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ORIGINAL ARTICLE

A study of different modes of disinfection and their effect on bacterial load in dental unit waterlines

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ABSTRACT

Objective: To compare the effect of disinfection of dental unit waterlines (DUWLs) on bacterial load using disinfection methods and agents like 0.12% chlorhexidine gluconate (CHX), time-dependent flushing (1 min, 2 min), and using distilled water and tap water as water sources. **Materials and Methods:** Four dental units were taken: Unit A contained 0.12% CHX, Unit B contained distilled water, Unit C contained tap water, and Unit D included flushing for 1 and 2 min. A total of 36 water samples were collected in 2 weeks. One sample of tap water from basin was taken as study control. One sample each from Unit A, B, and C and 2 samples from Unit D (1 min and 2 min flushing) were taken as baseline samples. Samples were collected three times a week and assessed for total viable count (TVC) and types of organisms present. **Results:** For Unit A, no growth of microorganisms was observed. Flushing for 1 min and 2 min showed variable TVC. No significant difference was seen in TVC of units B, C, and D in comparison to the baseline samples. **Conclusions:** It was found that 0.12% CHX was very effective in controlling DUWL contamination. Adhering to a recommended 2 min flushing regimen can reduce the bacterial counts, but is not a reliable means of disinfection.

Key words
Biofilms, dental equipment, infection control

INTRODUCTION

Effective infection control is one of the cornerstones of good practice and clinical governance. Dental unit waterlines (DUWL) are sites for the development of biofilms of aerobic, mesophilic, and heterotrophic microorganisms.[1] High counts of bacteria in DUWLs are well documented and have been described in several scientific articles during the last decade. The significance of DUWL and ultrasonic scaler waterline contamination lies in the reports of potential opportunistic pathogens such as *Streptococci* spp., *Enterococci* spp., *Pseudomonas aeruginosa, Legionella* spp., and other gram-negative rods isolated from these lines.[2-4] These organisms can cause pneumonia, other respiratory infections, or wound infections in immunocompromised people. Dental personnel have been shown to have altered nasal flora, with colonization of *Pseudomonas* spp. consistent with those found in their dental units.[5,6] Cross-infections between patients; chronic infection of dental personnel with long-term exposure to oral fluids, splatter, and aerosols; and direct infections of open surgical wounds should be a concern for any therapist. The microorganisms capable of forming biofilms on surfaces of DUWLs may also form biofilms on heart valves, creating endocarditis. Hospitalized patients are at risk of nosocomial infections from *Pseudomonads, Acinetobacter,* and other waterline bacteria.[7,8]

This study was conducted to compare the various means of disinfection of DUWL using 0.12% chlorhexidine gluconate (CHX), time-dependent flushing (1 min, 2 min), and using distilled water and tap water as water sources. Their effect on bacterial load in DUWLs was also assessed by evaluating the total viable count (TVC) and identification of microorganisms present in the waterlines.

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MATERIALS AND METHODS

Four dental units (A, B, C, and D) with self-contained water systems were selected for the study from the Department of Periodontology at Darshan Dental College

and Hospital, Udaipur. Out of the four dental units, Unit A was selected for instituting 0.12% CHX solution† as a disinfectant, Unit B contained distilled water in the self-contained water source, Unit C used tap water as the water source, and Unit D samples were collected after 1 and 2 min of flushing intervals. For Unit D, tap water was filled in the reservoir. One sample of tap water from the basin was also used as the study control. The sample collected from tap of the basin represents the municipal water supply (potable drinking water). A total of 5 samples (100 ml) were collected as baseline measures before the study began, one each from the 4 dental units selected. For the next 2 weeks, 100 ml of samples were taken directly from the end of ultrasonic tubing into sterile containers, three times per week as test samples [Figure 1]. The clinical day started at 8:30 AM and ended after the treatment of the last patient in the department.

The waterlines of Unit A were treated with 0.12% CHX solution (approximately 250 ml) overnight by introducing the disinfectant into the waterlines at the end of each clinical day. CHX solution without any dilution was filled into the reservoir, lines were flushed until CHX solution, which was blue in color, flowed out of the three-way water syringe, ultrasonic lines, and hand piece. This ensured that the disinfectant reached all the tubing in the waterline system. Next day, in the morning before the start of the clinical day, flushing was done till the colored (blue) CHX solution no longer flowed out, indicating removal of residual disinfectant from the lines. Flushing was done for additional 2 min in Unit A and then water sample was taken at the start of the clinical day.

For all dental chairs, the reservoirs were washed daily with sterile water and then filled with their respective water sources. Samples for Unit B and C were taken at the end of the clinical days. For Unit D, two samples were taken, one sample after 1 min of flushing and another after 2 min of flushing at the start of clinical days, and additional flushing was done for 30 s between each patient.

Microbiological assessment

Water samples of 100 ml were analyzed for TVC at 37°C incubation, and they were cultured on chromogenic culture media to identify microorganisms in them. On chromogenic media, colonies are well isolated and easy to identify by means of their differentiating colors.

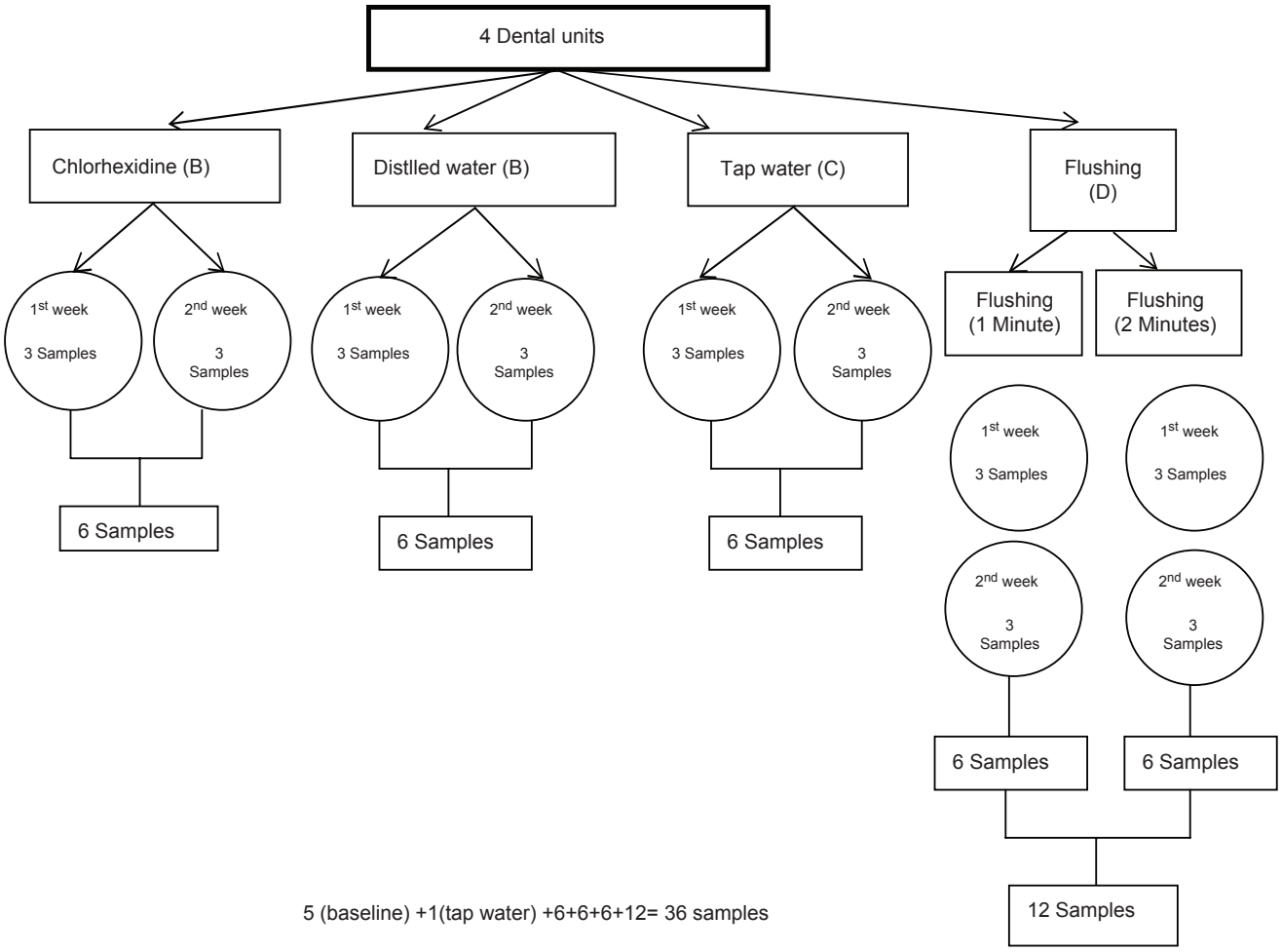

Figure 1: Flowchart showing study design

Microbial enumeration with colorless background optimizes colony counting. For culturing, each plate was divided into four equal parts, as shown in Figure 2. No dilutions of the samples were made. Using a sterile calibrated loop, 40 µl of the sample was inoculated on to culturing plate. First, the loop was passed down the middle of the chosen divided part of the plate, then side to side to distribute it evenly on the plate. Counting of the colonies was performed after 48 h of incubation at 37°C, however, the plates remained incubated for 1 week. The number of colony forming units counted on one part of the equally divided plate represented the number of colony forming units (cfu) per 40 µl of that sample. Colonies were named according to the color on the chromogenic media. Simultaneously, samples were also isolated for microorganisms using Biomerieux ID system. Biomerieux ID system[‡] is a standardized, automated system for identification of microorganisms. It uses miniaturized enzymatic tests, biochemical tests, as well as specific database to identify organisms. This system has a mini device including an automated reading unit, and it interprets the automated strips[§]. The strips allow identification tests to be performed within 4 to 24 h. These automated strips are comprised of 32 optimized biochemical tests that are extremely discriminating and helpful in confirmation of organisms. These strips are associated with extensive databases, which are stored in the device's software and regularly updated.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) to assess statistical difference between various modes of disinfection (units). Paired sample "*t*" test was used to study the difference within each group (unit), i.e., between 1st and 2nd week of the study. Results between 1st and 2nd week analysis showed no significant differences ($P>0.05$) within each group. All data are represented in Tables 1 and 2.

RESULTS

The number of cfu counted on each part of the plate represented the number of cfu per 40 µl of the sample. Thus, conversion factor to convert 1 µl to 1 ml was used to get the number of cfu per ml of the sample. Baseline samples revealed a wide range of microbial counts from 0 to 10^5 cfu/ml [Table 3]. Baseline sample of tap water from the basin (control) showed no growth of microorganisms. At the baseline, variations were seen in TVC in the samples from all the 4 dental units, ranging from 10^5 cfu/ml (in units C and D) to 10^4 (in units A and B). During the 1st week of study, as evident from Table 3, a gradual decrease in the counts from the baseline values was seen. For units B, C, and D, counts

[†]0.12% chlorhexidine gluconate solution, Colgate

[‡]bioMerieux SA, France

[§]API®ID 32 automated strips, bioMerieux

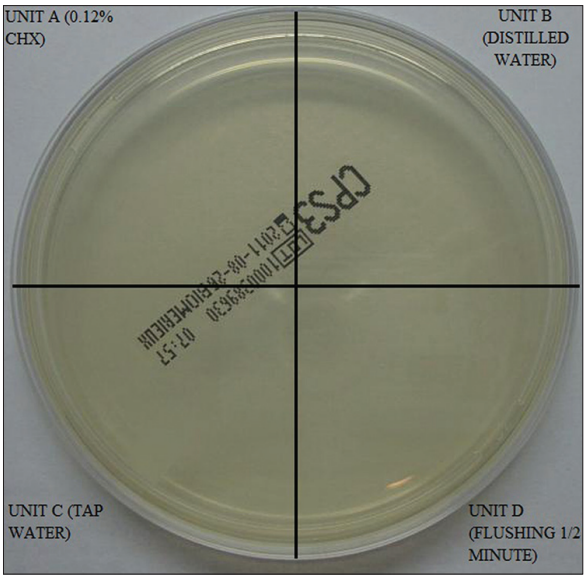

Figure 2: Water samples collected from dental unit waterlines were cultured on chromogenic culture media

Table 1: Analysis of variance test to assess differences between different modes of disinfection

	Sum of squares	DF	Mean square	F	Significance
First week					
Between groups	4.100E11	4	1.025E11	0.847	0.527 (not significant)
Within groups	1.210E12	10	1.210E11		
Total	1.620E12	14			
Second week					
Between groups	2.245E12	4	5.614E11	925.313	0.000 (significant)
Within groups	6.067E9	10	6.067E8		
Total	2.251E12	14			

DC – Degrees of freedom; ANOVA – Analysis of variance

Table 2: Comparison of mean and standard deviations of different modes of disinfection of DUWL

Mode of disinfection	1st Week	2nd Week
Chlorhexidine (Unit A)		
Mean	0	0
SD	0	0
Distilled water (Unit B)		
Mean	36666.67	36666.67
SD	55075.71	55075.71
Tap water (Unit C)		
Mean	370000	10000
SD	547448.6	0
Flushing (Unit D) 1 minute		
Mean	370000	1000000
SD	547448.6	0
Flushing (Unit D) 2 minutes		
Mean	70000	100000
SD	51961.52	0

DUWL – Dental unit waterlines; SD – Standard deviation

of 10^3 cfu/ml were observed in the last sample of 1st week. During the 2nd week, Unit D showed the most variation in counts. Flushing for 1 min did not show any significant decrease in the counts. Flushing for 2 min did show a 90% reduction in count from 10^5 to 10^4 cfu/ml. ANOVA test showed significant differences between and within the groups during 2nd week of the study. Paired sample test for individual units for 1st and 2nd week showed no significant difference. Unit A (0.12% CHX) showed the best results. From a baseline count of 10^4 cfu/ml, no growths of microorganisms were observed from the following day until the end of the study.

Samples were also assessed for the type of microorganisms present. Both gram-negative and gram-positive species were identified from the DUWL. In baseline samples [Table 3], gram-negative species such as *Enterobacter* spp., *P. vulgaris,* and *P. aeruginosa* and gram-positive species such as S*treptococcus* spp. were identified. Tap water sample from basin (control) showed no growth even after 48 h of incubation. In units B and D, *P. aeruginosa* and *P. vulgaris* [Figure 3a and c] and *Enterobacter* and *Streptococcus* spp. [Figure 3b] could be observed. Unit A (0.12% CHX) showed *P. aeruginosa* and *Enterobacter* spp. in the baseline samples, but,

Table 3: Total viable counts and organisms identified in the samples during the study

Water source	Total viable count (colony forming units/ml)			Organism identified
	1st sample	2nd sample	3rd sample	
Chlorhexidine (unit A)				
Baseline	10^4			*Pseudomonas aeruginosa*
				Enterobacter spp
1st week	No growth	No growth	No growth	
2nd week	No growth	No growth	No growth	
Distilled water (unit B)				
Baseline	10^4			*Proteus vulgaris*
1st week	10^4	No growth	10^3	*Enterobacter* spp
				Streptococcus spp
2nd week	10^4	10^3	No growth	*Enterobacter* spp
				Streptococcus spp.+contaminants grown
Tap water (unit C)				
Baseline	10^5			*Enterobacter* spp
1st week	10^5	10^4	10^3	*Enterobacter* spp
				Streptococcus spp
2nd week	10^3	10^3	10^3	*Enterobacter* spp
				Streptococcus spp
Flushing (unit D) (1 minute)				
Baseline	10^5			*Enterobacter* spp
				Streptococcus spp
1st week	10^5	10^4	10^3	*Enterobacter* spp
				Streptococcus spp
2nd week	10^5	10^5	10^5	*Enterobacter* spp
Flushing (unit D) (2 minutes)				
Baseline	10^5			*Pseudomonas aeruginosa*
1st week	10^4	10^4	10^3	*Pseudomonas aeruginosa*
				Proteus vulgaris
2nd week	10^4	10^4	10^4	*Enterobacter* spp

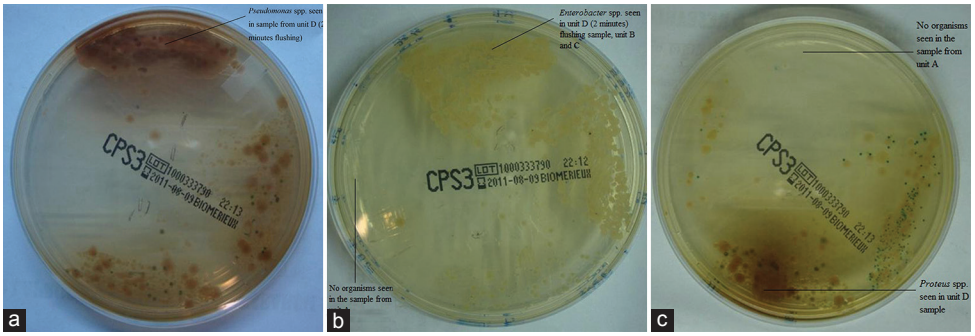

Figure 3 (a-c): Different types of organisms identified from different dental unit waterlines

from the following day and during the entire course of study, none of the samples showed any growth of microorganism [Figure 3b and c].

DISCUSSION

Many opportunistic bacteria were identified from DUWLs in our study such as *P. aeruginosa, P. vulgaris, Streptococcus* spp., *Enterobacter* spp. *P. aeruginosa* derived from DUWL has been shown to cause opportunistic infections. In a documented report, two patients with solid tumors were exposed to DUWL contaminated with *P. aeruginosa.* Both patients subsequently developed oral abscesses caused by the same strain isolated from the DUWL.[8] *Enterobacter* spp. and *Streptococcus* spp. are potential opportunistic organisms found to cause bacteremia, bacterial endocarditis, urinary tract infections, and respiratory tract infections. *P. vulgaris* is an opportunistic pathogen commonly causing wound infections.[7,9,10]

In 1996, the American Dental Association (ADA) set a limit for dental water to contain no more than 200 cfu/ml. In 2003, the Centre for Disease Control and Prevention (CDC) recommended ≤500 cfu/ml for non-surgical dental procedures. However, many studies have reported contamination of DUWL at the level from 1.5×10^2 to 1×10^6 cfu/ml.[11] As a result, various methods have been developed to reduce bacterial colonization and growth, including time-dependent flushing of waterlines, independent water reservoir systems, distilled or pasteurized water, ultraviolet light, micro pore filtration, electrolyzed water, and periodic or continuous chemical disinfection.[12-14]

According to CDC guidelines, flushing for 2 min in the morning and for 20-30 s between patients should be followed during dental procedures. Very conflicting results are seen in the studies conducted regarding flushing of DUWL, indicating flushing as a weaker means of disinfecting DUWL. It has been reported that 20 min of flushing reduced the number of detectable bacteria in water from dental school lines to zero, but the lines repopulated in the next 24 h.[15] Relatively high levels of bacterial endotoxin (LPS) in DUWL, ranging from 480 to 1,008 endotoxin units (EU)/ml, have been reported in DUWL.[16] However, waterline flushing of 5-10 minutes did not reduce LPS levels to zero. In our study, flushing the waterlines for 1 min had no significant difference in reducing the cfu/ml. Flushing for 2 min reduced the count by more than 90% (from 10^5 to 10^4 cfu/ml) by the end of 2nd week, but it still did not meet the CDC standards.

Chlorine, in the form of sodium hypochlorite, is the most commonly employed biocide in water and has proven efficacy in hospital cold water system, particularly for controlling *Legionella* proliferation. Similar results of chlorine application have been found for DUWLs.[17] Disadvantage of long-term exposure to chlorine include bacteria developing resistance, corrosion damage even at 1 ppm, and formation of trihalomethanes (potential carcinogens). Our study showed that 0.12% CHX to be the most effective mode of disinfection. In Unit A, *P. aeruginosa* and *Enterobacter* spp. were seen in the baseline samples, but, later, with the introduction of CHX, no growth was seen during the course of the study. No growth was seen even after 48 h, followed by 1 week, of incubation at 37°C. The bactericidal action of CHX inhibits the growth of microorganisms. Thus, regular use of easily available 0.12% CHX is a very convenient and hassle-free method for DUWL disinfection for daily usage. It is also not as sensitive a technique as other chemical treatments since the solution need not be prepared.

An excellent source of water for use in dental water systems is bottled sterile water for irrigation as it not only free of viable microorganisms but also has very low levels of minerals and organic compounds that can encourage re-establishment of biofilms. In our study, the dental unit with distilled water (Unit B) as a water source showed variance in counts. The water source was sterile, but the pathway was not; thus, intricacy and complexity of DUWL contribute to contamination.

The baseline samples of tap water, one from the basin and the other from dental Unit C using tap water as water source showed contrasting results. No growth was seen in the water sample from basin, this may due to the fact that municipal water supply, which is chlorinated and meet potable water standards comes through the faucet of tap. Unit C, though using same water source as municipal water supply, still showed counts up to 10^5 cfu/ml, with *Enterobacter* spp. being identified. This could be attributed to the biofilm formation in the complex system of DUWLs.

CONCLUSION

The results of our own studies as well as those of other authors conducted over the years show a high number of microorganisms found in DUWL Dahlén *et al.*,[18] Göksay *et al.*,[19] Walker.[20] Thus, disinfection of DUWL is an important step in infection control of dental offices. Our study demonstrates that, although adhering to a recommended 2-min flushing regimen may reduce bacterial counts, it is not a reliable means of disinfection. In fact, 0.12% CHX solution was found to be the most effective and reliable means of disinfecting DUWL. Further investigations need to be done to expand these findings and delineate additional implications for clinical practice.

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