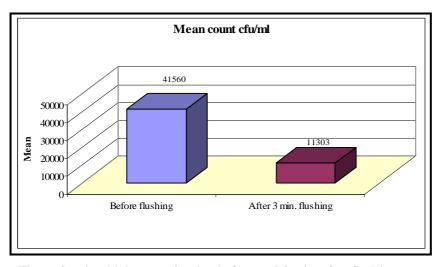


Figure 3: microbial contamination before and 3 min. after flushing, mean count (cfu/ml)

Discussion

Dental unit water contamination is a worldwide problem. A study aimed to assess the microbiology of dental unit waterlines (DUWL) and biofilms in general dental practices across seven European countries, concluded that water supplied by 51% of 237 dental unit water-lines exceeded the current American Dental Association's recommendations of ≤ 200 colony-forming units (cfu/ml) ⁽²⁴⁾. In another study, 83% of DUWL water samples exceeded American Dental Association standards, the microbial loading range from 500 to 10⁵ cfu/ml ⁽²⁵⁾.

In study of Williams and co-workers $1996^{(26)}$ the mean total plate count of colony-forming units (cfu/ml) for the waterline samples from 31 dental units was 2.7 x 10^5 cells per mL, with a range of 1.1×10^3 cells per mL to 5.3×10^6 cells per mL. In our study in Damascus, 97, 4% of the collected samples were contaminated in the range of 1000-350000 cfu/ml, with a median count of 25900 cfu/ml.

The highest contamination was found in samples taken from the oral surgery clinic of the dental college in Damascus (dental high speed handpiece 65000 cfu/ml, air-water syringe 350000 cfu/ml, and cup filler 230000 cfu/ml). Students in this clinic usually do not use water outlets when extracting teeth. This might be the reason behind this result. The same fact might also be

explained in part by the reduction of contamination at the beginning of the working day (before the dental unit was used) and later after using the dental unit ⁽²⁷⁾. Also the highest densities and frequency of Legionella pneumophila were observed in water samples from public institutions ⁽⁵⁾.

Analysis of data showed that levels of microbial contamination were highest in ultrasound scaler outlets and air/water syringes, while contamination levels of cup filler were the lowest, but without significant differences. Results from an earlier study showed that water from high speed handpieces was the most contaminated, followed by air/water syringes ⁽²⁾. Souza-Gugelmin et al 2003 ⁽²⁸⁾ in another study found that dental water contamination was highest in the air/water syringe (13 of 15) and in the high-speed handpiece (11 of 15); both levels were higher than those of the water reservoir; there was no significant statistical difference between the level of contamination in the air/water syringe and the high-speed.

In the current study all water samples from ultrasound scalers were contaminated in the range between 4000 - 260000 cfu/ml, with a median count of 55214 cfu/ml. Ultrasound scalers that used reservoirs of distilled water showed significantly less contamination than ultrasound scalers that used municipal water, but both were still contaminated.

In study of Williams and co-workers 1996 $^{(26)}$ the mean count from ultrasonic scalers was 4.2 x 10⁵ cells per mL. In another study $^{(29)}$, the highest level of contamination was identified in ultrasound scaling devices, then high-speed handpieces, indicating the necessity of using disinfectants $^{(23)}$.

In this study the presence of pseudomonas organisms was detected in 8 out of 25 dental units (32%) that used municipal water, no significant differences were found between dental college clinics and private clinics (33.3%, 30,8% respectively).

The presence of pseudomonas species was reported in many studies ^(9, 25). P.aeruginosa was detected in 86.7% of the dental units at a dental teaching center in Jordan at the beginning of the working day, and in 73.3% after 2 minutes of flushing and at midday ⁽³⁰⁾.

In another studies, no deleterious bacteria (Legionella, Streptococcus, Pseudomonas aeruginosa, and Esherichia coli) were detected $^{(4, 6)}$. It should be mentioned the limitation of culture-based techniques to detect and identify bacteria $^{(31)}$.

The results of this study indicate that flushing can significantly (P=0.003) reduce the level of bacteria present in dental waterlines, the contaminated samples reduced from 100% to 88%, and the largest amount of reduction was from 350000 to 150000 cfu/ml. These results confirm the latest CDC

recommendations that flushing alone is not a reliable procedure for improving dental waterlines quality ⁽¹⁵⁾.

Flushing water is a simple measure that should be undertaken as part of the dental infection control routine, because it was able to reduce the level of total aerobic bacteria in water from old and new dental units ⁽³²⁾.

The same result was reported in other studies that found bacterial reduction in total counts, but a relative ineffectiveness of the flushing procedure in reducing the occurrence of either Legionella spp. or protozoa⁽³³⁾.

Barbeau and coworkers 1996⁽²⁷⁾ tested 121 dental units located at the dental school of Universite' de Montre'al, and showed that none of the waterlines was spared from bacterial contamination, significant differences were recorded between samples taken at the beginning of the day and samples taken after a 2 minute purge. Ma`ayeh et al 2008⁽⁷⁾ sampled 10 dental units from a dental teaching center in Jordan, they concluded that a 2 minute flushing procedure can reducing Legionella counts, while the incorporation of disinfectants is recommended. Many disinfectants achieve a sufficient reduction in microbial count⁽³⁴⁾.

A questionnaire survey in several European countries tested the attitude of general dental practitioners to the microbial risk associated with dental unit water systems, the study showed that the majority of dentists did not clean, disinfect or determine the microbial load of their DUWL ⁽³⁵⁾.

Although the number of published cases of infection or respiratory symptoms resulting from exposure to water from contaminated DUWL is limited, there is a medico-legal requirement to comply with potable water standards and to conform to public health measures on water safety ⁽¹⁰⁾. It is difficult to measure the risks associated with aerosolized bacteria for the majority of patients seen in general dental practices. However, it seems prudent to eliminate this source of infection during treatment of compromised patients ^(19, 36, 37)

Conclusion

Our data demonstrate the microbial contamination of dental unit- and ultrasonic scaler waterlines in Damascus. Water samples from different dental clinics at the dental school were more contaminated than private clinics. The highest contamination level was found in samples taken from the oral surgery clinics at dental school. The highest contaminated source of dental water was from the ultrasound scaler and air/water syringe outlet respectively. The presence of pseudomonas organisms was detected in 32% of the dental units that used

municipal water. Flushing for three minutes can reduce the level of bacteria present in dental waterlines significantly.

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