Comparative Evaluation of Effectiveness of Different Antiseptic Agents into Reducing Microbial Load in Dental Chair Water Supply

Dr. Prashant Singhal, [1] Dr. Geetha K. Bhat, [2] Dr. Rahul Bhagabati, [3] Dr. Akanksha Swaraj, [4] Dr. Arushi Sharma, [5] Dr. Pallavi [6]

1. **Dr. Prashant Singhal**
   Professor, Department of Periodontics, Jaipur Dental College & Hospital, Dhand, Tehsil- Amer, Jaipur, Rajasthan, India
2. **Dr. Geetha K. Bhat**
   Professor & Head, Department of Periodontics, Jaipur Dental College & Hospital, Dhand, Tehsil-Amer, Jaipur, Rajasthan, India
3. **Dr. Rahul Bhagabati**
   Postgraduate Student, Department of Periodontics, Jaipur Dental College & Hospital, Dhand, Tehsil-Amer, Jaipur, Rajasthan, India
4. **Dr. Arushi Sharma**
   Postgraduate Student, Department of Periodontics, Jaipur Dental College & Hospital, Dhand, Tehsil-Amer, Jaipur, Rajasthan, India
5. **Dr. Pallavi**
   Postgraduate Student, Department of Periodontics, Jaipur Dental College & Hospital, Dhand, Tehsil-Amer, Jaipur, Rajasthan, India

CORRESPONDING AUTHOR

Dr. Prashant Singhal
Department of Periodontics, Jaipur Dental College
Dhand, Tehsil- Amer, Jaipur,
Rajasthan, India-302028
Mobile - 09887676756
Email - sprashantdoc@gmail.com

Abstract

Background: Dental unit water system harbor bacterial biofilms, which can be a source of microbial contamination via ultrasonic scalers and dental hand pieces and thus may be a potential source of contamination in the dental operatory.

Aims and objective: The aim of the study was to investigate the microbial load in dental unit water tubing and to study the effect of incorporating antiseptic (chlorhexidine) into the water source for the dental unit, and its effect on the microbial load.

Material and Methods: 20 dental unit waterlines were divided into 4 groups. Group 1 (control group) dental chair supplied with centrally RO treated water through self-contained water system, Group 2 dental chair supplied with distilled water through self-contained water system, Group3 water lines stagnated with 30 ml of 0.2% Chlorhexidine overnight in tubing system. Group 4 dental chair supplied with 1:10 dilution of 0.2% Chlorhexidine through self-contained water system. Sample of 100 ml were collected and used as the definitive measure of total microbial contamination.

Results: Out of 4 groups studied chlorhexidine 1:10 dilution showed maximum reduction in microbial load followed by stagnant chlorhexidine followed by distilled water and normal water.

Conclusion: Use of chlorhexidine either incorporating in water system or tubing reduces the contamination level (< 200 CFU mL-1), which is also recommended by the American Dental Association that water for dental procedures should not contain more than 200 CFU mL-1of aerobic bacteria.
Keywords: dentistry, dental unit waterlines, scaling & root planning microbiological load.

INTRODUCTION

Effective infection control is one of the cornerstones of good practice and clinical governance. Due to increased scientific knowledge of dental unit waterlines (DUWL) biofilms and their associated risks, contamination of dental unit waterlines has become a prominent infection-control issue. The perceived threat to public health from DUWL contamination comes from opportunistic and respiratory pathogens such as Legionella spp (causative agent of the pneumonia, legionnaires' disease), Mycobacteria spp and Pseudomonads. These organisms can be amplified in the biofilm to reach infective concentrations, with the potential for inhalation or direct contamination of surgical wounds.1

Dental equipment manufacturers have in turn responded with a variety of approaches to this complex problem. There is a plethora of automated flushing systems, filters, water disinfectants, independent bottle water systems, and even fully detachable autoclavable DUWL in the market.2

Dentists have a duty to care for their staff and patients. It is deemed ethically unacceptable to knowingly expose patients to contaminated water. Guidelines on preventive measures for reducing DUWL contamination have been issued by government agencies such as the CDC Atlanta, USA, the mainstay of which is flushing of dental units 3.

Dental water may be ingested, inhaled in the form of aerosols or directly contaminate surgical wounds. The ADA recommended to their members that dental unit water should comply with drinking water standards and contain <200 CFU mL-1of bacteria (equivalent to that permitted for drinking water as per WHO guidelines)4. Separate sterile water supplies are advised for surgical procedures. Devices used to deliver the sterile water must be sterilized before use for invasive procedures5.

The study will examine to investigate the microbial load in dental unit water tubing and to study the effect of incorporating antiseptic (chlorhexidine) into the water source for the dental unit, and its effect on the microbial load.

MATERIALS AND METHOD

This study was conducted in department of Periodontics & Implantology, Jaipur dental college, Jaipur, Rajasthan. 20 dental units for which tubing had been changed 8 months prior were selected for the study (acc. To ADA specification 2000)7. These 20 dental units were divided into 4 groups.

Group1 (control group) dental chair supplied with centrally RO treated water through self-contained water system.

Group 2 dental chair supplied with distilled water through self-contained water system.

Group 3 water lines stagnated with 30 ml of 0.2⁒ Chlorhexidine overnight in tubing system.

Group 4 dental chair supplied with 1:10 dilution of 0.2⁒ Chlorhexidine through self-contained water system.

A total number of 20 patients were randomly selected from the outpatient department of periodontics.

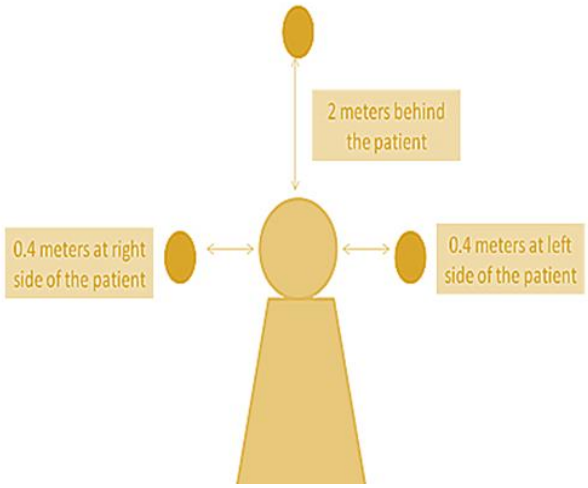
Inclusion criteria were,

1) a minimum number of 20 teeth present,
2) age ranging between 18 and 60 years,
3) systemically healthy patients,
4) a minimum oral hygiene score of 3–4 (Oral Hygiene Index Simplified),
5) pocket probing depth of ≥5 mm, and
6) nonsmokers and nonalcoholic patients.

Exclusion criteria were,

1) patient on systemic antibiotics in the past 6 months,
2) undergone oral prophylaxis within the last 3 months, and
3) pregnant or lactating women.

The patients were informed of the protocol and the written consent was obtained from the patients. Before starting the treatment, care was taken to maintain a clean sterilized environment with fumigation in the working room. A standardized location was used to place the nutrient agar (enriched with 5% sheep blood) plates to collect the airborne particles during the treatment. Two agar plates were used for each patient (one plate was kept at the center of the operatory room 20 min before the scaling procedure, and the other plate was kept 40 cm away from the working area near the patient's chest for 20 min during the scaling). The same clinician performed all the treatment procedures on all days and only one patient was carried out in a day to allow the operatory room to be free of aerosols.



The water sample collection was performed in morning prior to starting clinical work. Before treatment, group 3(0.2% Chlorhexidine overnight in tubing system) lines were flushed with water for 2 minutes to remove residual disinfectant from the lines. Water samples of 100 ml were collected in separate sterile containers using aseptic techniques from each scaler unit for microbial count. These were labeled and quantified for total mean CFU mL-1.

Total viable counts were carried out on decimal dilutions of the water samples of DUWL and were used as the definitive measure of total microbial contamination. Water samples of appropriate dilutions (for aerobic 1:10 dilution and anerobic without dilution) were plated on Columbia blood agar for oral aerobes (incubated for 37°c for 2 days) and oral anaerobes (incubated anaerobically at 37°c for up to 10 days under a gas phase of 80% [vol/vol] co2-10% [vol/vol] h2-10% [vol/vol] n2).6and CFU counts were determined by digital colony counter.

STATISTICAL ANALYSIS

Statistical analyses were carried out using SPSS (STSC Inc., Rockville, Md.). Bacterial loads in different groups were compared using a two-way analysis of variance (ANOVA) on log-transformed viable counts. Where significant differences ($P<0.05$) were indicated by ANOVA. Individual groups were then compared by the least-significant-difference method.

RESULT

20 DUWS samples taken during the study were grouped as Group1. (Control group) dental chair supplied with centrally RO treated water through self-contained water system, Group 2. Dental chair supplied with distilled water through self-contained water system, Group3. Water lines stagnated with 30 ml of 0.2% Chlorhexidine overnight in tubing system. Group 4. Dental chair supplied with 1:10 dilution of 0.2% Chlorhexidine through self-contained water system.

S.No.	Groups	Colony count (in CFUs)		F value	P value
		Mean	SD		
1	**Group 1**	48	2.933	399.54	0.0001
2	**Group 2**	38.8	2.234		
3	**Group 3**	16.2	0.815		
4	**Group 4**	11.8	1.012		

The geometric mean for aerobic microbial count for group 1,2,3,4 was 48×101, 38.8×101, 16.2×101 and 11.8×101 CFU ml-1 respectively. There was no significant difference when group 2 was compared with control (p=0.03) but there was significant difference found in group 3 and group 4 with control (p=0.002).

S.No.	Groups	Colony count (in CFUs)		F value	P value
		Mean	SD		
1	**Group 1**	26.2	0.815	457.17	0.0001
2	**Group 2**	18.2	1.084		
3	**Group 3**	11.6	0.836		
4	**Group 4**	7.18	0.679		

The geometric mean for anaerobic microbial count for group 1,2,3,4 was 26.2×101,18.2×101,11.6×101 and 7.2×101 CFU ml-1 respectively. There was no significant difference when group 2 was compared with control (p=0.04) but there was significant difference found in group 3 and group 4 when compared with control (p=0.003).

DISCUSSION

Group 1 (Control-Tap Water)

In the present study, the mean aerobic bacterial contamination level was 4.8×101 CFU mL-1 and mean anaerobic bacterial contamination level was 2.6×101 CFU mL-1, the mean CFU's was found to be higher than the recommended level by ADA7 (<200 aerobic CFU mL-1).

The bacteria in water interact with tubing system to form a biofilm. Bacteria adhere more readily to hydrophobic plastic tubing of the dental unit (William J et al 1994) thereby enhancing microbial growth.

The Result of the present study goes in accordance with the study done by Kettering et al. 19977 & Blake et al. in 1963, in which it was found that the dental units with the tap water showed CFU's in the range 5,00,000 to 5,000,000 while chlorhexidine solutions demonstrated no bacterial growth.

Another study done by Puttajah et al. 2001, in which comparison was made between three suction line cleaning agents, it was found that chlorhexidine solution containing dental units had bacterial growth in comparison to dental units containing sodium hypochlorite, while the units containing tap water showed the highest bacterial growth.

The contamination could be due to microorganism sloughing off into the flowing water, from the microbial growth along the inner surface of water tubing thereby a source of contamination for the patient. 11

Group 2 (Distilled Water)

The mean aerobic bacterial contamination level was 38.8×101 CFU ml-1 and mean anaerobic bacterial count was 1.8×101 CFU ml-1.

The aerobic microbial load was higher in present study than studies done by Williams, et al 199612.

The present study does not go in hand with the Study done by Williams et al and Kettering et

al. 19977 who also compared distilled water to normal water and found significant decrease in aerobic microbial load in distilled water when compared to normal water.

According to the study done by Reinhardt et al 1982 who used sterile and non-sterile water to examine the incidence of bacteremia after scaling found higher number of gram-negative bacteria in the non-sterile water. this can explain the lower number of microbial count in distilled water, as the opportunistic pathogens like pseudomonas species and klebsiella species which are gram negative microorganisms proliferate in lesser amount in distilled water.

The bacterial count in the present study was low as compared to the Distilled water as it is considered to be the purest form of water.

Group 3 (Chlorhexidine 0.2% Stagnant Overnight)

In the present study, the mean contamination level < 200 CFU /ml for aerobic and < 12 CFU ml-1 for anaerobic. The results were found to be in accordance to the study done M Ozcan et al13 1982, who used chlorhexidine stagnant in tubing overnight, which has been shown to effectively reduce microbial load.

Study goes in accordance to the study done by J. Kettering et al7 who also compared chlorhexidine (0.2% stagnant overnight) to tap water and found significant decrease in aerobic and anerobic microbial load.

Micro Organism in the dental unit waterline is derived from incoming water source & from microbial growth coating the water tubing. Planktonic organism is frequently released into the flowing water source. (Tall B D et al. 1995).

The reduction in the bacterial count could be due to the antibacterial activity exerted by the CHX on the dental tubing. It has been shown that CHX has an affinity for bacteria probably because of an interaction between the positively charged groups on the bacterial cell wall (phosphate groups).

The interaction increases the permeability of the bacterial cell wall and thus permits the agent to penetrate into the cytoplasm and cause the death of the microorganisms.

CHX is indicated to limit the operatory contamination by oral bacteria.

Group 4 (Chlorhexidine 1:10 Dilution)

The bacterial contamination level <150 CFU mL-1 for aerobic and < 7 CFU mL-1 for anaerobic, when CHX 1:10 dilution was used. A significant decrease in the CFUs was noted when compared to control.

Our study does not go in hand with the Study done by James T. Walker et al6 which did not find any significant reduction in microbial load when comparing with control.

Since the main water was treated with CHX, reduction is attributed to antimicrobial property of chlorhexidine which might have reduced the biofilms and eliminate the planktonic bacterial count.

CHX is referred to as a gold standard. Its superior antiplaque effect can be explained in terms of its superior degree of persistence of anti-bacterial effect (both bactericidal and bacteriostatic).

SUMMARY

Out of 4 groups studied chlorhexidine 1:10 dilution showed maximum reduction in microbial load followed by stagnant chlorhexidine followed by distilled water and normal water. Substantial decrease in microbial load is seen by incorporating an antimicrobial like chlorhexidine in to the water, or water tubing.

CONCLUSION

Use of chlorhexidine either in container or tubing reduces the contamination level (< 200 CFU /ml), which is also recommended by the American Dental Association that water for dental procedures should not contain more than 200 CFU/ml of aerobic bacteria. For routine use in dental colleges, clinics 1.10 dilution chlorhexidine is advocated. Chlorhexidine overnight stagnant in tubing can also be used for decreasing microbial load, with an added advantage of cost effectiveness.

BIBLIOGRAPHY

1. Pankhurst Cl et al. Microbial Contamination Of Dental Unit Waterlines: The Scientific Argument. Int Dent J 1998;48:359-68.
2. ADA Council on Scientific Affairs. Dental Unit Waterlines: Approaching The Year 2000. J Am Dent Assoc 1999;130:1653-64.
3. US Department Of Health And Human Services. Recommended Infection Control Practices For Dentistry. Mmwr 1993;42: (No.Rr-8) 7.
4. Shearer Bt. Biofilm and The Dental Office. J Am Dent Assoc 1996;127: 181–189.
5. Waggoner M B. The New CDC Surgical Water Recommendations: Why They Should Be Implemented and What They Require. Compend Contin Educ Dent 1996;17: 612–614.
6. James T. Walker et al. Microbial Biofilm Formation and Contamination Of Dental-Unit Water Systems In General Dental Practice. Applied and Environmental Microbiology 2000; Vol. 66, No. 8, P. 3363-3367.
7. J.Kettering et al. Comparison of Methods For Reducing Dental Unit Water- Line Bacteria and Biofilm. Journal of Dental Research 1977;76, Abstract # 3371, 435.
8. Blake, G. C et al. The Incidence and Control of Bacterial Infection in Dental Spray Reservoirs. Br. Dent. J 1963;19:413–416.
9. Mayo, J. A et al. Bacterial Biofilm: A Source of Contamination in Dental Air-Water Syringes. Clin. Prev. Dent 1990;12: 13–20.
10. 10 Puttajahr et al. Comparison of Microbial Contamination Control between Three Dental Suction Line Cleaning Agents. Journal of Dental Research 1999;78:Abstract # 600, 180.
11. Wirthlin Mr et al. Formation and Decontamination of Biofilms in Dental Unit Waterlines. J Periodontol 2003;74(11):1595-1609.
12. Wiluams et al. Microbial Contamination of Dental Unit Waterlines: Current Preventive Measures and Emerging Options. Compendium of Continuing Education in Dentistry 1996;17: 691.
13. M. 0 Z Can, Y. The Effect of Disinfectant Agents in Eliminating the Contamination of Dental Unit Water. Journal of Oral Rehabilitation 200;30: 290-294.