ORIGINAL

Study of the antimicrobial efficacy of chlorhexidine in dental unit water: Evaluation of microbial contamination in the dental office

Estudo da capacidade antimicrobiana da clorexidina na água do equipamento odontológico: avaliação de contaminação microbiana em ambiente odontológico

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ABSTRACT

Objective

The aim of this study was to count anaerobic bacteria before and after the use of dental equipment and to study the influence of chlorhexidine on the dental unit reservoir water.

Methods

Sterile swabs were used to collect bacterial samples from the cuspidor, lights, syringes, low- and high-speed handpieces, and dental chairs (arms and backrest) before and after the placement of barriers. Blood agar plates were placed on the patient's and dentist's forehead and by the patient's nose and shoulder and exposed to aerosols without (Group 1) and with 0.5% (Group 2) and 1.0% (Group 3) chlorhexidine generated by the high-speed handpiece. Ten aerosol samples were collected for each group. A sample of 1mL of the dental unit reservoir water was collected before and after the use

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of the high-speed handpiece. The anaerobic bacterial counts were compared by the Wilcoxon and Kruskal-Wallis tests.

Results

The contamination on the high-speed handpiece (p=0.0431) and cuspidor (p=0.0117) increased significantly after use. Contamination in the dental unit reservoir water also increased significantly after use of the high-speed handpiece. The most contaminated area was the patient's nose.

Conclusion

The addition of 0.5% and 1.0% chlorhexidine in the dental unit reservoir water reduced the microbial contamination in the dental office significantly.

Indexing terms: Bacteria, Anaerobic. Contamination. Dental equipment. Chlorhexidine.

RESUMO

Objetivo

O objetivo deste estudo foi quantificar as bactérias anaeróbicas, antes e após o uso de equipamentos odontológicos e estudar a influência da clorexidina na água do reservatório.

Métodos

Os seguintes itens foram avaliados: cuspideira, luzes, seringas, baixa e alta rotação, braço da cadeira e do encosto com coleções realizadas antes e após a colocação de barreiras. A contaminação microbiana causada pelos aerossóis de alta rotação também foi avaliada: Grupo 1 (controle): (100%) de água no reservatório; Grupo 2: água no reservatório contendo 0,5% de clorexidina, Grupo 3: água no reservatório contendo 1,0% de clorexidina. Dez amostras de aerossol foram recolhidos a partir de cada grupo: placas de ágar-sangue foram colocadas na testa do paciente e do dentista e no nariz e ombro do paciente. Amostra de 1mL a partir do conteúdo da água no reservatório foi medida antes e após a utilização de alta rotação. Comparações entre bactérias anaeróbias foram feitas com o uso de Wilcoxon e Kruskal-Wallis teste estatístico.

Resultados

Verificou-se um aumento significativo na contaminação antes e após o procedimento utilizando alta rotação (p=0,0431) e na cuspideira (p=0,0117). Foi possível observar um aumento significativo de contaminação microbiana na água do reservatório, após a sua utilização. O nariz do paciente era a área mais afetada.

Conclusão

A adição de 0,5% e 1,0% de clorexidina no reservatório representa uma redução significativa de contaminação microbiana gerado no ambiente de um consultório odontológico.

Temos de indexação: *Bactérias anaeróbicas. Contaminação. Equipamentos odontológicos. Clorexidina.*

INTRODUCTION

The use of antibiotics to treat infectious diseases leads to the selection of resistant microbial strains with potentially increased virulence[1]. Immunosuppressed patients, patients recently discharged from a hospital, and the elderly are more susceptible to cross infection[2]. Dentists should give special attention to the dental unit reservoir water because it may harbor microbes[3].

The aerosol generated by high-speed handpieces may infect the professionals performing

the dental procedure[4]. This infection is usually caused by gram-negative mesophilic, heterophilic, aerobic, and/or facultative anaerobic bacteria, including anaerobic bacilli[3]. In 1996, the American Dental Association[5] recommended that the bacterial count in water from dental units should not exceed 200cfu/mL, which is difficult to achieve even when antimicrobials and barriers are used.

The biosafety protocols for dental offices include the use of barriers on dental equipment, triple syringes, and high- and low-speed handpieces. Antimicrobials should also be used for disinfecting the equipment, accessories, cuspidor, water reservoir and dental office[6,7]. However, these measures are not enough to eliminate the contamination generated during clinical procedures[7].

Chlorhexidine is an antimicrobial widely used in dentistry because of its antibacterial and bacteriostatic activities[8]. The literature recommends its use to treat infections in the oral cavity. It damages the bacterial membrane, causing an irreversible loss of cytoplasmic constituents and enzyme inhibition. At high concentrations (0.5% to 1.0%), chlorhexidine causes extensive cell damage, coagulation of cytoplasmic constituents, and precipitation of proteins and nucleic acids. Chlorhexidine's antimicrobial activity is affected by pH, temperature, and certain substances[9].

Since clinical procedures may contaminate the dental office and equipment, the effect of adding the antimicrobial agent chlorhexidine to the dental unit reservoir water should be investigated. Therefore, the aim of this study was to count the anaerobic bacteria on the dental unit and high-speed handpiece aerosol before and after the use of the dental equipment, and to verify how the addition of 0.5% or 1.0% chlorhexidine to the dental unit reservoir water affects this count.

METHODS

The present study was approved by the Research Ethics Committee of the *Pontifícia Universidade Católica de Campinas* (PUC-Campinas) under Protocol number 107/07. The study complied with all the principles set forth by the Declaration of Helsinki. Anaerobic bacteria were collected from the cuspidor, lights, accessories (syringes, low- and high-speed handpieces), and chair (armrest and backrest) of ten dental units (*Kavo Amadeus, Joinville, Santa Catarina,* Brazil) of PUC-Campinas' dental clinic. The counts were done before and after the use of high-speed handpieces using water treated or not with chlorhexidine digluconate (Sipharma, *Campinas,* Brazil).

Dental units cleaning

The water in the dental unit reservoirs was replaced daily with fresh deionized water (*Fórmula & Ação, São Paulo,* Brazil). Unit waterlines were cleaned weekly at the end of the day. The unit was left undisturbed until the next morning, when a new reservoir was attached and handpieces, air/water syringe tips, and ultrasonic tips were flushed thoroughly with water. Syringes, cuspidor, and low- and high-speed handpieces were run for 20-30 seconds.

Samples were collected by the same individual early in the morning, before the first patient. Samples of each group were collected separately, always on Mondays.

Surface bacterial collection

Bacterial samples were collected by rubbing a sterile swab (*Consolab Comercial e Importadora Ltda, São Paulo,* Brazil) soaked with Brain Heart Infusion (BHI) (Acumedia Manufacturers, Inc. Lansing, Michigan) for one minute against the cuspidor, lights, accessories (syringe and low- and high-speed handpieces), and dental chairs (arms and backrest) in the morning, before barriers were placed, and at the end of the day, after the barriers were removed.

Three groups were created for assessing the antimicrobial efficacy of chlorhexidine (Sipharma, *Campinas*, Brazil) in the dental unit reservoir water:

- Group 1 (Control): water without chlorhexidine;

- Group 2 (Experimental): water with 0.5% chlorhexidine,

- Group 3 (Experimental): water with 1.0% chlorhexidine.

Aerosol sampling

Ten aerosol samples were collected for each group by placing blood agar plates (Labcenter, *Campinas*, Brazil) on patient's and dentist's forehead and by the patient's nose and shoulder. The agar on these plates was exposed to the aerosol generated by the high-speed handpiece for one minute. All patients agreed to the procedures and Signed a Free and Informed Consent Form.

Dental unit reservoir water collection

Disposable pipettes (Labcenter, *Campinas,* Brazil) were used for collecting 1mL samples of water from the dental unit reservoirs before and after the use of the high-speed handpiece. The samples were immediately transferred to test tubes containing BHI.

Inoculation

Surface samples and reservoir water samples were homogenized by a vortex mixer (Vortex-Wizard, *Porto Alegre,* Brazil) for 30 minutes and inoculated on blood agar plates (Labcenter, *Campinas,* Brazil) in a laminar flow cabinet (*Veco, Campinas*, Brazil).

Incubation

All blood agar plates were incubated anaerobically using envelopes (Anaerobac-Probac *do Brasil, São Paulo*, Brazil) containing 85% nitrogen (N_2), 10% carbon dioxide (CO_2) and 5% hydrogen (H_2). The samples remained in an incubator (*Nova Técnica, São Paulo,* Brazil) at 37° for five days. The colony-forming units were counted by a manual colony counter (Phoenix, *Araraquara,* Brazil).

Culture medium preparation

Brain Heart Infusion: Thirty-seven grams of BHI powder (Acumedia Manufacturers, Inc. Lansing, Michigan) were dissolved in 1 liter of distilled water by stirring the mixture for one minute. Next, the BHI solution was autoclaved at 121ºC for 15 minutes.

Blood agar plates: Blood agar was added to one liter of distilled water and stirred until complete dissolution. The solution was then sterilized by autoclaving it at 121ºC for 15 minutes. Once the solution had cooled to 45-50°C, 5% defibrinated sheep blood was added to it (*Biotério Boa Vista, Valinhos,* Brazil).

Statistical analysis

The number of colony-forming units per millimeter was converted into scores as follows: 0cfu/mL=0; 1-100cfu/mL=1; 101-200cfu/mL=2; 201-300cfu/mL=3; 301-400cfu/mL=4; 401-500cfu/mL=5; 501-600cfu/mL=6; 601-700cfu/mL=7; 701-800cfu/mL=8; 801-900cfu/mL=9; 901-1000cfu/mL=10; >1000cfu/mL (uncountable)=11.

The data were compared by the software Biostat 4.0 with a significance level of 5% ($p<0.05$). The anaerobic bacterial counts on the cuspidor, lights, accessories (syringe and low- and high-speed handpieces), and dental chairs (armrest and backrest) before and after the use of the high-speed handpiece were compared by the Wilcoxon's test. The anaerobic bacterial counts in the aerosols of the three groups (pure water, 0.5% chlorhexidine, and 1.0% chlorhexidine) were compared by the Kruskal-Wallis test.

RESULTS

The number of anaerobic bacteria on the high-speed handpiece ($p=0.0431$) and cuspidor ($p=0.0117$) increased significantly after their use. The bacterial

counts on the low speed handpiece, triple syringe, lights, dental chairs (armrest and backrest) did not change (p>0.05) (Table 1).

The microbial contamination in the dental unit reservoir water increased significantly after the high-speed handpiece was used (Group 1). The most susceptible area to microbial contamination was the patient's nose (Table 2). The addition of 0.5% or 1.0% chlorhexidine to the dental unit reservoir water (Group 2) reduced the microbial contamination of the dental equipment and office significantly and to a similar degree (Table 3).

Table 1. Arithmetic means, standard deviation, and *p*-values of the colony-forming unit scores of the dental equipment and accessories before and after the use of the high-speed handpiece.

Samples	Before	After	*p*-value
High-speed handpiece	0.90 (0.56)*	4.00 (4.64)*	0.0431
Low-speed handpiece	0.80 (0.63)	2.20 (3.19)	0.0935
Triple syringe	0.80 (0.63)	2.90 (4.30)	0.0935
Backrest	0.40 (0.51)	0.40 (0.51)	1.0000
Armrest	1.80 (3.29)	0.90 (0.56)	0.6858
Lights	0.50 (0.52)	0.60 (0.51)	0.6858
Cuspidor	1.00 (1.15)*	5.70 (4.99)*	0.0117

Note: *Statistically significant difference between the colony-forming unit scores before and after the use of the high-speed handpiece and cuspidor according to the Wilcoxon test.

Table 2. Arithmetic means, standard deviation, and *p*-values of the colony-forming unit scores of the blood agar plates placed on the locations listed below and dental unit reservoir water samples collected before and after the use of the high-speed handpiece - Group 1 (water without chlorhexidine).

Samples	Means and standard deviation	*p*-value	
Before (water) -1	0.50 (0.52)*†‡	1x2=0.0494	2x5=0.0960
After (water) - 2	1.10 (0.31)*	1x3=0.5138	2x6=0.7392
Dentist's forehead - 3	0.70 (0.48)‡	1x4=0.1026	3x4=0.3273
Patient's forehead - 4	1.00 (0.00)†	1x5=0.0003	3x5=0.0029
Patient's nose - 5	1.60 (0.51)†‡	1x6=0.0215	3x6=0.0999
Patient's shoulder- 6	1.20 (0.42)‡	2x3=0.1894	4x5=0.0458
		2x4=0.7392	5x6=0.1830

Note: Groups with the same symbol: statistically significant differences according to the Kruskal-Wallis test.

Table 3. Arithmetic means, standard deviation, and *p*-values of the colony-forming unit scores of the blood agar plates placed on the locations listed below and dental unit reservoir water samples collected before and after the use of the high-speed handpiece - Groups 2 and 3 (water with 0.5% and 1.0% chlorhexidine).

Samples	Means and standard deviation	
	0.5% chlorhexidine	1.0% chlorhexidine
Before (water)	0.00 (0.00)	0.00 (0.00)
After (water)	0.00 (0.00)	0.00 (0.00)
Dentist's forehead	0.00 (0.00)	0.30 (0.48)
Patient's forehead	0.10 (0.31)	0.30 (0.48)
Patient's nose	0.00 (0.00)	0.60 (0.51)
Patient's shoulder	0.30 (0.48)	0.30 (0.48)
p-value	0.8259	0.1770

Note: Values compared by the Kruskal-Wallis test.

DISCUSSION

The number of anaerobic bacteria before and after the use of the high-speed handpiece varied significantly, especially on the cuspidor and handpiece, because both have direct contact with the patient's oral cavity and/or saliva. The aerosol generated by the high-speed handpiece also contains bacteria. These findings agree with Cristina *et al.*[4], who report that this aerosol contains several pathogenic agents that survive on surfaces for long periods of time. In addition to bacteria, the aerosol may contain blood and saliva that may be inhaled by the patient and dentist. Cristina *et al.*[4] found hemoglobin in aerosol samples collected during dental procedures, indicating that transmission of hepatitis B and C viruses and Human Immunodeficiency Virus (HIV) from the patient to dentist is also possible.

Blood agar plates contaminated with chlorhexidine-free aerosols had significantly more colony-forming units. The area most exposed to the contaminated aerosol was the patient's nose, followed by his shoulder and forehead, and the dentist's forehead. These results are corroborated by Cristina *et al*.[4] and Miller[10], who associated aerosols with respiratory, eye, skin, and HIV infections, tuberculosis, hepatitis B, and hepatitis C. Therefore, according to Schneider *et al.*[11], Barbeau[12], and Epstein *et al.*[13], aerosols may pose serious risk to immunosuppressed patients.

The anaerobic bacterial counts on the cuspidor increased significantly because of its direct contact with the patient's saliva. The water flowing inside the cuspidor is not enough to prevent bacterial proliferation. On the other parts of the dental unit, such as lights and backrest, the number of anaerobic bacteria did not increase significantly. The unchanged bacterial counts on these areas show that the barriers can effectively prevent contamination[1,2,14]. Finally, the low-speed handpiece did not increase the contamination in the dental office significantly because it is usually used in less invasive procedures, does not have a cooling system, and is used for short periods of time.

The methods used herein followed current biosafety standards, which include lining the accessories with Polyvinyl Chloride (PVC) film. Although this barrier did not prevent a significant increase in the anaerobic contamination of the high-speed handpiece, its contamination may have been even greater had the barrier not been used, facilitating cross infection[4]. Meiller *et al.*[6] found that exposing the high-speed handpiece waterline with 10% bleach, Cavicide, 3% glutaraldehyde, Listerine Antiseptic, Peridex, or Sterilex Ultra for 18 hours did not prevent the formation of culture-negative biofilms.

The study reservoir water was contaminated even before the use of the high-speed handpiece, a finding corroborated by Souza-Gugelmin *et al.*[15], who concluded that the reservoir water is contaminated by the biofilm that forms on the waterline surfaces, which is constantly watered. Newly formed biofilm on the waterline surfaces is reversible and easily removed, once bacterial adhesion depends on hydrophobic interactions and aerodynamic forces. During the secondary bacterial adhesion phase, bacteria produce extracellular polysaccharides that help them to adhere to solid surfaces. At this point, the bacteria become irreversibly attached to the surface and biofilm maturation begins[16].

In accordance with Souza-Gugelmin *et al.*[15] and Schel *et al*.[17], the present study has found that the biofilm on the waterline surfaces may continuously contaminate the water. Patients and dentists may be infected by pathogenic bacteria, such as pseudomonas or legionella. According to the World Health Organization, 80% of infections are caused by waterborne microorganisms, so it is extremely important to keep the waterlines and reservoir water clean to reduce the risk of cross contamination in dental offices[3].

The addition of chlorhexidine to the reservoir water reduced the contamination of the office and equipment significantly. Likewise, Porteous *et al*.[8] has demonstrated that the addition of chlorhexidine to the reservoir water one night a week reduces the bacterial counts significantly, even after 12 weeks.

The antimicrobial agent chlorhexidine was chosen because of its antibacterial and bacteriostatic

properties[8]. It is released slowly, preventing microorganism growth and adhesion, and is one of the most widely used antiseptic agents[18]. It controls plaque and gingivitis effectively because no microorganism in the oral flora is resistant to it. Epstein *et al.*[13] and Ranganathan[19] have described chlorhexidine as a disinfectant with wide antibacterial activity, including gram-positive and gram-negative species, antifungal properties, and low toxicity.

The chlorhexidine concentrations used herein were high (0.5% and 1.0%) to ensure extensive cell damage, coagulation of cytoplasmic constituents, precipitation of proteins and nucleic acids, and microbial death. The expected results were achieved - contamination in the dental office decreased significantly. Chlorhexidine works by inverting the polarity on the cell wall, causing loss of cytoplasm, enzyme inhibition, and precipitation of proteins and nucleic acids[20,21]. Sreenivasan & Gittins[22] observed that its antibacterial activity is influenced by environmental factors, including pH and temperature. Ferraz *et al.*[23] found that 2.0% chlorhexidine in water is effective against *Staphylococcus aureus, Enterococcus faecalis, Streptococcus sanguinis, Streptococcus sobrinus, Actinomyces naeslundii, Prevotella gingivalis, Prevotella endodontalis, Prevotella intermedia,* and *Prevotella denticola.* Ferraz *et al.*[23] and Lobo *et al.*[24] stated that chlorhexidine reduces *Streptococcus mutans* numbers significantly, but they can regrow, especially if high numbers were present before disinfection. Du *et al.*[25] reported that chlorhexidine remains on oral surfaces for long periods of time because of its sustained release.

According to this and other studies, contamination of the dental office, equipment, and accessories is a fact, so dentists must search for more efficient means to prevent cross-contamination and cross infection. The use of antimicrobials in the reservoir water and periodic monitoring of its quality are essential. Additionally, dentists must always wear Personal P*rotective Equipment and place physical barriers on the equipment, such as* PVC film, to reduce the risk of cross-contamination in the dental office.

CONCLUSION

The addition of 0.5% chlorhexidine to the dental unit reservoir water is indicated to control microbial contamination in the dental office.

CONTRIBUTORS

All authors participated in all phases of the research article.

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