Analysis of the Antimicrobial Efficacy, *In Vitro*, of Oral Antiseptics on Fungi and Bacteria

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Abstract

Objectives: The study aimed to evaluate the action, *in vitro*, of hydrogen peroxide in the concentrations of 1%, 1.5% and 3%, of cetylpyridinium chloride, of benzalkonium chloride of 1.3% and of chlorhexidine 0.12% associated with 1.5% hydrogen peroxide over standardized strains of microorganisms *Enterococcus faecalis*; *Staphylococcus aureus*; *Candida albicans* and *Escherichia coli*, aspiring to determine which is the best product. Chlorhexidine 0.12% was used as a positive control and sterile distilled water as a negative control. **Methodology:** Standardized suspensions of the microorganisms were prepared, poured and spread over the culture media. Sterile 4 mm paper discs were placed on the seeded agar and soaked with 15 microliters (uL) of each of the antiseptics. The plates were incubated in a bacteriological oven at 37° Celsius for 48 hours. The results were obtained through the growth inhibition halo. **Results:** The antiseptic that obtained the greatest antimicrobial action was the association of hydrogen peroxide at 1.5% with chlorhexidine at 0.12%, followed by benzalkonium chloride 1.3%. Hydrogen peroxide at 1% did not show any antimicrobial activity. **Conclusions:** The 0.12% chlorhexidine associated with 1.5% hydrogen peroxide demonstrated the best antimicrobial action, indicating a possible synergistic action between the products, increasing the efficiency when compared to the isolated use of each active ingredient. Benzalkonium chloride 1.3%, also showed an effective antimicrobial action, indicating that these products or combinations have a great potential to be used as an active ingredient in mouthwashes.

Introduction

Mouthwashes are indicated with the objective of controlling the formation of dental biofilm, preventing postoperative infections in surgical wounds; reducing bacteremia in cases of invasive procedures, preventing the spread of infections to other organs [1-4] and, reducing the microbial load in aerosols generated during care, reducing the levels of environmental contamination and the chances of cross-infection [5,6]. In hospitalized patients, oral hygiene with antiseptics has been shown to be a significant measure to reduce ventilator-associated pneumonia (VAP) [7].

The Centers for Disease Control and Prevention (CDC) [8] recommends the use of mouthwashes to reduce the level of oral microorganisms in droplets, splashes and aerosols generated during dental procedures. This and other measures, during dental practice, minimize the risk of contamination of staff, professionals and patients, also reducing environmental contamination, as these aerosols generated from the patients' mouths can contain up to one million bacteria per foot cubic air [9-14].

Mouthwashes are the easiest way to deliver antiseptic substances. They present, in their composition, water and/or alcohol, surfactants, humectants, flavors and antimicrobial agents, which act against microorganisms, so their use should be indicated by the professional. Among the products available to consumers, products with effective formulations for microbial reduction are marketed, such as chlorhexidine, cetylpyridinium chloride, essential oils and triclosan [5,15-17].

Chlorhexidine is widely used and has a satisfactory antimicrobial action, broad spectrum of action and good substantivity. Substances containing cetylpyridinium chloride have also shown proven efficacy, as they limit biofilm formation and adhesion, helping to prevent oral diseases such as gingivitis, periodontitis, and tooth decay [18,19].

With the emergence of SARS-CoV-2, which causes Covid-19 disease, several authors have indicated hydrogen peroxide in different concentrations for oral antiseptics before dental procedures, reducing the chances of virus transmission during care, as it is present in fluids of the infected, such as saliva. The authors suggest that products such as hydrogen peroxide are oxidizing and thus reduce salivary viral load, as SARS-CoV-2 would be vulnerable to oxidation caused by this product. However, studies of the action of this product on fungi and bacteria, in different concentrations, are scarce. Furthermore, the authors suggested that the commonly used chlorhexidine might not act on SARS-CoV-2 [14,20-22].

In this perspective, this research aimed to evaluate the action of different oral antiseptics, *in vitro*, on standardized strains of the microorganisms *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in order to determine which product is most efficient in the control of these commonly found fungi and bacteria in the oral cavity.

Methodology

Choice of antiseptics

The oral antiseptics tested were: 1% hydrogen peroxide (PH1%), 1.5% hydrogen peroxide (PH1.5%), 3% hydrogen peroxide (PH3%), cetylpyridinium chloride (CC), 1.3mg benzalkonium chloride (CB1.3) and the association of 0.12% chlorhexidine with 1.5% hydrogen peroxide (CPH). 0.12% chlorhexidine was used as a positive control (C+) and sterile distilled water as a negative control (C-).

Microorganisms selected for the experiment

The following strains of microorganisms were selected for the sensitivity test: *Staphylococcus aureus* (NEWP 0038); *Enterococcus faecalis* (NEWP 0012); *Escherichia coli* (ATCC 2592) and *Candida albicans* (NEWP 0031), acquired at the Food Research Center (FRC) of the University of Passo Fundo, RS, Brazil.

Preparation of culture media

A total of 24 Petri dishes, previously sterilized, were used. Six with blood agar for the growth of *E. faecalis*; six, with mannitol agar, for the growth of *S. aureus*; six, with Sabourad agar, for the growth of *C. albicans*; and six with MacConkey agar for *E. coli* growth. After preparing the culture media, they were poured into Petri dishes and gelled.

Experimental draw

Microorganism suspensions were prepared and standardized to a turbidity corresponding to number 6 on the MacFarland scale, equivalent to 1.8×10^7 bacteria per ml. The suspensions were poured (0.4 ml) and spread on the appropriate culture media for the microbial type, using a sterilized Drigalski loop, which was lightly slid with the suspension over the Petri dish in three different directions, after that, the plate dried at room temperature for 5 minutes so that the inoculum was absorbed into the agar.

For the antiseptic sensitivity test, sterilized paper disks measuring 4 mm in diameter were placed equidistantly on the seeded agar and soaked with 15uL of each antiseptic solution (Figure 1). To test the association of 0.12% chlorhexidine with 1.5% hydrogen peroxide (CPH), two discs were soaked, one with each of the solutions, and placed one on top of the other in the Petri dish. Tests were performed in triplicate.

After that, the plates were incubated in a bacteriological incubator at 37°C for 48 hours.

Figure 1: Schematic of the division of the Petri dishes with the demarcation of the paper discs soaked with the tested antiseptics (PH1%, PH1.5%, PH3%, CC, CB1.3 and CPH) and with the positive control products (chlorhexidine 0.12%) and negative (sterile distilled water). Passo Fundo, 2021.

Reading the results

The effectiveness of the antiseptics was evaluated using the microbial growth inhibition halo (MGIH), which was measured using a dry point caliper and a millimeter ruler. Measurements were performed by two researchers. The result was the average of the measurements obtained, in millimeters. The effectiveness of antiseptics was categorized as shown in Table 1.

Table 1: Categorization of the effectiveness of mouthwashes according to the average diameter of the microbial growth inhibition (MGHI) halos obtained in millimeters. Passo Fundo, 2021.

Category	MGIH (in millimeters)
Null	$\geq 0 < 9$
Low	$\geq 9 < 12$
Moderate	$\geq 12 < 15$
High	≥ 15

Results

The results obtained, *in vitro*, by the antimicrobial sensitivity test showed that *E. faecalis* was the microorganism that presented the greatest resistance to the tested antiseptics, followed by *E. coli* and the fungus *C. albicans* (Table 2).

S. aureus was the microorganism with sensitivity to the highest number of antiseptic agents, being more sensitive to cetylpyridinium chloride, followed by chlorhexidine associated or not with hydrogen peroxide, and benzalkonium chloride. 3% hydrogen peroxide showed low efficacy against *S. aureus*, and hydrogen peroxide at concentrations lower than 1.5% were not effective for any microorganism tested (Table 2).

Table 2: Mean and variability of microbial growth inhibition halos (MGHI), in millimeters, obtained from the antimicrobial sensitivity test by the agar diffusion method, for each mouthwash and microorganism tested (PH1: 1% hydrogen peroxide; PH1,5: 1,5% hydrogen peroxide; PH3: 3% hydrogen peroxide; CC: cetylpyridinium chloride; CB: benzalkonium chloride; CPH: chlorhexidine associated with hydrogen peroxide). Passo Fundo, 2021.

Products	MGHI (variability)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>E. faecalis</i>
PH1	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
PH1,5	2,3 (0-7,0)	0(0-0)	0 (0-0)	0 (0-0)
PH3	10,0 (7,5-13,0)	11,8 (10,0-14,5)	4,6 (0-14,0)	0 (0-0)
CC	30,6 (22,0-43,0)	0 (0-0)	0 (0-0)	0 (0-0)
CB	22,0 (20,5-24,0)	0 (0-0)	8,5 (8,0-9,5)	6,5 (0-10,5)
CPH	22,5 (22,0-23,5)	6,8 (0-10,5)	8,0 (0-13,0)	0 (0-0)
C+	22,0 (19,0-24,5)	0 (0-0)	1,3 (0-8,0)	1,3 (0-8,0)
C-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)

C. albicans showed resistance against cetylpyridinium chloride and hydrogen peroxide at all three concentrations, except in one plate, where 3% hydrogen peroxide had action against the fungus, although the action was considered null (Figure 2). Benzalkonium chloride was the antiseptic that had the greatest action against *C. albicans*, followed by chlorhexidine associated with 1.5% hydrogen peroxide, however, both were insufficient.

Figure 2: *Candida albicans* growth inhibition halos after the paper discs are soaked with the hydrogen peroxide product, at concentrations of 1% (A), 1.5% (B) and 3% (C) showing resistance of the fungus in the three concentrations, being possible to observe the growth of fungal colonies in the halo. Passo Fundo, 2021.

As for *E. coli*, 3% hydrogen peroxide obtained the best antimicrobial performance, although considered low. Then, the association of 0.12% chlorhexidine with 1.5% hydrogen peroxide, but considered null. There was no antimicrobial action of other antiseptics on this microorganism.

Only 1.5% hydrogen peroxide did not show any action against *S. aureus*. Based on the average of the results, cetylpyridinium chloride had the best antimicrobial action on this bacteria, followed by the association of 0.12% chlorhexidine with 1.5% hydrogen peroxide, by benzalkonium chloride and chlorhexidine, which are considered to have high action, then, 3% hydrogen peroxide showed a low action and finally, 1.5% hydrogen peroxide, having an action considered null.

The antiseptic that obtained action against the largest number of microorganisms tested was the association of 1.5% hydrogen peroxide with 0.12% chlorhexidine, however the microorganism *E. faecalis* was resistant to this method. Then, with similar results, benzalkonium chloride showed good antimicrobial activity, however the microorganism *E. coli* showed resistance to this antiseptic. The 1% hydrogen peroxide was the only one that did not show antimicrobial activity on any of the microorganisms tested, followed by the 1.5% hydrogen peroxide, which had action on only one plate, on *S. aureus*.

Discussion

The performance of oral antiseptics prior to dental procedures contributes to reducing the microbial load in aerosols generated during care, reflecting in less environmental contamination and a reduction in the risk of cross-infection [5,6,23-25]. The present study evaluated the action of different oral antiseptics, *in vitro*, on standardized strains of the microorganisms *E. faecalis*, *S. aureus*, *E. coli* and *C. albicans*, aiming to determine which is the most efficient product in the control of these fungi and bacteria commonly found in the oral cavity.

S. aureus are frequently found in bacteremias and in cases of infective endocarditis. *E. faecalis*, a Gram-positive bacterium, is associated with endodontic infections in dentistry and is also associated with nosocomial infections. *E. coli*, a Gram-negative opportunistic microorganism, is associated with pathological processes such as gastroenteritis, urinary infections, and postoperative surgical wound infections. *C. albicans*, which is present in 25 to 50% of healthy patients, causes opportunistic infections, usually in immunocompromised people and users of total dentures. *Candida* species are among the microorganisms that most cause nosocomial infections [3, 26-29]. In view of the virulent potential of these microorganisms, it was decided to test the *in vitro* action of different antiseptics on them.

Oral antiseptics have in their composition the active component, water and/or alcohol, surfactants, humectants and flavorizers. There are a variety of products on the market with different active components. Chlorhexidine, a type of active component, is a dicationic biguanidine, has a broad bacterial spectrum and is active against Gram-positive, Gram-negative bacteria, fungi and yeasts. Chlorhexidine has its mechanism of action from the moment it binds to the bacterial cell wall, due to the possibility of exchanges of its positive charge with the negative charges on the surface of the microorganism, thus promoting an increase in the permeability of the cell wall, causing lysis of the microorganism. It is considered the "gold standard" in bacterial combat and has excellent substantivity, up to 12 hours. However, its prolonged use can cause tooth pigmentation, desquamation of the oral mucosa, taste alteration, increase in the number of dental stones and a burning sensation in the mouth [5,28,30].

Chlorhexidine-based solutions have been described as effective in reducing cases of ventilator-associated pneumonia (VAP) [31], and for oral antiseptics against several microorganisms, such as *E. faecalis* [30, 32], *S. Mutans* [3,33,34], *E. coli* [35], *C. albicans* [36] and *S. aureus* [16,29]. Their antimicrobial properties demonstrated, in one study, a better action on gram-positive microorganisms being more sensitive than gram-negative [37]. Given its antimicrobial excellence, we chose to test it as a positive control. Chlorhexidine 0.12% revealed a high action against *S. aureus*, for *C. albicans*, *E. faecalis* and *E. coli*, its action was considered null, obtaining low numbers in the inhibition halo.

Maekawa et al. [28], aimed to determine the Maximum Inhibitory Dilution (MID) of mouthwashes, based on chlorhexidine, with and without alcohol in the composition, on *Candida albicans*, through the microdilution test. The authors showed that 0.12% chlorhexidine-based antiseptics are effective in controlling *C. albicans*, and that alcohol potentiates the active component, thus, chlorhexidine-based antiseptics containing alcohol should be used, except in cases where alcohol is contraindicated. Our results showed a resistance of *C. albicans* over 0.12% chlorhexidine, which acted as a positive control, in disagreement with the results of Maekawa et al. [28] however, in the present study, we evaluated only 0.12% chlorhexidine without alcohol in its composition.

Simões et al. [38], evaluated the antimicrobial action, *in vitro*, of oral antiseptics based on chlorhexidine, triclosan, cetylpyridinium chloride, benzidamide chloride and based on thymol, eucalyptol and menthol, on the microorganisms *P. aeruginosa*, *E. coli*, *C. albicans*, *S. aureus*, *S. epidermidis* and *E. faecalis*, using the agar diffusion method. Chlorhexidine obtained, in millimeters, halos of 10.4 against *P. aeruginosa*, 18.4 against *E. coli*, 28.5 against *C. albicans*, 22 against *S. aureus*, 32 against *S. epidermidis* and 12.5 against *E. faecalis*. Cetylpyridinium chloride showed, also in millimeters, 9, 0, 22, 10.5, 17, and 11 mm respectively to the microorganisms. Triclosan presented 9, 29, 17.5, 46, 35 and 8 millimeters of inhibition halo to the respective microorganisms. The products based on thymol, eucalyptol and menthol and benzydamide chloride did not demonstrate any action against the tested microorganisms.

Compared to the study by Simões et al. [38], the results found in our study were 22 mm halos for *S. aureus*, 1.3 mm for *C. albicans* and 1.3 mm for *E. faecalis* with 0.12% chlorhexidine, whereas *E. coli* showed resistance to this product. For cetylpyridinium chloride, the halos found in our study were 30.6 mm against *S. aureus*, *E. coli*, *C. albicans* and *E. faecalis* were resistant to this active ingredient, obtaining 0 mm inhibition of growth. Our results with chlorhexidine are in agreement with the results of Simões et al. [38], for *S. aureus*, where we also found 22 mm of inhibition, however for *C. albicans* and *E. faecalis*, we found results inferior to those of the comparative study, yet *E. coli* did not show sensitivity to chlorhexidine in the this study, unlike the findings of Simões et al. [38], where chlorhexidine showed 18.4 mm of inhibition on this microorganism, showing a resistance of these strains in our results. When comparing cetylpyridinium

chloride, our results are in line with those of Simões et al. [38] on *E. coli*, where none of the studies had action of this product against this bacterium, on *S. aureus*, as shown by Simões et al. [38], this product had antimicrobial action, however, on *C. albicans* and *E. faecalis*, we disagree with Simões et al. [38] who showed action of cetylpyridinium chloride on these microorganisms, where in our findings, we demonstrate resistance of these microorganisms on this antiseptic.

Andrade et al. [39], analyzed, *in vitro*, the Minimum Inhibitory Concentration (MIC) of four mouthwashes: based on chlorhexidine, essential oils, triclosan and cetylpyridinium chloride on the bacteria *S. mutans*, *E. faecalis*, *E. coli*, *S. aureus*, *P. aeruginosa* and the fungus *C. albicans*. The tests were carried out using the microdilution method. In conclusion, the study showed that the tested antiseptics, even at lower concentrations than those found on the market, were effective and inhibited the growth of gram-positive, gram-negative and *Candida* bacterial samples. Our results showed a high action of 0.12% chlorhexidine against *S. aureus*, a very low action was observed against *E. faecalis* and *C. albicans*, however it was considered null, since *E. coli* showed resistance against this product, not developing a growth inhibition halo. Cetylpyridinium chloride also had a high action against *S. aureus*, while for *E. faecalis*, *C. albicans* and *E. coli* the action was considered null.

A widely used product is cetylpyridinium chloride, it is a positively charged molecule that binds to negatively charged microorganisms, has efficacy against gram-positive microorganisms, and its method of action promotes a degradation of the membrane's lipid bilayer, generating an increase in cell permeability and successively breaks the bacterial cell wall leading to its death. Thus, the use of products containing cetylpyridinium chloride limits the formation of biofilm and bacterial adhesion, contributing to the reduction of oral infections. Its action is similar to that of chlorhexidine, however it has less clinical efficacy because it has less substantivity. The adverse effects of this product include extrinsic pigmentation of the teeth and a burning sensation in the mouth [5,19,29,30,40].

Cetylpyridinium chloride has been reported to be effective against several microorganisms such as *S. aureus* [29,30,41], *C. albicans* [36], *P. aeruginosa* [29], *S. mutans* [30] and *E. faecalis* [30]. In the present study, Cetylpyridinium chloride showed high action only against *S. aureus*, being *E. coli*, *C. albicans* and *E. faecalis* resistant to this product, which had no action.

Solutions based on hydrogen peroxide, in various concentrations, are also used for oral antiseptics, this is an active agent that has antimicrobial activity against a wide variety of microorganisms, including yeasts, fungi, bacteria and viruses. Bacteria that do not have catalase activity are sensitive to this product, as they cannot break down the peroxide, although the catalase activity can protect the bacteria from oxidation, this defense mechanism is diminished at high concentrations of this product. Its mechanism of action is through oxidation, hydrogen peroxide involves hydroxyl radicals, which is a potent oxidant, it reacts with macromolecules such as membrane lipids and proteins resulting in the microorganism's death [42,43]. However, the penetration of hydrogen peroxide into the cell seems to be the main obstacle to its biological effectiveness [44]. Some of its adverse effects include the imbalance of the oral microbiota, burns and irritation of the oral tissues [5]. Mouthwashing with hydrogen peroxide at 3% or lower concentrations has been shown to be safe and beneficial for microbial reduction in the oral cavity [45].

Our results with 1% hydrogen peroxide showed that the product had no action against *S. aureus*, *E. coli*, *C. albicans* and *E. faecalis*. At a concentration of 1.5%, hydrogen peroxide showed action only against *S. aureus*, however, considered null, as well as against other microorganisms. At the concentration of 3%, hydrogen peroxide showed low action on *E. coli* and for *S. aureus*, a small action was observed against *C. albicans*, which was considered null, as well as against *E. faecalis*. Concentrations of 1%, 1.5% and in two plates at a concentration of 3% showed an initial action of this antiseptic on *C. albicans*, however, in the halo, there were resistant colonies of this fungus, indicating an action, but that the product it was not entirely effective, perhaps because of its lack of substantivity. Diverging from our results, a study showed that hydrogen peroxide based products have been effective against the bacteria *S. mutans*, *P. aeruginosa*, *E. faecalis* and *S. aureus* [30].

Matos et al. [46], evaluated the antimicrobial activity of mouthwashes on *Candida albicans*. The antiseptics evaluated were based on 1.5% hydrogen peroxide and another based on mallow extract (*Malva sylvestris*). As a control group, they used 0.12% chlorhexidine. The tested products showed an inhibitory effect on most of the *C. albicans* strains evaluated in the study, statistically, the chlorhexidine-based solution obtained greater efficacy, whereas the hydrogen peroxide and mallow extract-based solutions did not differ from each other. The study also evaluated the Maximum Fungicidal Dilution (MFD). Statistically there was a difference between the groups, chlorhexidine had lower mean MFD values compared to the other two products. In conclusion, the study showed that the chlorhexidine-based product had better antimicrobial activity on *C. albicans*, followed by the hydrogen peroxide-based solution, yet the mallow-based solution did not demonstrate fungicidal activity on most strains analyzed. Our results with 1.5% hydrogen peroxide were unsatisfactory, having no action against *C. albicans*, as well as at the 1% concentration, this product showed antifungal action at the 3% concentration in only

one plate, however, considered null. When analyzing the Petri dishes, we observed an initial action of the product, however, in the inhibition halo, fungal colonies appeared, suggesting that the product does not present substantivity, having an initial action, but not sustained. Still, 0.12% chlorhexidine had action against this fungus alone in only one sample, being considered null action.

In order to evaluate the antimicrobial activity of mouthwashes against *S. mutans*, *P. aeruginosa*, *E. faecalis*, *S. aureus* and bacteria obtained from the salivary sample of ten people, Moreira et al. [30] tested 0.12% chlorhexidine gluconate; 0.2% chlorhexidine gluconate; cetylpyridinium chloride with and without fluorine; thymol; triclosan with fluorine; mallow extract with fluorine and xylitol and hydrogen peroxide using the agar diffusion technique. Triclosan antiseptics with fluorine, hydrogen peroxide and chlorhexidine were the most effective. In millimeters, cetylpyridinium chloride showed 12 mm of inhibition against *S. aureus* and *E. faecalis*. Hydrogen peroxide showed 37 mm against *S. aureus* and *E. faecalis*. Chlorhexidine 0.12 presented 23 mm against *S. aureus* and 18 mm against *E. faecalis*. The results of Moreira et al. [30] diverge from our findings where cetylpyridinium chloride showed an average of 30.6 mm against *S. aureus* and no action on *E. faecalis*. Furthermore, we tested hydrogen peroxide at concentrations of 1%, 1.5% and 3%, the first concentration did not show any action against any microorganism, the concentration of 1.5% showed a small action on *S. aureus*, an average of 2.3 mm of inhibition, while the concentration of 3% showed a greater antimicrobial action, being 10.3 mm for *S. aureus*, 11.8 mm for *E. coli* and 4.6 mm for *C. albicans*. *E. faecalis* proved to be resistant to this concentration, and so again our results diverge from those obtained by Moreira et al. [30], where hydrogen peroxide showed action on *E. faecalis* and a better performance on *S. aureus*. On the other hand, chlorhexidine showed, in our results, an average of 22 mm on *S. aureus* and 1.3 mm on *E. faecalis*, for the microorganism *S. aureus*, the result was similar to that found by Moreira et al. (2009) [30], however for *E. faecalis*, the results differed. The difference in the results may be related to the methodologies used in the two studies, since the tests of oral antiseptics, *in vitro*, are adaptations of the agar diffusion procedures, the determination of the minimum inhibitory concentration (MIC) or of tests to determine the decimal reduction time, with no official methodology for evaluating these products in Brazil [15].

Caruso et al. [21] indicate 3% hydrogen peroxide for antiseptics of the oral cavity, even acting as a virus oxidant. Thus, it helps to prevent the progression of diseases from the upper airways to the lower tract. Zubko & Zubko [47] compared the inhibitory effects and mode of action of iodine and hydrogen peroxide used separately and associated, against bacterial and fungal species. Antiseptics, when used separately, allowed the growth of fungi, this growth was stopped when the products were associated. The results further suggested that *P. aeruginosa* and *S. aureus* cells were more effectively eliminated by the association of iodine and hydrogen peroxide, rather than individually, and that inhibitory effects were achieved at lower concentrations of antiseptics due to synergism. The authors' conclusions are that the synergistic and additive inhibitory effects of hydrogen peroxide associated with iodine may suggest the possibility of applying these antimicrobials on several occasions.

Some authors have indicated oral antiseptics with hydrogen peroxide, prior to dental care, to contain the spread of the SARS-CoV-2 virus, as the virus can be transported by aerosols produced during dental procedures, generating risks of contamination of the team [48]. Aerosol is a suspension of particles, which carry viruses, bacteria and fluids such as blood and saliva, being a potent means of infection in dental offices [49]. Thus, oral antiseptics before dental procedures reduces the proportion of aerosolized microorganisms [14]. Data on the use of 0.12% chlorhexidine against SARS-CoV-2 are inconclusive, so hydrogen peroxide is now indicated for this purpose, as it is known that the use of oxidizing agents can reduce viral load salivary, considering that SARS-CoV-2 is vulnerable to oxidation induced by this agent [14,20,22]. Still, reinforcing this hypothesis, the solution of hydrogen peroxide at a concentration of 0.5% was able to inactivate the SARS-CoV and MERS-CoV coronaviruses [50]. It is recommended to carry out mouthwashes with hydrogen peroxide at concentrations of 1.5%, 2% or 3%, for 1 minute, to obtain a reduction in the viral load [21]. Hydrogen peroxide demonstrates an increase in antimicrobial activity when combined with other compounds such as chlorhexidine, thus performing a better antiseptic action [51].

A study by Steinberg et al. [51] examined the possibility of a possible synergistic effect between chlorhexidine combined with hydrogen peroxide to inhibit bacterial growth. This synergistic effect between the products was effective against *E. faecalis* and *S. sobrinus* at concentrations lower than the minimal inhibitory concentration of each agent separately. The combination of the two products inhibited the growth of *S. sobrinus* by 97% compared to the control group, on *E. faecalis*, there was a growth inhibition by 90%, on *S. aureus*, the hydrogen peroxide together with the chlorhexidine had an antimicrobial action that resulted in a full bactericidal effect. The use of hydrogen peroxide separately from another agent, against catalase positive bacteria, such as *S. aureus*, seemed to be less effective, reinforcing the hypothesis that catalase positive bacteria would have resistance when exposed to this antiseptic. The study also showed that despite not knowing the synergistic mechanism between chlorhexidine and hydrogen peroxide, it is believed that exposure of bacteria to chlorhexidine makes the cell wall more permeable and thus

hydrogen peroxide could penetrate more easily and damage the intracellular organelles. In the present study, 1.5% hydrogen peroxide was associated with 0.12% chlorhexidine, our results showed an excellent action against *S. aureus*, the association also demonstrated to have a slight action against *E. coli* and *C. albicans*, however considered null, as well as against *E. faecalis*, which showed resistance to the association of the products. We observed that the use of chlorhexidine or hydrogen peroxide, alone, without being associated, demonstrated a lesser action against *S. aureus*, in agreement with the findings of Steinberg et al. [51]. The association of chlorhexidine with hydrogen peroxide also obtained greater action against *E. coli* and *C. albicans*, when the individual action of these products on these microorganisms was evaluated.

Benzalkonium chloride at a concentration of 1.3% showed better action against *S. aureus*, considered high action. For *C. albicans* and *E. faecalis*, the product obtained action, but considered null, as well as for *E. coli*, which showed resistance against this antiseptic. According to Busscher et al. [52], quaternary ammonium compounds are widely used as surface-active agents, the best known are benzalkonium chloride and cetylpyridinium chloride, used as active principles for mouthwashes.

Garrote et al. [53] evaluated the antibacterial effect of mouthwashes on *S. mutans*, *E. faecalis*, and *P. aeruginosa* bacteria using agar diffusion and direct exposure tests. The products tested were 0.07% cetylpyridinium chloride, 0.075% cetylpyridinium chloride, 0.12% chlorhexidine gluconate and 0.13% benzalkonium chloride. The results obtained showed that 0.07% cetylpyridinium chloride reached a halo of 11 mm over *S. mutans*, 13 mm over *E. faecalis* and 11 mm over *P. aeruginosa*. The 0.075% cetylpyridinium chloride obtained 12 mm of inhibition on *S. mutans*, 11 mm on *E. faecalis* and 10 mm on *P. aeruginosa*. The 0.12% chlorhexidine gluconate achieved 14, 14 and 17 mm inhibition on *S. mutans*, *E. faecalis* and *P. aeruginosa*, respectively. Furthermore, 0.13% benzalkonium chloride with lidocaine achieved halos of 14 mm over *S. mutans*, 13 mm over *E. faecalis* and 12 mm over *P. aeruginosa*. By direct exposure, benzalkonium chloride showed an antibacterial effect against biological indicators after 5 minutes, while cetylpyridinium chloride and chlorhexidine gluconate showed an antibacterial effect only after 10 minutes. In conclusion, the study showed that the antiseptic solutions studied showed an antibacterial effect by direct contact against *S. mutans*, *E. faecalis* and *P. aeruginosa*. Our results observed in the present research diverge from the findings of Garrote et al. [53] in relation to cetylpyridinium chloride, because, unlike the comparative study, we did not obtain action of this product on *E. faecalis*, whereas for products based on chlorhexidine and benzalkonium chloride, our results are in line with those of Garrote et al. [53], as we also obtained action of these products on *E. faecalis*, however the halos were smaller compared to the study cited. In the present study, antiseptics against *S. mutans* and *P. aeruginosa* bacteria were not analyzed.

The use of mouthwashes before dental procedures involving aerosols can reduce the proportion of microorganisms dispersed in the environment [54], considering that the splashes can reach up to 1.82m from the patients' mouths [10] and aerosols remain viable for hours suspended in the air, which can cause ophthalmological, dermatological, and respiratory infections, including tuberculosis and currently, Covid-19 [10-14]. The use of antiseptics based on chlorhexidine, cetylpyridinium chloride or essential oils has been shown to be effective in reducing colony-forming units (CFU) in dental aerosols [55,56]. Therefore the antiseptics tested in this study have the potential to reduce some species of fungi and bacteria in aerosols, however the impact of these mouthwashes on SARS-CoV-2 is unknown, considering that this microorganism was not tested in the present study.

The oral antiseptics used in preoperative antiseptics still play a fundamental role in reducing bacteremia that after invasive dental procedures, such as dental extraction, may occur in 100.0% of cases [57,58]. To avoid complications from this phenomenon, dentists can prescribe preoperative antibiotic prophylaxis, when indicated, and also pay attention to biosafety protocols and also use antiseptic solutions for the oral cavity that have good antimicrobial efficacy, as it is believed that reducing the number of bacteria in the oral cavity by using antiseptics decreases the chances of bacteremia during dental care [1-4].

Conclusion

Chlorhexidine 0.12% associated with hydrogen peroxide at 1.5% showed a better antimicrobial action on the microorganisms analyzed, indicating a possible synergistic action between the products, increasing the effectiveness when compared to the isolated use of each product. Benzalkonium chloride 1.3% also showed an effective antimicrobial action, however oral antiseptics based on chlorhexidine associated with hydrogen peroxide and neither on 1.3% benzalkonium chloride are available on the market, indicating that these products can be used in mouthwash solutions, having a superior performance in relation to current antiseptics.

Chlorhexidine 0.12% and hydrogen peroxide 3% also showed antimicrobial action. Cetylpyridinium chloride only acted on one microorganism. Hydrogen peroxide at concentrations of 1 and 1.5% were practically ineffective in this method.

The microorganism *E. faecalis* showed the greatest resistance to the tested antiseptics,

followed by *E. coli* and *C. albicans*, while *S. aureus* was resistant to only one antiseptic.

The study indicates that tests should be performed, *in vivo*, with products based on 0.12% chlorhexidine associated with 1.5% hydrogen peroxide as well as with products based on 1.3% benzalkonium chloride, to evaluate its clinical performance and the possibility of its use in oral antiseptics. Still, of the products available on the market, 0.12% chlorhexidine continues to be indicated by the study for practice.

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