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In vitro osjetljivost bakterije *Streptococcus oralis* na različite otopine za ispiranje usta

In vitro Susceptibility of Streptococcus oralis to Different Mouthwashes

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Sažetak

Svrha: Otopine za ispiranje usta često su prvi izbor u slučaju kemijske kontrole zubnog biofilma. Željelo se *in vitro* procijeniti kakva je osjetljivost bakterije *Streptococcus oralis* (ATCC 10557) na komercijalne otopine za ispiranje usta koje se prodaju na brazilskom tržištu. **Metode:** Procjenjivalo se sedam otopina: Equate®, Colgate Plax Overnight®, Sanifill®, Cepacol Cool Citrus® i Oral-B® (cetylpyridinium chlorid – cetilpiridinij klorid), Listerine® (thymol – timol) i Peroxyl® (hydrogen peroxid – vodikov peroksid). Otopina 0,12-postotnog klorheksidin diglukonata (Periogard®) bila je pozitivna kontrola. Određivao se antibakterijski učinak sedam različitih razrjeđenja (od 1:0 do 1:32) i najveće razrjeđenje s inhibitornim učinkom (MID) u Müller–Hintonovu agaru s dodatkom pet posto krvi. U svaku mikrojažicu s krutom hranjivom podlogom uliveno je 50 mikrolitara pripremljene otopine te je inkubirana 24 sata na temperaturi od 37°C. Analiza MID-a temeljila se na zonama inhibicije bakterijskog rasta (u milimetrima) stvorenima oko utora (razmatrale su se samo one s promjerom ≥10 mm). **Rezultati:** Dobiveni su sljedeći podaci o MID-u: za Equate® i Oral-B® – 1 : 2, za Sanifill® – 1 : 1, te za Plax®, Cepacol® i Periogard® – 1 : 4. Peroxyl® i Listerine® nisu antibakterijski djelovali na *S. oralis*. Zaključeno je da *Streptococcus oralis* predstavlja dokaz o osjetljivosti soja na otopine za ispiranje usta koje su korištene u ovom istraživanju, osim kad je riječ o Peroxylu® i Listerinu®. **Zaključak:** Rezultati pokazuju da su cetilpiridinijev klorid i klorheksidin glukonat (cetylpyridinium chloride i chlorhexidine gluconate), kao glavni aktivni sastojci ispitanih otopina za ispiranje usta, važni spojevi u kemijskoj kontroli zubnog biofilma.

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Ključne riječi
otopine za ispiranje usta; *Streptococcus oralis*; biofilm; cetilpiridinij; oralna higijena

Uvod

Oralni mikrookoliš važan je za održavanje oralnog zdravlja te u slučaju razvoja bolesti koje uzrokuju poremećaje u ekosustavu (1). Na to utječu ekološki, biokemijski, anatomski i okolišni čimbenici (2).

Među mikroorganizmima u oralnoj mikroflori važna je bakterija *Streptococcus oralis* jer sudjeluje u stvaranju zubnog biofilma, a on je primarni etiološki čimbenik karijesa i parodontnih bolesti (3 – 5). *S. oralis* ubraja se u *mitis*-grupu *viridans*-skupine streptokoka koji se nalaze u ljudskoj usnoj šupljini. Premda se tek treba točno odrediti njegov učinak u procesima nastanka karijesa, aciduričnost (svojstvo rasta u kiselim uvjetima niskog pH) smatra se odlučujućom za virulenciju bakterija u vezi s početkom i razvojem zubnog karijesa (6).

Introduction

The oral microbiota has an important role in the maintenance of oral health as well as in the development of diseases resulting from disturbances of this ecosystem (1) which is influenced by ecological, biochemical, anatomical and environmental factors (2).

Among the microorganisms that compose the human oral microbiota, *Streptococcus oralis* has an important role in the formation of dental biofilm, which is the primary etiological agent of caries and periodontal diseases (3-5). *S. oralis* is a member of the *mitis* group of the *viridans* group streptococci resident in the human oral cavity. Although the precise role of *S. oralis* in caries disease process has yet to be fully defined, its aciduricity, that is, the ability to grow under conditions of low pH, is considered a determinant of virulence for

Na površinama zuba, nakon uklanjanja četkicom i pastom za zube, kemijske razgradnje ili profilakse, odmah se stvara pelikula od sastavnih dijelova sline. Početna kolonizacija površina zuba kariogenim mikroorganizmima, posebice sojem streptokoka, dakle, i *S. oralis*, uključuje taloženje tih bakterija na vezna mjesta novostvorene salivarne pelikule (5). Ti se mikroorganizmi nalaze u slini pojedinaca s visokom karijesnom aktivnošću, čvrsto prianjanju na površine zuba, pa na njihovu kolonizaciju utječu čimbenici povezani s oralnom okolinom, posebice ako je loša higijena usne šupljine (7, 8). *S. oralis,* uz potencijalni utjecaj na karijes, dobro je istražen i kad je riječ o različitim ekstraoralnim bolestima, uključujući endokarditis, dentoalveolarne infekcije i septikemije (6, 9, 10).

Korištenje kemijskih spojeva za kontrolu zubnog biofilma dobro je istraženo (5, 8, 9), ali nema dovoljno studija o osjetljivosti bakterije *S. oralis* na ispiranje usta otopinama i o tome zašto je taj mikroorganizam potreban u usnoj šupljini. Dokazano je da je *S. oralis* otporan na neke sintetske antibiotike koje liječnici dentalne medicine rutinski propisuju, primjerice, na makrolide, tetracikline i peniciline (9, 11, 12).

U Brazilu su otopine za ispiranje usta svrstane među kozmetičke proizvode za osobnu higijenu i imaju posebne indikacije za uporabu kao antiseptici te su uvrštene u drugu skupinu proizvoda i zato moraju imati potvrdu o sigurnosti i antimikrobnoj učinkovitosti.

Očekivalo se da će otopine za ispiranje usta korištene u ovom istraživanju djelovati tako da će inaktivirati biofilm mikroorganizama ili ga ukloniti sa zuba.

Za potrebe *in vitro* istraživanja protumikrobnog učinka otopina za ispiranje usta odabrana je tehnika maksimalnog razrjeđenja. Uzelo se u obzir i to da su ispitivani proizvodi dostupni i da sadržavaju različite koncentracije kemijskih sastojaka s biološkom aktivnošću (13, 14).

Svrha istraživanja bila je procijeniti u uvjetima *in vitro* osjetljivost *S. oralis* na komercijalne otopine za ispiranje usta koje se mogu nabaviti u Brazilu.

Materijal i metode

Ovo *in vitro* istraživanje provedeno je u Laboratoriju za tropsku medicinu (NUMETROP) Centra za zdravstvene znanosti pri Federalnom sveučilištu u Paraibi u Brazilu.

Soj bakterija *Streptococcus oralis* (ATCC 10557) za potrebe istraživanja darovala je zaklada Oswalda Cruza (FIOCRUZ, Rio de Janeiro, RJ, Brazil).

Zatim je izabrano sedam komercijalnih otopina za ispiranje usta s različitim aktivnim komponentama koje se mogu nabaviti u Brazilu. Proizvodi su kupljeni u gradu Campini Grande u drogerijama i trgovinama, te označeni u skladu s komercijalnim imenima i aktivnim sastojcima (vidi tablicu 1.): Equate® (cetilpiridinijev klorid), Colgate Plax Overnight® (cetilpiridinijev klorid), Sanifill® (cetilpiridinijev klorid), Ce-

bacteria associated with the development and progression of dental caries (6)

Immediately after its removal by toothbrushing with dentifrices, chemical dissolution or prophylaxis, the salivary acquired pellicle is rapidly formed on dental surfaces. Initial colonization of the tooth surfaces by cariogenic microorganisms, especially streptococcal species including *S. oralis*, involves the attachment of these bacteria to adsorbed salivary components of the acquired pellicle[5]. These microorganisms are commonly found in the saliva of individuals with high caries activity, have high capacity to adhere to oral surfaces, and its colonization is influenced by factors related to oral environment, especially poor oral hygiene (7,8). In addition to the potential role of *S. oralis* in dental caries, it is well documented that *S. oralis* is associated with a range of extraoral diseases including endocarditis, dentoalveolar infections and septicemia (6,9,10).

The use of chemical agents for the control of dental biofilm is well documented (5,8,9). However, there is a lack of studies investigating the susceptibility of *S. oralis* to oral rinses with chemical compounds as well as the role of this microorganism in the oral cavity. Additionally, it has been demonstrated that *S. oralis* is resistant to some synthetic antibiotics commonly prescribed by dentists, such as macrolides, tetracyclines and penicilins (9,11,12).

In Brazil, mouthwashes are classified as cosmetic and personal hygiene products in such a way that the formulations which present specific indications as antiseptics are classified as degree-2 products and require confirmation of their safety and antimicrobial efficacy. Therefore, it is expected that these mouthwashes, including those evaluated in the present study, may act on the inactivation or elimination of dental biofilm microorganisms.

In order to investigate *in vitro* antimicrobial activity of mouthwashes, the maximum inhibitory dilution technique has been used. It is taken into account that the products under study are already available for use, and concentrations of the chemical components with biological activity are different (13,14).

The purpose of this study was to evaluate the *in vitro* susceptibility of *S. oralis* to commercial mouthwashes available on the Brazilian market.

Material and methods

This *in vitro* study was carried out at the Laboratory of Tropical Medicine (NUMETROP) of the Health Sciences Center of the Federal University of Paraíba, Brazil. The *Streptococcus oralis* (ATCC 10557) reference strain used in the study was kindly donated by the Oswaldo Cruz Foundation (FIOCRUZ, Rio de Janeiro, RJ, Brazil).

Seven commercial brands of mouthwashes based on different active ingredients and widely used in Brazil were selected for this study. The products were purchased from supermarkets and drugstores in the city of Campina Grande, PB, Brazil, and were labeled according to their commercial brands and respective active components, as summarized in Table 1: Equate® (cetylpyridinium chloride), Colgate Plax

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Tablica 1. Raspodjela otopina za ispiranje usta ovisno o komercijalnom imenu, kemijskom sastavu i proizvođaču
Table 1 Distribution of the mouthwashes according to the commercial brand, chemical composition and manufacturer

Komercijalno ime • Commercial brand	Aktivni kemijski sastojak • Active chemical component	Proizvođač • Manufacturer
Cepacol cool citrus®	Cetylpyridinium chloride	Sanofi-Aventis Farmacêutica Ltda.
Equate®	Cetylpyridinium chloride	Ind. Com. e Repres. Poli Products Ltda.
Oral-B®	Cetylpyridinium chloride	Gillette do Brasil Ltda.
Periogard®	Chlorhexidine digluconate	Colgate-Palmolive Argentina SA.
Peroxyl®	Hydrogen peroxide	Colgate-Palmolive Ind. Com. Ltda.
Colgate Plax Overnight®	Cetylpyridinium chloride	Colgate-Palmolive Ind. Com. Ltda.
Listerine Cool Citrus®	Thymol	Laboratórios Pfizer Ltda.
Sanifill®	Cetylpyridinium chloride	Facilit Odontológica e Perfumaria Ltda.

pacol® (cetilpiridinijev klorid), Oral-B® (cetilpiridinijev klorid), Listerine® (timol) i Peroxyl® (vodikov peroksid). Otopina za ispiranje usta s 0,12-postotnim klorheksidin diglukonatom (Periogard®) smatra se uobičajenom (15, 16) i zato je odabrana kao *zlatni standard*. Protumikrobni učinak otopina za ispiranje usta provjeravao se u čistom obliku i u razrjeđenjima od 1:1 do 1:32 na bakterijskom soju *S. oralis* i to testom difuzije agara (tehnika jažice u agaru). Pritom se određivao inhibitorni učinak maksimalnog razrjeđenja (MID) (13, 14, 17). Bakterijski soj reaktiviran je tijekom noći u infuzijskoj otopini mozga i srca (BHI; Difco, Detroit, MI, SAD) u mikroaerofilnim uvjetima (svijeća pod zvonom) i na temperaturi od 37°C. U skladu s preporukama Instituta za kliničke laboratorijske standarde (CLSI) inokulat je pripremljen u fiziološkoj otopini, a turbidnost je podešena prema McFarlandovoj ljestvici (0,5) koja odgovara absorbanciji od 0,08 do 0,10 pri 625 nm i 5 x 10^6 CFU/mL (18). Dobiveni inokulat u otopini zabrtvljen je u Petrijevoj zdjelici veličine 20 x 10 milimetara na Müller-Hintonovu agaru s pet posto krvi (Difco, Detroit, MI, SAD) korištenjem zasićenih briseva u bakterijskoj otopini. U svakoj zdjelici probijen je bunarić od šest milimetara i napunjen s 50 µL pripremljene otopine za ispiranje usta različitih razrjeđenja. Svaki se pokus ponavljao dva puta. Petrijeve zdjelice inkubirane su u mikroaerofilnim uvjetima 24 sata na temperaturi od 37° C. Izračunat je MID za najveće razrjeđenje proizvoda koji je sposoban sprječavati bakterijski rast u zoni inhibicije oko bunarića te je izmjeren u milimetrima digitalnom pomičnom mjerkom (Mitutoyo Corp., Tokijo, Japan) u uvjetima reflektiranog svjetla.

Analiza podataka

Kako bi se osigurala reproducibilnost, pokusi su obavljeni dva puta. Način je primijenjen kao parametar u deskriptivnoj statističkoj analizi.

Rezultati

U tablici 2. su vrijednosti MID-a testiranih otopina za ispiranje usta koje pokazuju osjetljivost soja bakterije *S. oralis* na određene proizvode i njihova razrjeđenja. Dobivene su sljedeće vrijednosti: za Equate® i Oral-B® – 1 : 2, za Sanifill® – 1 : 1 te za Plax®, Cepacol® i Periogard® – 1 : 4. Peroxyl® i Listerine® nisu imali protubakterijski učinak.

Overnight® (cetylpyridinium chloride), Sanifill® (cetylpyridinium chloride), Cepacol® (cetylpyridinium chloride), Oral-B® (cetylpyridinium chloride), Listerine® (Thymol) and Peroxyl® (hydrogen peroxide). A 0.12% chlorhexidine digluconate oral rinse (Periogard®), which is considered as a gold standard (15,16) served as a positive control. The antimicrobial activity of these commercial mouthwashes in their pure form and in six dilutions ranging from 1:1 to 1:32 against *S. oralis* was evaluated using the agar diffusion test (agar-well technique) by determination of the maximum inhibitory dilution (MID),(13,14,17).

The bacterial strain was reactivated in Brain Heart Infusion (BHI; Difco, Detroit, MI, USA) broth and incubated overnight in microaerophilia (candle jar system) at 37°C. In accordance with the recommendation of the Clinical and Laboratory Standards Institute (CLSI), the inoculum was prepared in saline solution; its turbidity was adjusted through McFarland scale (0.5), which is equivalent to the absorbance of 0.08-0.10 at 625nm corresponding to 5 x 10^6 CFU/mL (18). The inocula obtained in saline were seeded in 20 x 10 mm sterile Petri dishes containing Muller Hinton agar supplemented with 5% blood (Difco, Detroit, MI, USA) using swabs saturated in the bacterial suspension. In each dish, 6-mm-diameter wells were perforated and filled with 50 µL of the ready-to-use mouthwashes and their dilutions. All tests were performed in duplicate. The dishes were incubated in microaerophilia at 37°C for 24 hours. MID was considered to be the smallest range of product dilution capable of preventing bacterial growth as represented by the formation of inhibition zones around the wells measured in millimeters with a digital caliper (Mitutoyo Corp., Tokyo, Japan) under reflected light.

Data analysis

Tests were performed in duplicate in order to guarantee reproducibility. The mode was employed as a parameter for descriptive statistical analysis.

Results

Table 2 shows the MIDs for the evaluated mouthwashes, which indicate the susceptibility of *S. oralis* to the ready-to-use products and their dilutions. The following MIDs were obtained: 1:2 for Equate® and Oral-B®, 1:1 for Sanifill®, and 1:4 for Plax®, Cepacol® and Periogard®. Peroxyl® and Listerine® did not show antibacterial activity.

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Tablica 2. Minimalno inhibitorno razrjeđenje (MID-s) za procijenjene otopine za ispiranje usta
Table 2 Minimum inhibitory dilutions (MIDs) for the evaluated mouthwashes

Bakterijski soj • Bacterial strain	Razrjeđenje • Dilutions	Cepacol®	Oral-B®	Listerine Cool Citrus®	Peroxyl®	Plax Overnight®	Sanifill®	Equate®	Periogard®
S. Oralis (ATCC 10557)	01:00	-	-	+	+	-	-	-	-
	01:01	-	-	+	+	-	-	-	-
	01:02	-	-	+	+	-	-	-	-
	01:04	-	+	+	+	-	+	+	-
	01:08	+	+	+	+	+	+	+	+
	01:16	+	+	+	+	+	+	+	+
	01:32	+	+	+	+	+	+	+	+

(-) Odsutan bakterijski rasta • Absence of bacterial growth, (+) Prisutan bakterijski rast • Presence of bacterial growth

Rasprava

Kao i svako istraživanje *in vitro*, tako je i ovo imalo određena ograničenja. To se, primjerice, odnosi na reproducirani učinak stečene salivarne pelikule, puferski kapacitet sline i ostale uvjete u usnoj šupljini koji utječu na adheziju i kolonizaciju mikroorganizama (19). Dodatni nedostatak difuzijskog testa u agaru jest da rezultat ne ovisi samo o toksičnosti korištenog materijala na određeni mikroorganizam, nego i o njegovoj sposobnosti za difuziju kroz agar. Kemijska svojstva otopina za ispiranje usta mogu povećati ili smanjiti njihovu difuziju kroz medij za kultiviranje te tako neposredno utjecati na doticaj s mikroorganizmima, što je ključno za njihovo djelovanje (20). Testovi za difuziju agara, kojima se određuje antibakterijski učinak otopina za ispiranje usta, odabrani su u ovom istraživanju zbog činjenice da je to prva metoda probira u određivanju farmakološke aktivnosti novih sredstava. Naime, tako se učinkovitije otkrivaju i manje otrovne tvari u neprekidnoj borbi protiv rezistencije i pojave patoloških mikroorganizama (21, 22).

Multifaktorijalna priroda oralnog biofilma nije potanko opisana. Različiti mikroorganizmi, većinom iz soja streptokoka, povezani su s prianjanjem mikroorganizama na površine zuba (23).

Odabir bakterije *S. oralis* za ovo istraživanje temelji se na njezinu sudjelovanju u inicijalnoj kolonizaciji zubnog biofilma i na važnoj ulozi u bakterijskoj koagregaciji jer stvara zreli i patološki biofilm. Mora se istaknuti da postoje i znanstveno potvrđeni podaci o djelovanju kemijskih tvari. Zubni biofilm ima nizak patološki potencijal i njegove dominantne mikrobne vrste nisu neposredno povezane s pojavom oralnih bolesti (24). No kada se na površini zuba u biofilmu nakupe debeli slojevi bakterijskih zajednica, stvaraju se povoljni uvjeti za razvoj infekcija (25).

Otopine za ispiranje usta često su prvi izbor za kemijsku kontrolu zubnog biofilma. Idealna otopina za ispiranje usta trebala bi imati nisku toksičnost, dobru permeabilnost i ne bi trebala utjecati na komenzalsku mikrofloru (26).

Kao što je uočeno u ranijim istraživanjima (27), otopine koje sadržavaju cetilpiridinijev klorid (Cepacol®, Periogard®, Colgate Plax Overnight® i Equate®) učinkovitije su uništavale *S. oralis*, što pokazuje da je taj spoj vrlo važan sastojak otopina za ispiranje usta. Herrera i suradnici (28) istaknuli su antibakterijski učinak cetilpiridinijeva klorida na *Lactobacillus*

Discussion

Just like every *in vitro* investigation, the present study has some limitations. It does not reproduce, for example, the effects of the salivary acquired pellicle, the buffering capacity of the saliva and other oral conditions that influence the adhesion and colonization of microorganisms (19). In addition, a disadvantage of the agar diffusion test used in the present study is the fact that the result of this method does not depend only on the toxicity of the material for the particular microorganism, but it is also highly influenced by the diffusion of the material across the medium. The chemical properties of the mouthwashes may either increase or reduce their diffusion through the culture medium and consequently affect their direct contact with the microorganism, which is key point for their action (20). The choice for the agar diffusion method to evaluate the antibacterial activity of the mouthwashes in the present study was based on the fact that this is the first screening method used in determining the pharmacological activity of new agents, having a significant contribution in the development of health sciences, since more effective and less toxic substances are revealed in the continuous efforts against the resistance and appearance of pathogenic microorganisms (21,22).

The multifactorial nature of the oral biofilm has been extensively demonstrated. A variety of microorganisms, mainly streptococcal species, are associated with the initial adherence of microorganisms to the dental surfaces (23). The use of *S. oralis* in the present study is justified by its participation in the initial colonization of the dental biofilm and its important role in bacterial co-aggregation, producing a mature and pathogenic biofilm. The scarcity of research-based information about the action of chemical agents against this microorganism should be mentioned.

The dental biofilm has low pathogenic potential and its predominant microbial species are not directly related to the occurrence of oral diseases (24). However, when bacterial communities in biofilms accumulate on the dental surfaces, thick layers are formed generating favorable conditions for the development of infections (25).

The use of mouthwashes represents, in many cases, the first option for the chemical control of dental biofilm. An ideal mouthwash should have low toxicity, good permeability and should not disturb the resident microbiota (26).

– drugi mikroorganizam neposredno uključen u početak i napredovanje karijesa, a Lessa i njegovi kolege (29) u kliničkom su istraživanju zabilježili da cetilpiridinijev klorid antibakterijski djeluje na streptokoke.

Klorheksidin snažno djeluje na streptokoke povezane s infekcijama usne šupljine, uključujući i bakteriju *S. oralis* (30), pa je često sredstvo izbora za kemijsku kontrolu zubnog biofilma (31, 32).

I na kraju je važno istaknuti da ostali sastojci u nekim otopinama za ispiranje usta, kao što su triclosan, esencijalna ulja i cinkov klorid, mogu sinergizmom pojačati djelovanje pojedinih komponenti, što opravdava istodobno korištenje različitih tvari u proizvodnji komercijalnih otopina za ispiranje usta.

Zaključak

S. oralis je osjetljiv na otopine za ispiranje usta rabljene u ovom istraživanju, osim na Peroxyl® i Listerine®. Ti rezultati pokazuju da su cetilpiridinijev klorid i klorheksidin glukonat, kao najvažniji aktivni sastojci u testiranim proizvodima, važni u kemijskoj kontroli zubnog biofilma.

Zahvala

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In the same way as observed in the previous study (27), mouthwashes that present cetylpyridinium chloride in their composition (Cepacol®, Periogard®, Colgate Plax Overnight® and Equate®) were more effective against *S. oralis*, which indicates the importance of this compound in mouthwashes. Herrera et al (28). observed antibacterial activity of cetylpyridinium chloride against *Lactobacillus*, which is another microorganism directly involved in the development and progression of caries disease, while Lessa et al (29), in a clinical study, showed that cetylpyridinium chloride presents antibacterial activity against streptococcal species.

Chlorhexidine has great efficacy against streptococci involved in oral infections, including *S. oralis (30)*, and is usually the substance of choice for the chemical control of dental biofilm (31,32).

Finally, it is important to emphasize that the presence of other compounds in some mouthwashes, such as Triclosan, essential oils and zinc chloride, may promote a synergism of the effects observed, which justifies the association of different compounds in the formulations of commercial oral rinses.

Conclusion

S. oralis presented susceptibility to the mouthwashes evaluated in this study, apart from Peroxyl® and Listerine®. These results suggest that cetylpyridinium chloride and chlorhexidine gluconate, which are the most important active components of the tested brands of mouthwashes, are important agents in the chemical control of dental biofilm.

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Abstract

Aim: The use of mouthwashes represents, in many cases, the first option for the chemical control of dental biofilm. To evaluate *in vitro* susceptibility of *Streptococcus oralis* (ATCC 10557) to commercial mouthwashes available on the Brazilian market. **Methods:** Seven mouthwashes were evaluated: Equate®, Colgate Plax Overnight®, Sanifill®, Cepacol Cool Citrus® and Oral-B® (cetylpyridinium chloride), Listerine® (Thymol) and Peroxyl® (hydrogen peroxide). A 0.12% chlorhexidine digluconate oral rinse (Periogard®) served as a positive control. The antibacterial activity of the mouthwashes in 7 dilutions (1:0 to 1:32) was evaluated by determining the maximum inhibitory dilution (MID) in Muller Hinton agar supplemented with 5% blood. Fifty microliters of each ready-to-use solution and their dilutions were poured into wells perforated in the solid culture medium and the dishes were incubated in bacteriological incubators at 37°C for 24 hours. The analysis of MID data was based on the measurement of the zones of bacterial growth inhibition (in mm) formed around the wells (only inhibition zones with a diameter ≥10 mm were considered). **Results:** The following MIDs were obtained: 1:2 for Equate® and Oral-B®, 1:1 for Sanifill®, and 1:4 for Plax®, Cepacol® and Periogard®. Peroxyl® and Listerine® did not show antibacterial activity against *S. oralis*. In conclusion, *S. oralis* presented susceptibility to the mouthwashes evaluated in this study, except for Peroxyl® and Listerine®. **Conclusion:** These results suggest that cetylpyridinium chloride and chlorhexidine gluconate, which are the most important active components of the tested brands of mouthwashes, are important agents in the chemical control of dental biofilm.

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Key words
Mouthwashes; *Streptococcus oralis*; Biofilms; Cetylpyridinium; Oral Hygiene; Chlorhexidine Gluconate

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