

Effectiveness of Home Disinfection of Dental Brushes through Chemical Methods

Eficácia da Desinfecção Domiciliar de Escovas Dentárias através de Métodos Químicos
Eficacia de la Desinfección Domiciliar de Cepillos Dentales mediante Métodos Químicos

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Abstract

Oral hygiene gained a great deal of space in the daily lives of the population, with that the concern with the correct way to perform the hygiene and storage of the toothbrush. Bacterial plaque is one of the main causative agents of caries and periodontal disease, along with bacteria, fungi and viruses that are found within the oral cavity. The accumulation of biofilm and the lack of oral hygiene provide an ideal environment for the proliferation of microorganisms, increasing the risk of causing these diseases. In this regard, the toothbrush will serve as an important tool for removing plaque and these organisms. However, right after the first use of the brush, these microorganisms become lodged on the surface of the brush. In order to reduce the number of microorganisms, physical and chemical methods have emerged for disinfecting toothbrushes. Thus, this study aims to analyze the effectiveness of home disinfection of toothbrushes using chemical substances. This is an in vitro laboratory study in which brush hygiene was evaluated by immersion and spraying (n=3), with chlorhexidine, hydrogen peroxide, essential oils and distilled water, as a negative control, to serve as a comparison for microbial growth in Brain Heart Infusion Broth (BHI) culture medium. The result was evaluated according to the transparency of the culture medium following the McFarland turbidity scale. The result of the experiments was that the group by immersion of essential oils showed the best result in the absence of bacterial growth, followed by chlorhexidine and then hydrogen peroxide. The group by spraying both substances was the one that proved to be the most flawed, with a high rate of bacterial growth. Thus, essential oils were the most effective substance, however, none of the methods proved to be totally efficient in combating *Streptococcus mitis*.

Descriptors: Toothbrushing; Disinfection; Contamination.

Resumo

A higiene bucal ganhou muito espaço no dia a dia da população, com isso a preocupação com a forma correta de realizar a higiene e o armazenamento da escova dental. A placa bacteriana é um dos principais agentes causadores de cáries e doenças periodontais, juntamente com bactérias, fungos e vírus que se encontram na cavidade oral. O acúmulo de biofilme e a falta de higiene bucal proporcionam um ambiente ideal para a proliferação de microrganismos, aumentando o risco de causar essas doenças. Nesse sentido, a escova de dente servirá como uma importante ferramenta para a remoção de placa bacteriana e desses organismos. Porém, logo após o primeiro uso da escova, esses microrganismos se alojam na superfície da escova. Para reduzir o número de microrganismos, surgiram métodos físicos e químicos para a desinfecção de escovas de dente. Assim, este estudo tem como objetivo analisar a eficácia da desinfecção domiciliar de escovas dentais com uso de substâncias químicas. Este é um estudo laboratorial in vitro em que a higiene da escova foi avaliada por imersão e pulverização (n = 3), com clorexidina, peróxido de hidrogênio, óleos essenciais e água destilada, como controle negativo, para servir de comparação para o crescimento microbiano no cérebro Meio de cultura Heart Infusion Broth (BHI). O resultado foi avaliado de acordo com a transparência do meio de cultura seguindo a escala de turbidez de McFarland. O resultado dos experimentos foi que o grupo por imersão dos óleos essenciais apresentou o melhor resultado na ausência de crescimento bacteriano, seguido da clorexidina e depois do peróxido de hidrogênio. O grupo que aplicou as duas substâncias foi o que se mostrou mais falho, com alto índice de crescimento bacteriano. Assim, os óleos essenciais foram a substância mais eficaz, porém nenhum dos métodos se mostrou totalmente eficiente no combate ao *Streptococcus mitis*.

Descritores: Escovação Dentária; Desinfecção; Contaminação.

Resumen

La higiene bucal ganó mucho espacio en la vida diaria de la población, con eso la preocupación por la forma correcta de realizar la higiene y almacenamiento del cepillo de dientes. La placa bacteriana es uno de los principales agentes causantes de caries y enfermedades periodontales, junto con bacterias, hongos y virus que se encuentran en la cavidad bucal. La acumulación de biofilm y la falta de higiene bucal proporcionan un entorno ideal para la proliferación de microorganismos, aumentando el riesgo de provocar estas enfermedades. En este sentido, el cepillo de dientes servirá como una herramienta importante para eliminar la placa y estos organismos. Sin embargo, inmediatamente después del primer uso del cepillo, estos microorganismos se alojan en la superficie del cepillo. Para reducir el número de microorganismos, han surgidos métodos físicos y químicos para desinfectar los cepillos de dientes. Así, este estudio tiene como objetivo analizar la efectividad de la desinfección domiciliar de cepillos de dientes mediante sustancias químicas. Se trata de un estudio de laboratorio in vitro en el que se evaluó la higiene de los cepillos mediante inmersión y aspersión (n = 3), con clorhexidina, peróxido de hidrógeno, aceites esenciales y agua destilada como control negativo, para que sirva de comparación para el crecimiento microbiano en el cerebro. Medio de cultivo Heart Infusion Broth (BHI). El resultado se evaluó según la transparencia del medio de cultivo siguiendo la escala de turbidez de McFarland. El resultado de los experimentos fue que el grupo por inmersión de aceites esenciales mostró el mejor resultado en ausencia de crecimiento bacteriano, seguido de clorhexidina y luego peróxido de hidrógeno. El grupo que aplicó las dos sustancias resultó ser el más defectuoso, con una alta tasa de crecimiento bacteriano. Por tanto, los aceites esenciales fueron la sustancia más eficaces, pero ninguno de los métodos demostró ser totalmente eficaz para combatir el *Streptococcus mitis*.

Descriptores: Cepillado Dental; Desinfección; Contaminación.

INTRODUCTION

Dental biofilm or bacterial plaque is a concentrated mass, enriched with non-calcified polysaccharides and salivary glycoproteins,

firmly adhered to dental surfaces or the oral mucosa. However, the accumulation of plaque together with the lack of proper hygiene makes the oral cavity a favorable environment for the

proliferation of pathogens. Biofilm is also the main cause of periodontal disease and caries¹.

The toothbrush is the main instrument for mechanical removal of dental biofilm. Its mechanism of action consists of disorganization and removal of microorganisms, such as bacteria and fungi, present in the oral cavity, which are adhered to the plaque. With regular use of this instrument, it is possible to maintain an oral flora free of oral diseases².

The microorganisms most commonly found in toothbrushes are *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Pseudomonas* and fecal coliforms, as well as pathogens related to respiratory, intestinal and other diseases. Researches show that after the first use of the toothbrush, there is already contamination from the oral environment or from the external environment. Thus, the extreme importance of the correct form of storage, preventing cross contamination both inside the oral cavity and for other people or objects³⁻⁵.

According to the American Dental Association (ADA) brushes should be stored in an upright position, in an airy place, free of moisture and separate from other brushes. Also, they should be washed with plenty of water, eliminating the remaining dentifrices. It is extremely important not to share the brushes, as well as changing them every three to four months, or when the bristles are frayed⁶.

Thus, disinfection plays an essential role in the health-disease process, considering that the level of contamination acquired in a toothbrush is high. Several protocols have been proposed to carry out home disinfection of brush bristles, from physical processes, such as disinfection by ultraviolet light and microwaves, to the use of chemical agents. In this context, chemical agents stand out for being easy to perform technically, and can be efficiently applied in different environments^{7,8}.

The use of antiseptic solutions such as chlorhexidine, essential oils, hydrogen peroxide, among others, as well as antimicrobial sprays, has become an alternative in an attempt to reduce the rate of pathogens present, as these are shown to have a significant reduction rate of cross contamination².

However, chlorhexidine proved to be the main means of disinfection, as it is a cationic agent, presenting a broad-spectrum antimicrobial effect, being a reference in the control of results⁹.

Although it is of great importance, there is no well-defined protocol on the correct way to

disinfect toothbrushes at home. Thus, this study aims to analyze the effectiveness of home disinfection of toothbrushes using chemical methods, including chlorhexidine, essential oils and hydrogen peroxide, using different forms of applications (immersion and spraying).

MATERIAL AND METHOD

○ *Experimental Design*

This is an in vitro laboratory study. It was conducted in the Microbiology laboratory of the Dentistry course at the University of Fortaleza, CE, Brazil. There was no use of specimens of human or animal origin, making the approval of the Ethics Committee unnecessary.

The study presented as dependent variables: different disinfection substances (distilled water, chlorhexidine, H₂O₂ and essential oil solution) and application methods (immersion and spraying). To verify the antimicrobial activity, the specimens were submitted to microbiological evaluation through suspension in culture medium (BHI).

○ *Specimen Confection*

27 (n=3) adult toothbrushes (Dental K®, Taboão da Serra, Brazil) were used, which underwent a sterilization process in an autoclave. After autoclaving, the brushes were evaluated for the integrity of the bristles, being discarded in case of noticeable visual damage.

Subsequently, the brushes were immersed in culture broth rich in *Streptococcus mitis* for 1 minute, then they were washed with sterile distilled water for 15 seconds, in order to remove excess components on the brush. Except for the negative control group, which was not immersed in any solution with the presence of bacteria.

○ *Disinfection Methods*

Three previously contaminated brushes from each group (n=3) were immersed or sprayed with the respective disinfectant solutions (distilled water, chlorhexidine, H₂O₂ and essential oils solution). Immersion was carried out for 1 minute, the brush head was completely submerged without agitation, then the brushes were gently dried with sterile absorbent paper. For the spraying procedure, an operator held the brush with a sterile glove 10 centimeters away from the spray tip and performed 6 applications, with an interval of 10 seconds between them. For this purpose, a standardized spray bottle was used.

○ *Decontamination Assessment*

Afterwards, all brushes were immersed in sterile BHI environment for 10 seconds, then the samples in BHI were placed in the

bacteriological incubator at 37°C for 48 hours.

The result was evaluated according to the transparency of the culture medium following the McFarland turbidity scale. Then, the bacteria were stained following the Gram stain to verify the microbial specimens present, confirming only the presence of bacteria in chains.

Table 1. Description of tested substances

| Substance | Brand |
|-------------------|--|
| Hydrogen peroxide | H ₂ O ₂ solution 3%, Rioquímica, São José do Rio Preto, Brazil |
| Chlorhexidine | Chlorhexidine Digluconate 0,12%, Periogard®, Colgate-Palmolive, São Paulo, Brazil |
| Essencial oils | Listerine Cool Mint®, Johnson & Johnson, São Paulo, Brazil |

RESULTS

After microaerophilia in the oven for 48 hours, the samples were removed from the incubator and analyzed for their level of turbidity and transparency of the BHI culture environment, following the McFarland scale. The presence of sediment at the bottom of the tube was also analyzed, so when the tube is cloudy, it would be a trace of the presence of microbial growth, and when clear, it would be a trace of the sterility of the medium.

Then, the following results were obtained: in the Negative Control group, which had no contact with the bacteria and was taken directly to the culture environment, there was no bacterial growth and remained clear; the Positive Control group, which was previously contaminated with the bacteria and then used distilled water for decontamination, by immersion and by spraying, there was bacterial growth, with the presence of sediment; the Chlorhexidine group by immersion, there was slight turbidity, without the presence of sediment, and by spraying, there was bacterial growth, but without sediment; in the group of Essential Oils by immersion there was no bacterial growth, without the presence of turbidity, and by spraying there was bacterial growth, with the presence of sediment; in the Hydrogen Peroxide group, by immersion and by spraying, there was bacterial growth, with the presence of turbidity and sediment (Table 2).

Table 2. Degree of contamination of the brushes after the substances used

| Groups | Bacterial Growth | Presence of sediment |
|-------------------------------|------------------|----------------------|
| Negative Control | ∅ | Absence |
| Positive Control - Immersion | +++ | Presence |
| Positive Control - Spray | +++ | Presence |
| Chlorhexidine - Immersion | + | Absence |
| Chlorhexidine - Spray | ++ | Absence |
| Essentials Oils - Immersion | ∅ | Absence |
| Essentials Oils - Spray | +++ | Presence |
| Hydrogen peroxide - Immersion | +++ | Presence |
| Hydrogen peroxide - Spray | +++ | Presence |

+++ high bacterial growth; + slight growth; ∅ slight bacterial growth

DISCUSSION

The scarcity of dissemination of information on contamination and disinfection of toothbrushes is great, especially within the academic environment, which should be the main means of disseminating guidance on the cleaning and conditioning of these brushes. In addition, the mouth is one of the primary sites for contamination and infection of brush bristles, as it contains a high number of microorganisms, whether bacteria, fungi and virus. Because there is this exacerbated flora in the oral cavity and other forms of contamination, such as cross contamination, it is extremely important that oral health education on this subject is passed on and recognized¹⁰.

The *Streptococcus* class causes some infections, depending on the species that is in disharmony, such as sinusitis, bacteremia, respiratory infections, pharyngitis, endocarditis, among others¹¹. Thus, cleaning is essential, especially in cases where the individual has one of these infections, and it is most advisable to change the brush after a peak of infection⁶.

With the start of research on the levels of microorganisms found in toothbrushes, more attention began to be paid to ways of disinfecting and storing toothbrushes. Substances that could be used to disinfect increased, such as sodium hypochlorite, cetylpyridinium chloride, chlorhexidine, among others. The one that stood out the most was 0.12% chlorhexidine gluconate, which was considered the ideal antiseptic of choice in Dentistry, as it has a high antimicrobial efficacy, great substantivity and antiseptic efficiency¹².

In the study carried out by Neves et al.¹³, in which 100 brushes were contaminated and separated into groups of 25 brushes for contamination with different microorganisms, involving *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Escherichia coli* and *Candida albicans*, it was used as a substance for disinfection with bleach 1% and 0.12% chlorhexidine gluconate as disinfectant substances, through immersion. As a result, bleach was less effective when compared to chlorhexidine.

According to Nascimento et al.¹⁴ who conducted a research using Periogard® and Periobio®, both containing 0.12% chlorhexidine gluconate, Cepacol®, with 0.05% cetylpyridinium chloride, and sterile water, as a positive control, tested on used brushes by sixteen participants, three times a day for 7 days, it was more effective in mouthwashes with

the presence of chlorhexidine gluconate, containing little bacterial growth. As in the present study, the chlorhexidine, when used in the form of immersion, presented an excellent result in combating the group of *Streptococcus mitis*, already in the form of spraying, it presented a greater microbial environment, making this form of use inefficient.

In another study, hydrogen peroxide and hot water were used for disinfection, in which brushes were given to the participants and they would use them at three times of the day (morning, afternoon and evening) for 7 days. After analyzing the study results, it was proven that hydrogen peroxide had a greater efficiency than hot water¹⁵. The study of the current work, in the hydrogen peroxide group, both in the form of immersion and in the form of spraying, for the bacterium *Streptococcus mitis*, showed poor results, similar to the positive control group.

As for the use of Listerine®, belonging to the group of essential oils, it was shown in a survey, in which a questionnaire was made about the knowledge of nursing students, the way of storing and disinfecting toothbrushes. It was found that in a group of 129 students, 71 have knowledge about disinfection and 117 consider disinfection important. When asked about the use of substances for disinfection, most answered that they do not use precisely 117 students, and of the remaining 12, the most used disinfectant was Listerine®, with a percentage of 41.7%¹⁶. However, compared to the present study, the group of essential oils, in the form of immersion, proved to be the most efficient method, without the presence of turbidity or sediments in the BHI medium, with better results than chlorhexidine.

A more current study, carried out by Ralephenya et al.¹⁷ in which a total of 98 toothbrushes were used, distributed among patients for contamination and after 24 hours they were collected, using Andolex C® (chlorhexidine gluconate 0, as a form of disinfection). 12%), Brushtox® (0.2% chlorhexidine gluconate), Listerine® (essential oil) and distilled water (positive control), the brushes were submerged in 15 ml of the liquid of each group and left for one night. The result of this experience was that 78% of the samples had growth of microorganisms, and the other 22 brushes did not show growth. Andolex C® and Listerine® presented a reduced number of contaminants, around 74%, and Brushtox® presented in 90%, as the water presented a poor performance, not achieving any

disinfection. In other words, statistically analyzing Brushtox®, Listerine® and Andolex C® did not show significant differences, but when compared with the effectiveness of using only water, disinfection is significantly higher.

Although we have important evidence, a greater amount of in situ, in vivo and clinical studies is needed, in addition to the evaluation of other bacterial specimens, in view of the great complexity of the oral biofilm. It is also necessary to establish clear brush disinfection protocols, enabling the dissemination of information based on the literature.

CONCLUSION

In view of the aspects analyzed in the present experiment, the substance that showed the greatest effectiveness against bacterial growth was the essential oil, in the immersion form, followed later by chlorhexidine, also in the immersion form. Hydrogen peroxide and distilled water did not show good performance, in both forms used for decontamination, allowing the growth of microorganisms. The form of spraying proved to be inefficient in all substances used. However, none of the substances were effective in completely destroying the *Streptococcus mitis* bacteria.

Thus, it is extremely important that the dentist is aware of the microorganisms that colonize the brushes and the substances that can be used efficiently for their disinfection, when used properly.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

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