ORIGINAL ARTICLE

Comparison of chlorine dioxide and chlorhexidine 2% antiseptic in reducing bacterial colony counts as an alternative to DUWLs cleaning: a quasi-experimental study

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ABSTRACT

Introduction: The inner surfaces of dental unit waterlines (DUWLs) possess an accumulation of any bacteria. Microorganisms can enter dental unit waterlines from water reservoirs. Antiseptics are substances that inhibit the growth of bacteria. Chlorine dioxide is effective in decontaminating microbes in the DUWLs and has a beneficial effect on reducing nosocomial infections. Chlorhexidine effectively prevents the growth of *Streptococcus* bacteria. The addition of antiseptic agents to the water source contributed to a significant reduction of the cultivable microbial counts in the aerosol. The purpose of this study was to analyze the difference between Chlorine Dioxide 0,1% and Chlorhexidine 2% antiseptics on the number of bacterial colonies in the Dental Unit Waterline. **Methods:** This study was a quasi-experimental study with a total sample size of 8 dental units that have water tanks in the oral surgery clinic. The sampling technique was total sampling, where the sample was divided into 2 groups, group I using Chlorine Dioxide and group II using 2% Chlorhexidine calculation of colony counts unit using the plate count method. **Results:** The difference in the number of colonies before being given Chlorine Dioxide was 13,153 CFU/mL and after being given Chlorine Dioxide antiseptic was 6,070 CFU/mL, while before being given 2% Chlorhexidine antiseptic was 12,917 CFU/mL and after being given 2% Chlorhexidine antiseptic was 2,823 CFU/mL. There is a significant difference in the number of bacterial colonies before and after being given Chlorine Dioxide and Chlorhexidine 2% antiseptic with ρ=0.001; **Conclusion:** Chlorhexidine 2% reduces bacterial colony forming unit in DUWLs much more than using Chlorine dioxide for the alternative of DUWLs Cleaning Agents.

KEYWORDS

DUWLs, chlorine dioxide, chlorhexidine 2%, bacterial colony counts

INTRODUCTION

Dental units are generally utilized for oral health services. Dental Unit is an important part, which consists of several main devices, including a high-speed handpiece, three way syringe and ultrasonic scaler. Dental unit waterlines (DUWLs) drain water into the dental unit. The water is used to operate several dental tools available in the dental unit such as threeway water syringe, contra angle, ultrasonic scaler, etc.[1] Water is important in dental treatment, because it functions as a coolant that works when the contra angle is being used to avoid excessive heat and for irrigation during dental treatment. In general, dental units' water must conform to drinking water standards. The water provided can be directly from the well and can be stored in the reservoir/water tank in the dental unit.[2]

Dental procedures using high-speed handpieces can produce liquid splashes and liquid sprays that usually contain pathogenic microorganisms, therefore, many studies have stated the importance of reducing water pollution with microorganisms found in waterways in dental units.[3] Dental unit waterlines (DUWLs) have been shown to harbor the development of aerobic, mesophilic, and heterotrophic microorganisms commonly found in drinking water systems.[4] High-speed handpieces are used for many dental treatments such as: scraping tooth tissue during restorative procedures, removing and polishing restoration materials, removing bone during surgical procedures. Tooth extractions and surgeries using high-speed handpieces can produce aerosols containing blood and allow airborne exposure so that operators (dentists) and dental nurses can be exposed to microorganisms.[5] Healthcare staff in the clinic area such as dentists and nurses can also inhale airborne aerosols generated from dental treatment.[6]

The prevention of infections should be performed by health care providers worldwide to protect patients from hospital-acquired infections, both healthcare workers and patients visiting healthcare

facilities (dental clinics).[7] The Centers for Disease Control and Prevention (CDC) recommends that, various measures used to minimize biofilm levels in dental unit waterways include adding a dose of water chemistry (e.g., with hydrogen peroxide, peroxygen compounds, silver ions, iodine, chloramine or silver nanoparticles). The contamination rate of DUWLs with *L. pneumophila* and *P. aeruginosa* is very high. Therefore, it is recommended to use high-quality water, implant filters in water reservoirs, and to regularly monitor water resources, as the best measures that can be taken, to prevent bacterial colonization and biofilm formation in the DUWLs and avoid many infections caused by opportunistic pathogens:[8] Gram-positive cocci contamination in eight samples and Gram-negative bacilli contamination in sixteen samples. Klebsiella pneumoniae was the bacteria found in two samples of water flowing as irrigation flow in the water syringe. DUWLs is a potential source of microorganisms.[9]

The Centers for Disease Control and Prevention (CDC) recommends that any device that enters the patient's mouth (e.g. handpiece, ultrasonic scaler, or air/water syringe) should be connected to a water line and flushed for at least 20 seconds after each patient. Flushing lines after each patient and at the start of the day, reduces biofilm accumulation overnight or over the weekend. This is especially important after periods of non-use (such as vacations and long weekends). Daily flushing has been shown to reduce bacteria levels in dental unit drains.[10]

Research on water contamination in dental units was first conducted in the 1960s by Blake GC. The results of this study stated that water from the DUWLs contained many microorganisms.[11] Some studies reveal that DUWLs is often contaminated by several microorganisms (bacteria, fungi, protozoa, viruses). Based on the literature, there have been cases and reports of proven links between local or systemic infection from exposure to contaminated DUWLs.[12] The goal of cross-infection control is to minimize potential exposure and create a safe workplace for patient care*. Legionella sp, Streptococcus sp, Mycoplasma sp, Staphylococcus sp, Haemophilus spp are* bacteria found to cause pneumonia, a respiratory infection. British dental association recommends for infection control in DUWLs, in that DUWLs channels should be cleaned by flushing before starting work which is necessary to reduce the number of microbes that arise due to water deposits when the dental unit is not in use.[12] In one such case, an 82-year-old Italian woman died of pneumonia due to *Legionella spp* after exposure to dental units contaminated with *L. pneumophila* serogroup 1.[6] Therefore, the use of appropriate antiseptic is recommended to reduce the prevalence of contamination and reduce the probable cross-infection.

Most studies have tested the efficacy of disinfectants as flushing solutions with intermediate use of a normal water supply. However, disinfectants may only be effective when the development of biofilm is minimized and the intermittent exposure of normal water is also eliminated. Disinfectants used in DUWLs do not only have to eliminate heterotrophic bacteria but also common pathogens. In addition, the disinfectant has to be safe and biocompatible.[13] The addition of antiseptic agents to the water source contributed to a significant reduction of the cultivable microbial counts in the aerosol and hence can be used to reduce the risk of cross-infection during ultrasonic scaling.[14]

The results of Mamajiwala's 2018 study on Cinnamon (CIN) and Chlorhexidine 2% (CHX) both used in tanks installed in dental units effectively help reduce the number of bacteria. Chlorhexidine is more effective in preventing the growth of *Streptococcus* bacteria.[15] Chlorine dioxide is a safe and effective disinfectant, even at concentrations as low as 20-30 mg/L.[16] Chlorine dioxide mouthwash is effective in controlling contamination in the DUWLs for ultrasonic scalers installed in dental units. At low concentrations, Chlorine Dioxide with and without flux significantly reduced mixed culture biofilms cultured in vitro on DUWLs tube sections. Therefore, it has the potential to be used in patient treatment water as it is potable at this concentration, while decontaminating and limiting biofilm formation in the waterway.[17] In the study, we used two antiseptics to look for alternative materials for minimizing bacterial colonies that are safe for oral mucosa and widely available on the market for daily regular DUWLs cleaning procedures.

The addition of antiseptic agents to the water source contributed to a significant reduction of the cultivable microbial counts in the aerosol. And also safer for the mucosa. The researcher planned to research on Comparison of Chlorine dioxide and Chlorhexidine 2% Antiseptics on the Number of Bacterial Colonies in Dental Unit Waterline. This study is expected to reduce the number of bacteria found in the dental unit water container while reducing the risk of cross infection. The purpose of this study was to analyze the difference between Chlorine Dioxide 0,1% and Chlorhexidine 2% antiseptics on the number of bacterial colonies in the Dental Unit Waterline.

METHODS

The type of research was the Quasi Experiment. The sample used was all dental units in the Oral Surgery clinic since only those dental units had a water reservoir connected directly to the dental unit in RSGM UNPAD and removed through a high speed contra angle. Dental units were divided into 2 groups Group I, 4 dental units using Chlorine Dioxide 0,1% and Group II, 4 dental units using Chlorhexidine 2%. The materials and tools used for this study consisted of distilled water, measuring cups, Chlorine dioxide antiseptic (Oxyfresh,Stabilized Chlorine Dioxide), Chlorhexidine 2% antiseptic (Hexidine, Onemed), Colony Counter, sterile tubes, denatured alcohol, micro pipettes, incubators, agar media, sterile cups.

Microbiology test was conducted at the Analyst Medical Technology Laboratory of the Bandung Health Polytechnic. Distilled water was put into the tube and released through the contra angle. The first day the samples that have been obtained are put into sterile tubes and taken for bacterial cultivation. The samples that were brought were samples before being given antiseptic. The tube was replaced with distilled water mixed with antiseptic in a ratio of 1:10. The water was removed from the contra angle for 5 minutes and then it was let it to stand for 24 hours.[4]

This Microbiology test used the pour method. Petri dish had been poured as much as 1000 µL sample and pouring nutrient agar into petri into the petri dish repetition was done 3 times. Petri dishes were placed in an incubator for 24 hours at 37°C. Calculation of colony counts used the plate count method. Counting the number of bacterial colonies used Colony Counter. The same thing was done as the first day for sampling.[18] Data analysis was measured using the normality test using Shapiro Wilk because the number of samples was less than 50 and continued with Mann-Whitney test.

RESULTS

Comparison of the number of bacterial colonies before and after antiseptic treatment

Table 1. Comparison of the number of bacterial colonies before and after giving Chlorine Dioxide and Chlorhexidine 2% antiseptic

Antiseptic	Colony count before (CFU/ml)	Colony count after (CFU/ml)	Average decrease (CFU/ml)	Decrease presentation (CFU/ml)
Chlorine Dioxide	13.153	6.076	589.75	53.81 %
Chlorhexidine 2%	12.917	2.823	841.17	78.15 %

The results of the number of bacterial colonies show that there is a difference in the decrease that occurs between the two antiseptics where the Chlorhexidine 2% antiseptic is more decreased with an average decrease of 841.17 CFU/mL (78.15%), compared to the Chlorine Dioxide antiseptic with an average decrease of 589.75 CFU/mL (53.81%).

Table 2. Mann-Whitney test results before and after Chlorine Dioxide and Chlorhexidine 2% treat to DUWLs

	n	Median (Minimum-Maksimum)	ρ-value
Chlorine Dioxide	24	1086.25 (11 – 2528)	0.001
Chlorhexidine 2%	24	370.79 (0 – 1100)	

Mann Whitney test showed a significant difference in the number of bacterial colonies before antiseptic administration and after antiseptic administration of Chlorine Dioxide and Chlorhexidine 2%.

DISCUSSION

Based on the results of the above research, before and after being given Chlorine Dioxide antiseptic there is a decrease in the number of bacterial colonies. In line with the results of this study according to Noszticzius et al, Chlorine Dioxide is an antimicrobial agent that can kill micron-sized organisms quickly and can also be used in patient care water, because it is very safe when ingested by patients at this concentration, and to decontaminate and limit the number of bacteria in waterways.[19] Chlorine Dioxide has advantages over other chlorine products. Controlling DUWLs bacteria may have a very good effect on nosocomial infection control.[20] Chlorine dioxide water line cleaners will be most effective in preventing DUWLs contamination.[21] Chlorine Dioxide belongs to the Halogen and Halogenophore family where this compound is often used for antiseptics and disinfectants.

Chlorine Dioxide has many advantages besides reducing the number of bacterial colonies in the dental unit. This Chlorine Dioxide is used to eliminate halitosis according to research.[22] Chlorine dioxide can also reduce plaque and gingival indices and reduce the number of bacterial colonies in the oral cavity.[23] Chlorine dioxide penetrates bacterial cell walls and reacts with vital amino acids in the cell's cytoplasm to eliminate the organism.[24] Since chlorine dioxide is derived from chlorine, it is possible for some microorganisms, like mycobacteria, to acquire resistance to chlorine dioxide. Chlorine resistance may result from the composition of the mycobacteria cell membrane, as the study explains.[16]

The results of the study before and after being given Chlorhexidine 2% showed that there was a decrease in the number of bacterial colonies. In line with this study, according to research by Agahi et al, it showed that Chlorhexidine 2% can reduce the number of microbes in the dental unit waterline.[25] Chlorhexidine is better at reducing the number of bacterial colonies in the dental unit waterline compared to povidone iodine.[26] Chlorhexidine has broad-spectrum antimicrobial activity. Chlorhexidine is potent in reducing gram positive and gram negative bacteria including aerobes and anaerobes, yeast fungi, viral lipid envelopes. Chlorhexidine increases cell membrane permeability

followed by coagulation of cell macromolecules. Chlorhexidine does not interact with microbial enzymes or receptors so it does not cause resistance in the organism.[27] Chlorhexidine belongs to the biguanide group whose mechanism of action damages the microbial cell membrane by coagulating cytoplasmic proteins.[28] Antimicrobials include vegetative bacteria, fungi and viruses. Bactericidal concentrations cause bacterial cell destruction. Chlorhexidine is very useful for reducing the number of bacterial colonies.[29]

The results of this study showed a significant difference between Chlorine Dioxide and Chlorhexidine 2%, namely by looking at the decrease in the number of bacterial colonies in the Dental Unit waterline that occurred. The number of bacterial colonies in the Dental Unit Waterline which was greater in the group given Chlorhexidine 2% compared to the group given Chlorine Dioxide. Chlorhexidine is more effective than Chlorine Dioxide. The weakness in this study was using Chlorine Dioxide which is a lower percentage than Chlorhexidine, as Chlorine Dioxide was already available in this study.

All the research that has been conducted, shows that the research on the comparison of antiseptics Chlorine Dioxide and Chlorhexidine 2% on the number of bacteria in the dental unit waterline has a significant difference in the number of bacterial colonies between the Chlorine Dioxide group and the Chlorhexidine 2% group. The decrease in the number of colonies is more in Chlorhexidine 2%. CHX can be used as an irrigation agent in the DUWLs, this antiseptic is safe to use within 1 week.

This study's limitation was that the concentrations of chlorhexidine and chlorine dioxide differed. Compared to chlorhexidine, the concentration of chlorine dioxide was lower.

CONCLUSION

Chlorhexidine 2% reduces bacterial colony forming unit in DUWLs much more than using Chlorine dioxide for the alternative of DUWLs Cleaning Agents

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