Evaluation of a protocol for reducing the microbial contamination of dental unit water

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ABSTRACT

Biofilm on dental unit waterlines can spread microbial contamination in the water. The aim of this study was to investigate microbial contamination of water from supplies and dental units before and after the implementation of a protocol for microbial quality improvement and maintenance of dental unit water. The microbial load was evaluated in water from 27 taps and dental units (reservoirs, air-water syringes and high-speed outputs without handpieces) using the Petrifilm™ system (total aerobic bacteria and fungi) and conventional culture media (enterobacteria and Legionella spp.). The bacterial load in water samples from taps and reservoirs was within the parameter established by Brazilian legislation (<500CFU/mL); but the bacterial load in samples from air-water syringes and high-speed outputs without handpieces was not. The implementation of the protocol for the maintenance of microbial quality in dental unit water reduced bacterial load in high-speed outputs without handpieces (p=0.004). Enterobacteria and Legionella spp. were not isolated from any of the water samples from taps and dental units.

Key words: *Biofilms, dental equipment, water microbiology.*

Avaliação de um protocolo para redução da contaminação microbiana da água de equipos odontológicos

RESUMO

Biofilme nas linhas d'água de equipos odontológicos pode propagar contaminação microbiana na água. O objetivo deste estudo foi investigar a contaminação microbiana da água de abastecimentos e equipos odontológicos antes e após a implementação de um protocolo para melhoria e manutenção da qualidade microbiológica da água de equipos odontológicos. Avaliou-se a carga microbiana da água de 27 torneiras e equipos (reservatórios, seringas tríplice e alta rotação sem as peças de mão) de uma clínica odontológica por meio do sistema Petrifilm™ (bactérias aeróbias totais e fungos) e meios de cultura convencionais (enterobactérias e Legionella spp.). A carga bacteriana em amostras de água das torneiras e reservatórios estava dentro do parâmetro estabelecido pela legislação brasileira (<500 UFC/mL), mas a carga bacteriana das seringas tríplices e das saídas dos alta rotação sem as peças de mão não estava. A implementação do protocolo para manutenção da qualidade da água dos equipos reduziu a carga bacteriana nas saídas dos alta rotação sem as peças de mão (p=0,004). Enterobactérias e Legionella spp. não foram isoladas de qualquer das amostras de água das torneiras e dos equipos odontológicos.

Palavras chave: *Biofilmes, equipamento odontológico, microbiologia da água.*

INTRODUCTION

Over the past century, dental units have evolved from the original pedal-powered pulley models to the current versions with technology that provides safety and reduces biological risk. The greatest transformation took place in the early 1950s, with the emergence of air-water syringes and high-speed handpieces. Because this kind of equipment generates heat that can cause thermal injury, it requires water-cooling, so includes long, thin flexible tubes to channel water and air to it. But neither the inventors nor dental professionals imagined that those long, thin waterlines could conceal a great number of microorganisms from water, despite the implementation of basic principles of asepsis.[1]

Dental units are supplied with drinking water, which contains small microbial load. In Brazil, Ministry of Health ordinance No. 2,914 establishes

the limit as 500 colony-forming units (CFU) per milliliter (mL) of water.[2] In 1996, the American Dental Association (ADA)[3] recommended that the water in dental units should contain no more than 200 CFU/mL.

Dental unit water can pose a risk to oral and general health due to microbial contamination and biofilm formation on waterlines.[4-10] The first signs of microbial contamination of dental unit water and biofilm formation on waterlines were described by Blake[11] and Kelstrup et al.[12], respectively. The literature includes reports of infectious diseases due to contamination in dental unit waterlines.[13,14] This is a matter of concern, since the infections caused by microorganisms resistant to antimicrobials can be fatal, mainly in immunocompromised patients.

Once biofilm is formed, it is difficult to remove. Various strategies have been reported for controlling it on dental unit waterlines, such as development of surfaces with antibiofilm activity[15], supply of sterilized water for dental units[16], and physical-chemical treatments.[17,18] However, most of the strategies used for biofilm formation control on waterlines have limitations, often related to high cost and difficulty in implementation. There is thus a need for the development and use of an efficient protocol for biofilm control on dental unit waterlines based on easy implementation, short execution times and low cost.

The aim of this study was to evaluate the microbial load of water from taps and dental units (reservoirs, air-water syringes and high-speed outputs without handpieces) before and after the implementation of a protocol for improvement and maintenance of the microbiological quality of the water in dental units.

MATERIALS AND METHODS

Samples were collected aseptically from dental unit waterlines [reservoirs (R), air-water syringes (AWS) and high-speed outputs without handpieces (HSWH)] from 27 dental units at the School of Dentistry of Ribeirão Preto (SP, Brazil). Samples of tap water (TW) used to supply the dental units were also collected. The samples were collected at baseline ("T0") and seven months after baseline ("T1"). The "T1" samples were collected after the implementation of a protocol for improvement and maintenance of the microbiological quality of water in dental units as a daily routine. The protocol consisted of supplying and draining the dental unit reservoirs at the beginning and end of the work, respectively, and recommended flushing AWS and HSWH for 30 seconds before and after each patient.[3,19] The protocol did not include any chemical agents for disinfecting reservoirs and dental unit waterlines.

TW, AWS and HSWH samples were collected after water flushing (30s). In addition, samples were collected from reservoirs after rinsing three times with TW. All samples were collected in an approximate volume of 10mL in sterile test tubes (25x150mm). The samples were placed in a cooler, and microbiological processing began no longer than 30 minutes after collection.

The experiment was conducted in a Class II Type A1 Biological Safety Cabinet (VECO, Campinas, SP, Brazil). A 50µL aliquot of 2% sodium thiosulfate was added to each sample. The samples were homogenized (Phoenix, Araraquara, SP, Brazil), diluted up to 10^4 and seeded on Petrifilm™ AC and YM (3M, St Paul, USA) plates to evaluate total aerobic bacteria and fungi (filamentous fungi and yeasts), respectively. In addition, Petri plates (60x15mm) with conventional culture media for detection of *Legionella* spp. (Legionella Agar Base® supplemented with Legionella Agar Enrichment® – BD Difco, Sparks, MN, USA) and enterobacteria (MacConkey Agar – BD Difco, Sparks, MD, USA) were employed. The plates with water samples were incubated at 37°C for 48 h (total aerobic bacteria and enterobacteria), 23°C for 5 days (filamentous fungi and yeasts) and 37°C for 48 h (*Legionella* spp.). After the incubation periods, the colonies were counted using a trinocular stereomicroscope (Tecnival) under reflected light. The number of colony forming units per milliliter (CFU/mL) of water *in natura* was determined.

The statistical tests were performed using IBM SPSS Statistics 20.0 software (IBM Corp Armonk, NY, USA). As the distribution was non-normal, non-parametric Wilcoxon test was used to compare T0 and T1. Differences between contamination of the TW, R, AWS and HSWH in T0 and T1 were analyzed by Kruskal-Wallis. Since there was no count of total aerobic bacteria in T1 for TW and R, Mann-Whitney test was used for the comparison between AWS and HSWH. Relative frequency of presence and absence of contamination for the evaluated groups in T0 and T1 was performed through Pearson Chi-square test. The significance level was set at 0.05.

RESULTS

Tables 1 and 2 show the results of this study for loads of total aerobic bacteria and fungi (filamentous fungi and yeasts).

Of 27 TW samples, 6 (22.2%) were contaminated by total aerobic bacteria at T0. No TW sample was contaminated at T1, having a reduction of 16.0 times the bacterial load (CFU/mL). Filamentous fungus and yeast counts showed that 8 (29.6%) of TW samples were contaminated at T0, and 10 (37.0%) at T1, presenting an increase of 2.2 times of CFU/mL (p=0.407).

Of 27 reservoirs (R), 1 (3.7%) was contaminated by total aerobic bacteria at T0 and none at T1, presenting a reduction of 1.9 times of CFU/mL. The filamentous fungus and yeast count showed that 13 (48.1%) R were contaminated at T0 and 8 (29.6%) at T1, a reduction of 1.8 times of CFU/mL (p=0.351). It is worth noting that only 2 R remained contaminated at T1, while the other 6 R had new

Table 1: Median and confidence interval of CFU/mL for the evaluated groups: before (T0) and after (T1) the protocol implementation for reduction of the microbial contamination of dental unit water. Ribeirão Preto, SP, Brazil, 2018.

	Total aerobic bacteria		p***	Filamentous fungi and yeasts		p***
	T0	T1		T0	T1	
TW	0.0 (0.0; 35.4)^{ab}	0.0 (-;-)	-	0.0 (0.0; 7.9)^{a,A}	0.0 (0.6; 15.7)^{a,A}	0.407
R	0.0 (0.0; 5.7)^{a}	0.0 (-;-)	-	0.0 (0.6; 2.6)^{a,A}	0.0 (0.2; 1.5)^{a,A}	0.351
AWS	0.0 (0.0; 294.4)^{a,A}	0.0 (0.0; 0.7)^{a,A}	0.225	0.0 (0.0; 60.7)^{a,A}	0.0 (0.0; 1.3)^{a,A}	0.098
HSWH	0.0 (0.0; 316.8)^{b,A}	0.0 (0; 1.5)^{a,B}	0.004	4.0 (0.0; 279.0)^{b,A}	0.0 (0.0; 34.7)^{a,A}	0.131
p	0.001*	0.096**		0.001*	0.133*	

CFU/mL: colony forming units per milliliter of water; T0: baseline; T1: after implementation of the protocol for reduction of microbial contamination in dental unit water; TW: tap water; R: reservoirs; AWS: air-water syringes; HSWH: high-speed outputs without handpieces. *Kruskal-Wallis followed by Dunn test; **Mann-Whitney; ***Wilcoxon; ab Same lowercase letters indicate statistical similarity among collection sites; AB Same uppercase letters indicate statistical similarity between T0 and T1.

Table 2: Relative frequency of presence and absence of contamination for the evaluated groups in T0 and T1. Ribeirão Preto, SP, Brazil, 2018.

	Total aerobic bacteria				Filamentous fungi and yeasts			
	T0		T1		T0		T1	
	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence
TW	21 (77.8%)	6 (22.2%)	27 (100.0%)	0 (0.0%)	19 (70.4%)	8 (29.8%)	17 (63.0%)	10 (37.0%)
R	26 (96.3%)	1 (3.7%)	27 (100.0%)	0 (0.0%)	14 (51.9%)	13 (48.1%)	19 (70.4%)	8 (29.6%)
AWS	23 (85.2%)	4 (14.8%)	26 (96.3%)	1 (3.7%)	16 (59.3%)	11 (40.7%)	22 (81.5%)	5 (18.5%)
HSWH	14 (51.9%)	13 (48.1%)	22 (81.5%)	5 (18.5%)	6 (22.2%)	21 (77.8%)	16 (59.3%)	11 (40.7%)
Total	84 (77.8%)	24 (22.2%)	102 (94.4%)	6 (5.6%)	55 (50.9%)	53 (49.1%)	74 (68.5%)	34 (31.5%
p*	0.001		0.007		0.003		0.307	

T0: baseline; T1: after implementation of the protocol for reduction of microbial contamination in dental unit water; TW: tap water; R: reservoirs; AWS: air-water syringes; HSWH: high-speed outputs without handpieces; *Pearson Chi-square test.

contamination. Consequently, the protocol for improvement and maintenance of the microbiological quality of water in dental units as a daily routine showed a reduction in fungal contamination in 11 R.

Of 27 AWS, 4 (14.8%) were contaminated by total aerobic bacteria at T0 and 1 (3.7%) at T1, with a reduction of 539.7 times of CFU/mL (p=0.225). Moreover, this AWS contamination at T1 was considered new. The filamentous fungus and yeast count showed that 11 (40.7%) AWS were contaminated at T0 and 5 (18.5%) at T1, having a reduction of 143.7 CFU/mL (p=0.098). Thus, only 3 AWS remained contaminated at T1, and the other 2 AWS had new contamination, with a reduction in fungal contamination of 3 AWS.

Of 27 HSWH, 13 (48.15%) showed contamination by total aerobic bacteria at T0 and 5 (18.52%) at T1, presenting a reduction of 33.6 times of CFU/mL (p=0.004). Moreover, 4 HSWH remained contaminated at T1, and only one case of new contamination was reported. The filamentous fungus and yeast count showed that 21 (77.8%) of HSWH were contaminated at T0 and 11 (40.7%) at T1, with a reduction of 6.9 times of CFU/mL (p=0.131). Thus, only 9 HSWH remained contaminated at T1, and the other 2 HSWH had new contamination, with a reduction in fungal contamination of 12 HSWH.

The comparison among loads of total aerobic bacteria and filamentous fungi and yeasts from the different collection sites at T0 showed that the bacterial and fungal contamination in HSWH was greater than in AWS and R (p=0.001).

In relation to the relative frequency of cases with presence and absence of contamination at T0, HSWH contamination for total aerobic bacteria (48.1% / p=0.001) and filamentous fungi and yeasts (77.8% / p=0.003) was found to be greater than at the other evaluated sites (TW, R and AWS).

Moreover, in this study, the presence of enterobacteria and *Legionella* spp. was not detected in any of the samples (TW, R, AWS and HSWH) analyzed.

DISCUSSION

Dental units consist of reservoirs that supply water through waterlines (diameters 2 to 3 mm) to air-water syringes and high-speed handpieces.[20] Biofilm on these thin waterlines is an alarming source of microbial contamination of water[7,9,10] and can spread pathogenic microorganisms, thereby posing a threat to public health by causing respiratory infections and surgical site infections. Moreover, dentists and professional staff may become infected by aerosols generated in the dental office.[21,22] Since the microbiological quality of water for human consumption is directly related to human health, dental unit water must meet the drinking standard determined or suggested by national and international legislation or organizations.

In this study, water samples from AWS (7.4%) and HSWH (7.4%) presented a total aerobic bacterial load greater than 500CFU/mL. On the other hand, none of TW samples showed contamination above the limit permitted by Brazilian legislation.[2] According to ADA recommendations (1996), at T0, 2 AWS and 4 HSWH samples showed bacterial contamination greater than 200CFU/mL. In agreement with our results, other authors have reported contamination of water samples from dental units with counts above 200CFU/mL and 500CFU/mL.[23,6]

No national and/or international parameter has yet been established with regard to the fungal contamination of water intended for human consumption and dental units. Nevertheless, water should be monitored and controlled frequently for biosafety in dentistry, since filamentous fungi and yeasts have been isolated from dental unit water in other studies[24, 25] as well as in the current study.

Enterobacteria and *Legionella* spp. were not isolated from the water samples analyzed in the current study. Although the traditional culture technique is the main evaluation method for bacterial contamination, false-negative results or underestimated counts have been reported for *Legionella* spp. Some authors have therefore suggested the use of molecular techniques, such as polymerase chain reaction (PCR), to avoid these problems.[26,27]

Biofilm is composed of microorganisms protected by an extracellular polymeric matrix. When it develops on dental unit waterlines, it causes problems which may be resolved by applying physical/ mechanical strategies such as flushing water, as was done in the current study and in others[4, 28, 29], to partially remove microorganisms that are loosely adhered to the biofilm. Antimicrobial chemical agents are also used for this purpose, but they can compromise the structural integrity of dental unit waterlines[14], thereby increasing the

contact area for microbial adhesion and biofilm formation. Moreover, the use of chemical agents for biofilm control may present limitations related to microbial phenotypic changes[30], the difficulty of reaching the innermost microbiota in the biofilm[31], and residual toxic effects on individuals and the environment.

In this study, a protocol was implemented to improve and maintain the microbial quality of dental unit water. The protocol was inexpensive and simple to implement, created no risk to human health or the environment, and provided a partial solution to biofilm contamination on dental unit waterlines. Nevertheless, the problem remains as one of the greatest challenges in dentistry and requires further studies for better understanding, with the aim of providing a biologically safe environment in dentistry.

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