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ABCDE OF DUWL (Alternate Biofilm Chair side Disinfection Efficacy of Dental Unit Waterline)

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Abstract

Introduction: Bacterial Biofilm in Dental Unit Waterlines (DUWLs) is a widespread problem, especially in third world countries like India and poses a potentially significant risk of infection to dental staff and patients, particularly those who are medically compromised or immune compromised.

Aim: The purpose of the study is to investigate the level of bacterial contamination of each DUWL at Government Dental College & Hospital, Hyderabad. And also to investigate the efficacy of three Traditionally tried (Distilled water, 0.2% Chlorhexidine, 3% Sodium Hypochlorite) and two newer easily available household disinfectants (Common salt, Turmeric) in reducing bacterial loads to < or = 200 CFU/mL as recommended by the American Dental Association & other standardizations.

Materials & Method: Water samples were collected from hand piece outlet of DUWLs from 50 dental chair units and were subjected to bacteriological analysis. Three traditionally used disinfectants Distilled water, 0.2% Chlorhexidine, 3% Sodium Hypochlorite, were taken as standards and two newer easily available household disinfectants Common Salt, Turmeric were used to treat the DUWLs. Treated water samples were collected & sent for microbial analysis.

Results: The base line TVC (Total Viable Count) of water samples ranged between 33 -43 x 10^4 CFU/mL Before and after treatment difference in TVC (Total Viable Count) was significant for all the groups except when treated with distilled water. 2% Chlorhexidine has shown maximum reduction in the TVC (Total Viable Count) after overnight disinfection.

Conclusion: 2% CHX solution was found to be the most effective and reliable means of disinfecting DUWL. Further research with higher concentrations & prolonged duration of time has to be done for making 2% Common salt solution and 2% Turmeric extract/ DMSO solution in achieving acceptable levels of disinfection.

Keywords: *Keywords: Dental Unit Water line; Biofilm; Sodium Hypochlorite; Chlorhexidine; Turmeric; CFU/ml*

Abbreviations: DUWLs: Dental Unit Waterlines; ABCDE: Alternate Biofilm Chair side Disinfection Efficacy; DUWL: Dental Unit Waterline; CFU/ml: Colony Forming Units per milliliter; TVC: Total Viable Count

Introduction

Research has shown that in newly installed dental unit waterlines, microbial counts can reach a count of 200,000 colony-forming units per milliliter (CFU/mL) within five days [1]. In fact, counts as high as 10^6 CFU/mL of dental unit water have been found in unmonitored dental unit waterlines [2,3]. The plastic tubing of dental waterlines are very narrow of about 1/8 inches in diameter with a very slow flow rate in the hand piece of 2-10 ml/minute. This hydrophobic surface and slow flow allow the initial attachment and colonization of microbes to the tubing leading to the formation of biofilm. These bacteria and other microorganisms that form a biofilm inside the tubing

that supplies water to the dental instruments act as a primary reservoir for the continues contamination because as water moves through the tubing, or waterline, microorganisms slough off into the water, thus contaminating it. These biofilm provide an environment for the growth of other microbes like algae, fungi etc [4].

There is no evidence that dental unit water is harmful to patients [5]. Nevertheless, the CDC (Centers for Disease Control and Prevention) states that, "Exposing patients or dental health care personnel to water of uncertain microbiological quality, despite the lack of documented adverse health effects, is inconsistent with generally accepted infection control principles."

When compared with the general population it was found that the dentists and dental staff have higher chances of respiratory infections [4]. Aerosols from high speed drills act as a source of spreading these infections, because of presence of microbes like Legionella and Pseudomonas in the stagnant water of the dental units, Legionella species are pathogenic microorganism which results in two forms of diseases like Pneumonic form like Legionnaires disease and non-Pneumonic form like Pontiac fever [6,7]. Another important pathogen seen in the dental unit water line is *P. aeruginosa* is present in the oral cavity and can be aspirated into the dental unit water line through the defective values and may lead to development of nosocomial infections [1,8,9].

These microbes present in the DUWL may be ingested, inhaled and directly contaminate the surgical site which may definitely cause certain infections like development of systemic infections or oral abscess. Different standards and strategies have been adapted to control DUWL transmitted infections. According to American Dental Association (ADA), dental water should not have more than 200 colony forming units per milliliter (CFU/ml) of aerobic, mesophilic, heterotrophic bacteria. Different methods like anti-retraction valves and retrograde aspiration of oral fluid, UV light treatment of water, independent water systems, filtration, air purging, flushing of biocides, sterile water delivery systems, use of biocides/chemical disinfectants, ozone have been evaluated previously.

Aim

The aim of the study is to investigate the level of Bacterial contamination in the DUWLs at GDC&H, Hyderabad and to Compare the efficacy of two traditionally tried (2% Chlorhexidine, 2% Sodium Hypochlorite) and two easily available cheaper alternate disinfectants (2% Common Salt, 2% Turmeric Extract/DMSO) in reducing bacterial loads to acceptable levels, recommended by ADA (American Dental Association)/CDC (Centers for Disease Control)/EPA (Environment Protection Agency).

Materials and Methods

50 dental chairs were selected that were used most often for the dental treatments in the Department of Conservative Dentistry and Endodontics, Government Dental College, Hyderabad. The study was approved by the ethical committee of Osmania General Hospital and Institutional level ethics committee at GDC & H, HYD. Noneof the selected units had ever been treated for removal of bio-film or reduction of planktonic bacteria. Random water samples of 20 ml were collected in sterile containers from the outlet of hand piece line. Before sample collection, the end of each hand piece was disinfected with alcohol to avoid other sources of contamination. Water splashing was minimized when filling the sample container and any contact between the hand piece and the container was avoided. Samples were stored in a refrigerator and processed in the laboratory within two hours. The samples were centrifuged at 3000 rpm for 5 minutes, supernatant was discarded and centrifuged deposit was used for cultures. The collected samples were subjected to aerobic cultures. Viability count study was made using pour plate and most probable number technique (MPN).

50 Chairs were divided into 5 groups of 10 chairs. Each group of 10 dental chairs was treated with the particular disinfectant overnight. In Group A, Distilled water was added to the booster bottle of the unit. This served as the control. In Group B, 2% Sodium Hypochlorite was used and the dilution used to achieve 2% Sodium Hypochlorite was achieved 330 ml of 3% NaOCl & 660 ml of Distilled water. In group C, 2% Chlorhexidine was used. In Group D, 2% Common Salt solution was used. 20 gms of Common salt was diluted in 1 litre Distilled water to obtain 2% Common Salt solution. In group E, 2% Turmeric extract/DMSO (Dimethyl Sulfoxide) solution was added. 20 ml of Turmeric extract in 1 litre DMSO solution gave 2% Turmeric extract.

After adding the disinfectant to the reservoir bottle, the solution was run through the system for two minutes. Once the disinfectant was flushed till it came out of the other ends, the unit was then turned off and the disinfectant was left in situ overnight for 18 hours. At the beginning of the next workday, remaining disinfectant solution was discarded and the external water bottle was rinsed with hot water. The bottle was filled with hot water, each of the dental unit water lines (air/water syringe, hand piece lines) were flushed till froth disappeared and clear water could be seen. Water from aqua guard classic was made to run through the tubing. Water samples of 20 ml from each treated unit's aerotar hand piece were collected in separate sterile containers under aseptic conditions and labeled before treating the first patient of the day and quantified for total viable counts. The treated water Samples with microbial colonies were shown in figure 1.

Results

The study included collection of water samples from each unit beginning with baseline collection and after DUWL exposure to the disinfectant. Results obtained were the mean TVC in the treated water samples. Statistical Analysis was done for both Intra& Inter Group, before and after treatment of Dental water line booster bottles.Wilcoxon signed rank test was used for intra group comparison. Mann Whitney U test was used for inter group comparison ($P < 0.05$).

The base line TVC of water samples ranged between 33-43 x 10^4 CFU/ml. Before and after treatment difference in TVC was significant for all the groups except when treated with distilled water. 2% Chlorhexidine has shown maximum reduction in the TVC after overnight disinfection. Table 1 depicts the intra group comparison of mean CFU/ml before and after treatment. Table 2 depicts intergroup comparison of mean CFU/ml after treatment

Groups	Before Treatment	After Treatment
Group A	35 x 10^4 CFU/ml	35 x 10^4 CFU/ml
Group B	32 x 10^4 CFU/ml	10 CFU/ml
Group C	38 x 10^4 CFU/ml	Nil CFU/ml
Group D	43 x 10^4 CFU/ml	29 x 10^4 CFU/ml
Group E	32 x 10^4 CFU/ml	10^4 CFU/ml

Table 1: *The intra group comparison of mean CFU/mL before and after treatment.*

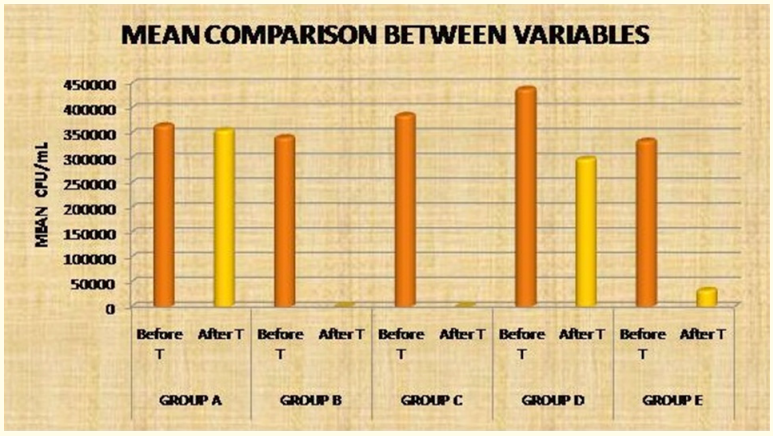

Chart 1: *The intra group comparison of mean CFU/mL before and after treatment.*

Groups	Before Treatment
Group A	10^2 CFU/ml
Group B	32 x 10^4 CFU/ml
Group C	38 x 10^4 CFU/ml
Group D	13 x 10^4 CFU/ml
Group E	30 x 10^4 CFU/ml

Table 2: *The intra group comparison of mean CFU/mL before and after treatment.*

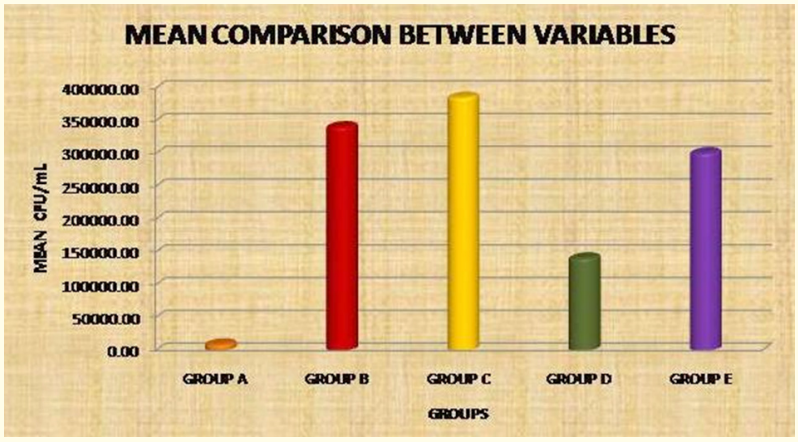

Chart 2: *Intergroup comparison of mean CFU/ml after treatment.*

Discussion

The biofilm on the inner surface of the tubing of dental units provides a continuous reservoir for microorganisms [10,11]. Not only patients but also dentists and dental personnel are at risk of being infected with opportunistic pathogens such as *Pseudomonas* or *Legionella* species by means of cross-infection or following aerosol formation from water emanating from DUWL [12,13]. The contaminates adhere to luminal walls of the small bore tubing in the dental units, and over a period of weeks or months, become a dense accumulation of biofilm resulting in dense growth of microorganisms [14]. Microorganisms suspended in an aqueous medium become attached to an available surface and multiply, forming micro colonies. Biofilm contamination of the dental unit water systems is a universal problem. *Pseudomonas, Klebsiella, Nocardia*, *Streptococcus, Micrococcus, Flavobacterium, Staphylococcus, Legionella*, are some of the organisms identified in dental unit waterlines. The relationship between biofilm organisms is often symbiotic with one species providing key cofactors required by another.

As water moves through the tubes of a working dental unit, the water flow is faster at the center of the lumen and more slowly away from the center as a result of friction with the walls. It is this part of the tubing where water is virtually stagnant, allows bacteria to colonize the internal surface. As the diameter of the waterline decreases, an increasingly larger surface area relative to volume becomes available for colonization allowing the bacteria to be in contact with the water line surface for a longer period of time.

There are certain studies which mainly aim at providing certain options for maintaining the water lines and reducing the chances of cross infection. Biofilm can be prevented by periodical flushing of the waterlines. The water line should be flushed for several minutes before starting so as to reduce microbe contamination that might have happened due to water stagnation. The mechanical flushing

compared with the use of disinfectants used in the present study will only temporarily reduce the number of microbes but does not have any significant effect on the adherent biofilm [3,15]. Installation of 0.22 microns filters as closely as possible reduced the number of microbes in the post filtration scanning electron microscope section [15].

Use of sterile water irrigating delivery system provided sterile solutions directly to the dental operating handpiece was found to be effective compared to DUWL but the main disadvantage include high cost and need for packed sterile solutions.

The various disinfectants used are Sodium Hypochlorite, Chlorhexidine Gluconate, Hydrogen Peroxide based solution and Citric Acid based products. Various authors have reported the use of biocides/disinfectants as effective decontamination methods to control DUWL contamination [16].

Biocides are non-antibiotic, antiseptic, disinfecting chemical compounds, having both bactericidal and bacteriostatic properties [17]. Other properties include that these should be effective at low concentrations, should be non-toxic and biodegradable [17]. The biocidal action depends on (i) chemical properties (e.g. optimum pH and temperature of activity, reactivity), (ii) micro-organism (e.g. tolerance/resistance, metabolic status, number of organisms in the population), (ii) environment (e.g. surface type, water activity, presence of other reactive compounds). The biocide should therefore have a wide range of activity; both in terms of type of microorganisms susceptible and conditions of action [17]. An ideal biocide should be easily available, low cost, prevent the reformation of biofilm and protect the internal parts of water line from any corrosion or degradation. These compounds may be intermittently or continuously added to the waterline. The main disadvantage with these compounds is that there are aerosolized leading to a chronic exposure of these chemicals to the dental staff and may also have certain effect on the bond strength of adhesive resins [3,18].

Compounds such as H_20_2, Ozone and UV when introduce into the waterline were found to effective in reducing the microbes. Later on the efficacy of H_20_2 and Ozone was found to be limited and UV alone had a significant effect in lowering the number of planktonic microbes throughout. These compounds have been avoided to be introduced in the water line due to its potential for pollution and its destructive effects on environment.

The present study aimed at evaluating the efficacy of two traditionally tried disinfectants; 0.2% Chlorhexidine and 2% Sodium Hypochlorite and & two easily available cheaper alternate disinfectants 2% Common Salt, 2% Turmeric extract/DMSO in reducing bacterial loads to acceptable levels, recommended by ADA,CDC,EPA. In the first part of the study, prior to the use of disinfectants for flushing dental unit waterlines, water samples were collected at random intervals. These samples were sent to the laboratory for microbiological analysis.

Sodium hypochlorite has a wide spectrum antimicrobial efficacy, particularly controls. Sims., *et al*. [19] reported the effects of using varying concentrations (0.5-5.25%) of bleach in dental settings. According to him, although bleach is effective in biofilm from tubing samples but it also causes (i) slow corrosion of metal fitting in dental units (ii) compliance problems in private practice dental settings (iii) reacts with matrix to create chlorinated by products [19]. According to a study done by CL Pankhurst higher dose of 3-5 ppm of Sodium Hypochlorite was effective in controlling the Legionella proliferation. But prolonged exposure to Chlorine showed an increase in their Chlorine resistance often leading to failure in eradication of microbes [20].

Chlorhexidine is among one of the most tested Compounds. At high concentration it is bactericidal and in regular concentration (0.12-0.2%) it is bacteriostatic. Chlorhexidine Protects against microbial colonization and subsequently Biofilm development. It disrupts the cell membrane. Upon application *in vitro*, Chlorhexidine can kill nearly 100% of Gram- positive and Gram-negative bacteria within 30 seconds. Common Salt Solution causes cell death by osmosis. Its hyper tonicity pulls the water out of the cell causing its death. Curcumin is the active ingredient in turmeric responsible for its antimicrobial activity. It causes Bacterial cell wall perturbation. It is active against Pseudomonas, Staphylococcus, and Bacillus. It is not water soluble. Therefore, DMSO (Dimethyl Sulfoxide) Universal solvent is used. However it causes staining of the equipment. 0.2% Chlorhexidine and 2% sodium hypochlorite recorded nil TVC which is in accordance with the previous studies.

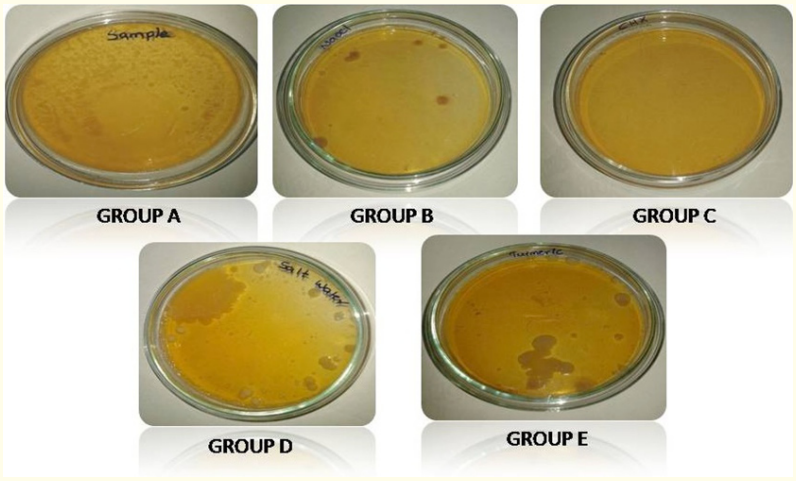

Figure 1: *Post treatment water samples showing CFU in Groups A-E.*

Conclusion

From this study it can be concluded that overnight disinfection with 0.2% Chlorhexidine and 2% Sodium Hypochlorite is a suitable method of disinfecting Dental unit water lines. Further research with higher concentrations and prolonged duration of time has to be done for making 2% Common Salt solution and 2% Turmeric extract/DMSO solution in achieving acceptable levels of disinfection. A reasonable protocol is mandatoryfor disinfecting and monitoring the water supply in busy dental practices, so that water used for dental patient treatment satisfies accepted safe public health standards.

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