Original Article

Evaluation of Hydrogen Peroxide and Cetylpyridinium Chloride as Bacterial Decontaminants of Dental Unit Water Lines at a Private Peruvian Dental School

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Received : 19-05-20
Revised : 27-06-20
Accepted : 09-07-20
Published : 24-11-20

ABSTRACT

Aim: The aim of this study was to evaluate the hydrogen peroxide and cetylpyridinium chloride as bacterial decontaminants of dental unit water lines at a private Peruvian dental school. **Materials and Methods:** Water samples were obtained from 66 dental units of a University Dental Clinic before decontamination treatment and at days 3 and 7 thereafter. The biofilm treatments were applied equitably among the two treatment groups ($n = 22$) and one negative control (distilled water). The samples obtained on each collection day were taken to the biochemical laboratory in thermal boxes and then diluted, seeded, and incubated at 37°C for 24 h to count colony forming units per milliliter (CFU/mL). **Results:** The samples to which hydrogen peroxide were applied had a mean of 1.53×10^5 CFU/mL before application, 0.04×10^5 CFU/mL at day 3, and 0.03×10^5 CFU/mL at day 7, whereas the samples undergoing cetylpyridinium chloride treatment had a mean of 1.74×10^5 CFU/mL before application, 615.38 CFU/mL on day 3, and 307.69 CFU/mL on day 7. Distilled water treatment showed a mean of $1.72 \times 10^5 \pm 0.39 \times 10^5$ CFU/mL at baseline, $1.51 \times 10^5 \pm 1.40 \times 10^5$ CFU/mL at day 3, and a mean of $1.74 \times 10^5 \pm 0.47 \times 10^5$ CFU/mL at day 7. Statistically significant differences were found among the three treatment groups at days 3 ($P \leq 0.001$) and 7 ($P \leq 0.001$) but not at baseline ($P = 0.306$). **Conclusions:** The antibacterial effect of cetylpyridinium chloride was significantly greater than that of hydrogen peroxide and distilled water, and can, therefore, be used for bacterial control in the water lines of dental units.

KEYWORDS: *Decontamination, dental school, dental unit, microbial profile*

INTRODUCTION

Advances in diagnostic methods and treatment alternatives have led to improvements to preserve the health of individuals over time. These improvements are continuously evolving as new concepts are developed in both the health sector and in general. One of the areas that has witnessed important changes has been biosecurity, with the need to reduce the risk of either patients or health personnel becoming ill or adversely affected by medical treatments. Relevant measures must be taken to avoid possible contamination during health-care procedures. Therefore, various protocols and recommendations, such as the sterilization of materials and the use of aseptic environments, among others, have been combined.[1-4]

Access this article online

Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_265_20

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How to cite this article: Munive-Degregori A, Mayta-Tovalino F, Gallo W, Luza S, Mauricio F, Ilizarbe S, *et al.* Evaluation of hydrogen peroxide and cetylpyridinium chloride as bacterial decontaminants of dental unit water lines at a private Peruvian dental school. J Int Soc Prevent Communit Dent 2020;10:731-5.

These advances are not alien to the field of dentistry, with biosecurity processes having been adopted for use in dental care. However, particular events in dentistry, such as the accumulation of biofilm in the water lines of dental units, differ from those of the medical profession, and they should be monitored and measured periodically according to the recommendations of the American Dental Association.[5,6]

It is important to identify that there are various chemical agents made from different active ingredients that are used for antimicrobial control. Among them, hydrogen peroxide and cetylpyridinium chloride have effectiveness due to their broad spectrum of action and substantivity.[2-6]

Water quality in a dental unit is of considerable importance as patients and health-care personnel are regularly exposed to the spray generated from dental units. Therefore, control of the level of water contamination is mandatory in the daily routine procedures of a dental office. Despite the lack of epidemiological evidence, some studies have described water lines of dental units as a source of infection. At a public health level, pathogens, which may be potentially harmful to humans, such as *Pseudomonas*, *Legionella*, and some strains of *Mycobacterium*, have been isolated in the water of dental units. Microorganisms found in the water lines of dental units can generate a biofilm that adheres to the walls of the water pipes, causing a level of bacterial contamination above permissible levels.[7-10] Nonetheless, to date there is little knowledge about the latent risk of contamination due to biofilm production in the pipes through which water is supplied to dental units.[1-6]

Therefore, the aim of this investigation was to evaluate hydrogen peroxide and cetylpyridinium chloride as bacterial decontaminants of dental unit water lines at a private Peruvian dental school.

Materials and Methods

Study design and participants

This was an experimental *in vitro*, longitudinal, comparative study. The unit of analysis was made up of the water lines of 66 dental units of the Stomatology Clinic of the Inca Garcilaso de la Vega University, Lima, Peru. The sample size was estimated using the means comparison formula with the Stata version 15.0 program (College Station, TX, USA). A confidence level (α) of 0.05 and a test power (β) of 0.8 were used.

The selection criteria are as follows:

- Dental units of a private dental clinic
- Dental units with irrigation systems in good condition
- Dental units without biofilm control of the water lines

Preparation of culture media

For the preparation of the culture medium, a proportional amount of MacConkey agar powder and distilled water was poured into a beaker. The sample was homogenized with a metal spoon and then placed on a metal container with water reaching three-fourth of the height of the beaker at the time of immersion. The metal vessel with the submerged beaker was then placed on an electric laboratory cooker at 300°C for 40 min. After heating, homogenization was considered successful if the mixture has the same color. Following the mixing, the beaker was placed along with the test tubes, which were used to collect the water samples and make the solution, covered, and wrapped with aluminum foil, into the autoclave for a complete cycle. Then, the liquid agar solution was poured into the petri dishes. To guarantee the ideal aseptic conditions, this procedure was performed with a laboratory burner or inside a gas hood. Finally, the petri dishes were refrigerated at 10°C until use. Regarding the test tubes, these were removed from the autoclave, then numbered, and placed in a thermal container under aseptic conditions, so that they are ready for sample collection.[5,8,10]

Collection of the biofilm

The samples were collected before the biofilm treatment and at 3 and 7 days after biofilm treatment. The distilled water, hydrogen peroxide, and cetylpyridinium chloride biofilm treatments were randomly assigned to the dental units. All the samples were obtained at the end of the clinical shift, using adequate protection (protective glasses, mask, and gloves). A total of 5 mL of water was collected using a triple syringe and injected into the test tubes, which were then immediately capped to avoid possible contamination.[5,11]

Biofilm treatment

The biofilm treatment consisted of removing the water supply bottle from the dental unit and activating all the water outlets of the dental unit until the remaining water was emptied. The water supply bottle was then filled with the assigned biofilm treatment and then placed back in the unit. The water outlets were activated for 1 min, and the bottle was removed. Finally, the content of the bottle was emptied, and it was refilled with the type of water that is commonly used. The water outlets of the dental unit were then activated until there was no biofilm treatment substance remaining inside the unit. The same sample collection procedure was performed on the days 3 and 7 of biofilm treatment.[5,11]

Sample processing

The water samples in the test tubes were transported in a thermal container to the laboratory for preparation. The test tubes with the samples were diluted at a 1:10

ratio in other test tubes and then placed in a vortex centrifuged to ensure homogenization. Then, we seeded the petri dishes, previously prepared with MacConkey agar, using the striatum technique. Finally, the petri dishes were incubated at 37°C for 24 h to stimulate colony formation. Thereafter, the plates were removed to count the colony forming units (CFU).[2,3,8]

Statistical analysis

The CFU/mL of all samples was registered in the data collection table. This table was digitized and transcribed in a Microsoft Excel file to convert the data into a numerical format, which was sent to the Stata, version 15.0 program database, for statistical analysis. For the univariate analysis, the descriptive statistics (mean, median, standard deviation, minimum value, and maximum value) of the variables under study by the treatment of the applied biofilm were obtained. In addition, the normal distribution of the sample was evaluated using the Shapiro–Wilk test. Finally, the Kruskal–Wallis test was used to establish statistical differences with a confidence level of $P < 0.05$.

Results

Univariate analysis of the dental units undergoing hydrogen peroxide treatment showed a mean of $1.53 \times 10^5 \pm 0.37 \times 10^5$ CFU/mL at baseline, $0.04 \times 10^5 \pm 1.11 \times 10^5$ CFU/mL at day 3, and $0.03 \times 10^5 \pm 0.05 \times 10^5$ CFU/mL at day 7, whereas distilled water (negative control) had a higher mean of $1.74 \times 105 \pm 0.47 \times 105$ on day 7 [Table 1].

On the contrary, univariate analysis of cetylpyridinium chloride treatment showed a mean of $1.74 \times 10^5 \pm 0.36 \times 10^5$ CFU/mL at baseline, $615.38 \pm 0.02 \times 10^5$ CFU/mL at day 3, and $307.69 \pm 0.01 \times 10^5$ CFU/mL at day 7, whereas distilled water (negative control) had a higher mean of $1.74 \times 105 \pm 0.47 \times 105$ on day 7 [Table 1].

Lastly, univariate analysis of distilled water treatment showed a mean of $1.72 \times 10^5 \pm 0.39 \times 10^5$ CFU/mL at baseline, $1.51 \times 10^5 \pm 1.40 \times 10^5$ CFU/mL at day 3, and a mean of $1.74 \times 10^5 \pm 0.47 \times 10^5$ CFU/mL at day 7 [Table 1].

Before the application of the biofilm treatments, there were no statistically significant differences among the treatments regarding the degree of bacterial contamination: hydrogen peroxide 1.5%, cetylpyridinium chloride 0.05%, and distilled water (negative control) ($P = 0.306$). However, at days 3 and 7, statistically significant differences were observed among the three treatments ($P < 0.05$) [Table 2].

Discussion

The purpose of this study was to evaluate the antibacterial effectiveness of hydrogen peroxide and cetylpyridinium chloride as biofilm treatment in the water lines of dental units and determine their effectiveness over time (before application and at days 3 and 7). To obtain samples that truly reflect the level of contamination present in the internal part of the water lines of the dental units, water samples were obtained from the terminals of the water lines (triple syringe) rather than surface samples, as the latter can be contaminated with aerial bacteria, altering the veracity and accuracy of the state of internal contamination. The samples were obtained according to the studies of Monteiro *et al.*,[11] Szymańska,[12] and Nikaeen *et al.*[13]

A direct count of the CFUs would be impossible due to the exponential growth of bacteria, which yields high levels of CFUs. Taking this into account, preprocessing of the water samples was necessary. After performing the CFU count, the values obtained by the conversion factor were multiplied to obtain the final results.[1-5]

In this study, the mean CFU counts before the application of the different biofilm treatments were 1.53×10^5 CFU/mL in the hydrogen peroxide group, 1.74×10^5 CFU/mL in the cetylpyridinium chloride, and 1.72×10^5 CFU/mL in the distilled water group. These values were similar to those reported by Barbeau

Table 1: Antibacterial effect of biofilm treatment on water lines of dental units over time

	Time	Mean (CFU/mL)	Median	SD	Min	Max	*P**
Hydrogen peroxide 1.5%	Baseline	1.53×10^5	1.40×10^5	0.37×10^5	0.92×10^5	2.16×10^5	0.362*
	Day 3	0.04×10^5	0	1.11×10^5	0	0.40×10^5	<0.05
	Day 7	0.03×10^5	0	0.05×10^5	0	0.16×10^5	<0.05
Cetylpyridinium chloride 0.05%	Baseline	1.74×10^5	1.72×10^5	0.36×10^5	1.20×10^5	2.40×10^5	0.670*
	Day 3	615.38	0	0.02×10^5	0	0.04×10^5	<0.05
	Day 7	307.69	0	0.01×10^5	0	0.04×10^5	<0.05
Distilled water	Baseline	1.72×10^5	1.76×10^5	0.39×10^5	1.20×10^5	2.52×10^5	0.635*
	Day 3	1.51×10^5	1.40×10^5	0.35×10^5	1.12×10^5	2.12×10^5	0.117*
	Day 7	1.74×10^5	1.64×10^5	0.47×10^5	1.20×10^5	2.60×10^5	0.027

*Shapiro–Wilk test; only groups with an asterisk presented a normal distribution ($P > 0.05$)

Table 2: Comparison of hydrogen peroxide 1.5%, cetylpyridinium chloride 0.05%, and distilled water (negative control) as biofilm treatment in the water lines of the dental units included in the study

Time	Hydrogen peroxide 1.5%		Cetylpyridinium chloride 0.05%		Distilled water		*P**
	Mean	SD	Mean	SD	Mean	SD	
Baseline	1.53×10^5	0.37×10^5	1.74×10^5	0.36×10^5	1.72×10^5	0.39×10^5	0.306
Day 3	0.04×10^5	1.11×10^5	615.38	0.02×10^5	1.51×10^5	0.35×10^5	<0.05**
Day 7	0.03×10^5	0.05×10^5	307.69	0.01×10^5	1.74×10^5	0.47×10^5	<0.05**

* Kruskal–Wallis test, **Level of significance ($P < 0.05$)

et al.,[14] in a quantitative analysis of the level of bacterial contamination, obtaining a mean value of 1.13×10^5 CFU/mL. In a study by Souza-Gugelmin *et al.*,[15] the baseline values of treatment with hydrogen peroxide, cetylpyridinium chloride, and distilled water were 0.46×10^5, 0.99×10^5, and 1.37×10^5 CFU/mL, respectively, which are similar to those obtained in this study. On the contrary, some studies have obtained baseline values of 3.65×10^5, 3.32×10^5, 3.06×10^5, and 3.92×10^5 CFU/mL, which are higher compared to this study. All the values mentioned previously were high and well above the values accepted by the American Dental Association. This may be due to the fact that water is an ideal milieu for the proliferation of bacteria, and that the internal ducts of water lines in dental units not adequately maintained with antibacterial agents are susceptible to biofilm formation.[12-15]

In this investigation, the antibacterial effect of 1.5% hydrogen peroxide was evaluated at three time points: before the application of the biofilm treatment and at days 3 and 7 after the treatment, showing a reduction in the mean values from 1.53×10^5 CFU/mL at baseline to 0.04×10^5 CFU/mL at day 3 and 0.03×10^5 CFU/mL at day 7. This progressive decrease was also evident in a study by Linger *et al.*,[16] who used hydrogen peroxide as an antibacterial agent for the water lines and reported a significant decrease to levels below those recommended by the American Dental Association. This antimicrobial effect of hydrogen peroxide on the water lines of dental units was also described in a study by Szymańska,[12] in which a reduction in fungal populations was observed after obtaining water samples and a 15-mm long fragment of the water lines.

In a study by Lin *et al.*,[17] the effect of 2%, 3%, and 7% hydrogen peroxide treatment in the water lines of dental units was determined showing a similar decrease in CFU values from a mean of 4.0×10^5 CFU/mL to levels below 500 CFU/mL after the application of the different concentrations of hydrogen peroxide and with a control at up to 12 weeks after treatment. These reductions in bacterial load described in the present and other studies may be due to the antimicrobial potential of the oxidizing effect of hydrogen peroxide. Therefore, this chemical compound is also used as an antiseptic agent in hospitals, medical centers, and clinics.

In this study, the antibacterial effect of 0.05% cetylpyridinium chloride was also evaluated at three time points, obtaining a mean of 1.74×10^5 CFU/mL before the application of the antimicrobial agent, followed by a mean of 615.38 and 307.69 CFU/mL at days 3 and 7, respectively, representing a substantial decrease in the microbial load in the water lines of the dental units. This antibacterial effect was also described in a study by Ramalingam *et al.*,[18] in which cetylpyridinium chloride 0.05% was used, showing a reduction to acceptable values in 99.8% of the samples. These antimicrobial effects have also been reported in several studies evaluating the disinfectant action of quaternary ammonium, the component from which cetylpyridinium chloride is derived, on *Staphylococcus aureus*, present in the microflora found in the irrigation systems of dental units.[18-20]

The results of this study are of clinical importance because they show the need to include an additional procedure to control the accumulation of bacterial biofilm in the irrigation systems of dental units to reduce the risk of infecting patients and dental professionals. Furthermore, there is a lack of knowledge regarding the importance of water contamination in dental clinics, and this study provides important information regarding disinfection protocols to improve biosecurity standards regarding the disinfection of the water lines of dental units, and thereby reducing the risk of immunosuppressed populations contracting diseases and ensuring safe dental care with fewer postoperative complications.

Finally, further studies are needed to evaluate the levels of contamination of other areas of potential bacterial accumulation, increase the number of controls over time, and assess whether the amount of water terminal use in dental units during the days of antibacterial treatment influence the bacterial count. In addition, we recommend that teaching clinics should progressively include a similar procedure to control the contamination of the water lines of the dental units. Lastly, bacterial decontamination procedures can be used as a protocol

before the care of patients by dental staff in their daily practice.

Conclusion

In summary, the antibacterial effect of cetylpyridinium chloride was significantly greater than that of hydrogen peroxide and distilled water, and can, therefore, be used for bacterial control in the water lines of dental units.

Acknowledgement

We wish to thank Universidad Cientifica del Sur (UCSUR) for constantly supporting us in the elaboration of this article.

Financial support and sponsorship

Not applicable.

Conflicts of interest

There are no conflicts of interest.

Authors contributions

Study conception (AMD, AMM), data collection (AMD, AMM), data acquisition and analysis (FMT, WG, SL, FM), data interpretation (WG, SL, SI, AMD, FMT), manuscript writing (FMT, SL, SI, FM, WG).

Ethical policy and institutional review board statement

Because it was an experimental *in vitro* study, it has no ethical implications, therefore, it was exonerated from review by the ethics committee of the university.

Patient declaration of consent

Not applicable.

Data availability statement

The data that support the study results are available from the author (Dr. Frank Mayta-Tovalino, e-mail: fmaytat@cientifica.edu.pe) on request.

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