Antimicrobial effectiveness of different trademarks mouthwashes with and without alcohol against different organisms: *in vitro* study

Efetividade antimicrobiana de diferentes marcas comerciais de enxaguatórios bucais com e sem álcool sobre diversos microrganismos: estudo *in vitro*

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Abstract

Purpose: The aim of this *in vitro* study was to evaluate the effect of mouthwashes with and without alcohol on some microorganisms.

Methods: Periogard®, Cepacol®, Plax Classic® and Oral-B® were the substances tested. For this study, 40 plates of Petri with medium blood agar were used for the following microorganisms: *Staphylococcus aureus, Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa*. To measure the inhibition zones, a single trained examiner used a stereomicroscope and a digital caliper. After data collection, the medias were compared using the ANOVA (analysis of variance) with Bonferroni test post-hoc correction for 5% significance level.

Results: Periogard®, with and without alcohol, showed the best results. Plax® without alcohol presented the poorest results. Plax® with alcohol was the best substance in relation to *S. aureus*. The others results remained with better effectiveness in relation to control substance.

Conclusion: The alcohol-free mouthwashes did not have the same efficacy of antimicrobial rinses with alcohol in relation to microorganisms tested in this study.

Key words: Mouthwash; chlorhexidine

Resumo

Objetivo: O objetivo desse estudo *in vitro* foi de avaliar a ação antimicrobiana de enxaguatórios bucais com e sem álcool.

Metodologia: Usaram-se no estudo o Periogard®, o CEPACOL®, Plax Classic® e o ORAL-B®, anti-séptico sem álcool. Para a realização desse estudo foram utilizadas 40 placas de Petri com meio de cultura de Ágar Sangue para os seguintes microrganismos: *Staphylococcus aureus, Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa*. Para a mensuração dos halos de inibição, um único examinador treinado utilizou uma lupa estereoscópica e um paquímetro digital. Após a coleta dos dados, as médias foram comparadas utilizando-se do teste estatístico ANOVA (análise de variância) com teste corretivo de Bonferroni, para nível de significância de 5%.

Resultados: O Periogard® com álcool e o sem álcool apresentaram os melhores resultados. O Plax® sem álcool obteve os piores resultados. O Plax® com álcool foi melhor substância em relação ao *S. aureus*. Os demais resultados mantiveram-se com melhor efetividade em relação à substância controle.

Conclusão: Os enxaguatórios sem álcool não têm a mesma eficácia antimicrobiana comparada aos enxaguatórios com álcool em relação aos microrganismos testados neste estudo.

Palavras-chave: Antissépticos bucais; clorexidina

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Introduction

Generally, patients on intensive care do not have adequate bucal higienization (1). This condition can cause periodontitis and gingivitis, ear infections, chronic rhinopharyngitis, xerostomy, possible outbreaks of nosocomial infections prone to pneumonia (2). The bacterial dental plaque, also designated as dental biofilm, produces irritant substances (acids, endotoxins and antigens) that potentially destroy teeth and support tissues (3).

The mouthwash use is an excellent tool in the dental biofilm control. Can be used as an auxiliary way for the dental biofilm mechanical removal by the dentist or in complementation to toothbrushing of the patient (4). Many times, the chemical resource used can be prolonged. Besides this fact, these substances have in their composition the presence of detergent, alcohol and dye, elements which can prejudice the bucal mucosis (5).

The alcohol is considered as risk factor for oral and oropharynx cancer development. Its pathogenesis is not still properly clear, requiring care in the approach of these lesions origin (6,7). Extensive discussion has been conducted about the prolonged use of alcohol-free mouthwash. However, it is not clear if the alcohol added to mouthwash can give rise to problems (8).

Based on findings which report the relation of the alcohol present in bucal mouthwashes and eventual lesions in oral cavity, alternative products with greater substantivity and alcohol free are researched. In this context, it is important to evaluate the mouthwash antimicrobial ability, with and without alcohol, marketed in Brazil, against to *Staphylococcus aureus, Candida albicans, Enterococcus faecalis* and *Pseudomonas aeruginosa*

Methodology

Periogard® (Colgate-Palmolive, Indústria Brazileira, São Bernardo do Campo, SP, Brazil), Cepacol ® (Sanofi Aventis Farmacêutica Ltda, Suzano, SP, Brazil), Plax Classic® (Colgate-Palmolive, Indústria Brasileira, São Bernardo do Campo, SP, Brazil) and Oral-B® non-alcohol anti-septic (Procter & Gamble do Brazil, São Paulo, SP, Brazil) were selected for the tests, as described in Table 1. Distilled water was used as control substance. These products were acquired in specialized stores in Cuiabá-MT, Brazil.

For the completion of this study, had been used 40 Petri dishes (48x12 mm, without division) with agar-blood medium (Newprov, Produtos Médicos e Hospitalares, São José dos Pinhais, PR, Brazil) for the microorganisms: *Staphylococcus aureus (*ATCC 6538), *Candida albicans* (ATCC *10231), Enterococcus faecalis* (ATCC 10231*)* and *Pseudomonas aeruginosa* (ATCC 27853). Four Petri dishes with the agar-blood medium were selected for the microorganism growth evaluation, designated as positive control. In other 4 dishes, the sowing of the microorganism was not done, aiming to evaluate the medium contamination absence, designated as negative control.

The microorganisms were inoculated in 7 mL of BHI (Brain Heart Infusion- Difco Laboratories, Detroit, MI, United States) and taken to bacterial incubator (Fanem model 502 – Industrias Fanem Ltda, São Paulo, SP, Brazil) at constant temperature of 37°C, for 24 hours, for replication.

The aim was that, at the end of this phase, the microorganism concentration was close to $3x10^8$ cels/ mL, similar to tube #1 in *MacFarland* scale. The microorganisms used in this study were tested only in aerobiosis conditions.

For the diffusion test, 0.1 mL of the suspension was inoculated, with the aid of sterile swabs (Rayswab Indústria Brasileira, Diadema, SP, Brazil).

The next step was the insertion of the absorbent paper disks, with 5 mm-diameter, obtained by patterned perforation of coffee filter paper (Melitta do Brazil, Ind. e Com. Ltda, São Paulo, SP, Brazil). These disks were properly sterelized and moistened in each substance test, as known: Cepacol® (Hoechst Marion Roussel, Indústria Brasileira, São Paulo, SP, Brazil) Periogard® with and without alcohol (Colgate-Palmolive, Indústria Brasileira, São Bernardo do Campo, SP, Brazil), Plax® with and without alcohol (Colgate-Palmolive, Indústria Brasileira, São Bernardo do Campo, SP, Brazil) Oral B® without alcohol (Procter & Gamble do Brazil, São Paulo, SP, Brazil) and water, distilled and dionized, as control. All of disks were immersed in equal time (superior to 1 min) in respective substances and in sequence, deposited neatly on sterile gauze to remove liquid excess. At the end of this phase, the dishes were to incubator at 37°C for 48 hours.

For the inhibition hales mensuration, in millimeters, a single trained examiner used a stereoscopic magnifying and milimetric regua (Jon, Com. de Produtos Odontológicos, São Paulo, SP, Brazil). The medium values and standard

Table 1. Description of the tested substances with respective composition and manufacturer identification.

Substance	Composition*	Manufacturer
CEPACOL®	Cetilperidine chlorete (0.500 mg)	Sanofi Aventis Farmacêutica Ltda, Suzano, SP, Brazil
ORAL B® antiseptic	Timol	Procter & Gamble do Brazil, São Paulo, SP, Brazil
PLAX®	Triclosan	Colgate-Palmolive, Ind. Bras., São Bernardo do Campo, SP, Brazil
PLAX® (non-alcohol)	Triclosan	Colgate-Palmolive, Ind. Bras., São Bernardo do Campo, SP, Brazil
PERIOGARD®	0.12% chlorhexidine gluconate	Colgate-Palmolive, Ind. Bras. São Bernardo do Campo, SP, Brazil
PERIOGARD® (non-alcohol)	0.12% chlorhexidine gluconate	Colgate-Palmolive, Ind. Bras. São Bernardo do Campo, SP, Brazil

* according to the manufacturers.

deviation were determined for all substances and, in sequence, compared each other. ANOVA statistical test was conducted with *Bonferoni* corrective test, at 5% significance level.

Results

Table 2 shows the means and standard deviation of inhibition hales, in millimeters, formed by the tested substances. In comparisons related to microorganism as *S. aureus, C. albicans, E. faecalis and P. aeruginosa,* cetilpiridine chloride with (Cepacol®) and without alcohol (Oral-B®) did not presented significant statistically differences (p>0,05), related to inhibition hales mensuration.

In the Plax® substances analysis, the product with alcohol demonstrated inhibition hale with minor diameter (p<0.05) for *S. aureus, C. albicans* and *E. faecalis*. Referring to *P. Aeruginosa,* Plax® without alcohol did not demonstrated statistically difference in relation to control substance (p>0.05), considering the size of the inhibition hale.

For Periogard®, the substance without alcohol presented the greatest inhibition hales (*P*<0.05) for *S. aureus*. In comparision with *C. albicans, E. faecalis* and *P. aeroginosa,* there were no statistically significant differences (*P*>0.05) between these two tested substances. For Periogard®, with and without alcohol, can be observed that only *S.aureus* had result with better antimicrobial action and with statistically significance (*P*<0.05) for the substance with alcohol. In comparison to others microorganisms, *S. aureus, C. albicans, E. faecalis* and *P. aureginosa* did not presented statistically differences (*P*>0.05) between these two tested substances.

For the microorganism *E. faecalis,* Cepacol®, with and without alcohol, Periogard®, with and without alcohol and Plax® with alcohol did not demonstrated statistically differences (*P*>0.05) between the groups. Plax® with alcohol showed antimicrobial effectiveness similar to the control substance (*P*>0.05).

In relation to *C. albicans,* Plax® with alcohol presented the greatest values of antimicrobial effectiveness (*P*<0.05). The others substances evidenced medium antimicrobial effectiveness, however, better results than the control substance (*P*<0.05). For this microorganism, Plax® without alcohol presented the minor values of antimicrobial activity (*P*<0.05), similar to control substance (*P*>0.05).

In relation to microorganism *P. aeruginosa,* Periogard®, with and without alcohol, demonstrated the greater antimicrobial activity compared to others substances (*P*<0.05).

Discussion

Until to the moment, it is not consensus the role of the alcohol, present in bucal mouthwashes, can predispose to neoplasic lesions appearance in oral cavity and oropharynx (6,7). The literature provides conflicting results. Blank et al. (8) report that does not exist evidences correlating these lesions to mouthwashes with alcohol. This statement was reinforced by experiments in which rats were subjected for 20 weeks to mouthwashes with alcohol in their composition, and after the test period, the animals showed no indications of carcinogenesis (9). By other side, in case-control study was observed a greater risk of patients who used mouthwashes with alcohol in developing neoplasic diseases in oral cavity and pharynx (10).

At same time that bucal antiseptics are been considered indispensable tools in infection control (11), another point to be discussed is the fact that this infection focus can be collaborator in the systemic diseases development (12,13). The dentist is considered an important member in the health promotion team (12). Patients, in critic health conditions, moreover in Intensive Care Unity (ICU), present a deficient oral hygiene with great amount of dental biofilm (13). Some cases of ICU stay are long term, increasing the virulence over the time (2,14). For the control of microorganisms linked to systemic nosocomial complication and opportunist diseases, bucal antiseptics alcohol-free are been used (6-8), raising the necessity to investigate the effectiveness of alcohol-free products for this purpose.

In this study, chlorhexidine, with and without alcohol, evidenced the best results, in agreement to others *in vitro* investigations (15,16). This substance, in dentistry research, is considered as gold-pattern in comparisons to others bucal antiseptics (4,17), although is so relevant that others studies should confirm the antimicrobial action of this product, with alcohol in its composition. The chlorhexidine without alcohol seems to maintain the antimicrobial action, *in vitro* essay. Certainly, the substantivity, great antimicrobial spectrum and the action in organic medium shall remain, even without alcohol. Probably, chlorhexidine without alcohol will have

Table 2. Means* and standard deviation of inhibition hales (in millimeters) formed by the tested substances.

Substances	Microorganisms			
	S. aureus	*C. albicans*	*E. faecalis*	*P. aureginosa*
CEPACOL®	9.25±1.00 a,f	7.75±0.65 ad	8.00±1.30 a	5.00±0.00 a
ORAL B (non-alcohol)	8.87±1.72 a	8.50±0.75 d	8.31±0.65 a	5.00±0.00 a
PLAX®	15.68±0.79 d	6.56±0.56 c,a	7.31±0.68 a	5.00±0.00 a
PLAX® (non-alcohol)	5.00±0.0 g	5.00±0.00 e	5.43±0.82 b	5.00±0.00 a
PERIOGARD®	13.10±2.09 c	10.37±1.18 b	9.00±1.00 a	7.50±0.46 b
PERIOGARD® (non-alcohol)	10.56±0.41 f	9.75±1.16 f,b	8.87±1.27 a	7.18±0.45 d,b
Distilled and dionized water	5.00±0.00 g	5.00±0.00 e	5.00±0.00 b	5.00±0.00 a

* Different letters in columns mean statistically difference (*P*<0.05, ANOVA with Bonferroni pos-hoc test).

importance as bucal antiseptic as the ones with alcohol have (18-22). One of the interesting point in the study is related to the fact that Plax® without alcohol demonstrated absence of antimicrobial efficiency compared to the control substance. By other side, Plax® with alcohol showed the best results in relation to *S. aureus,* evidences in agreement to others studies (15,16).

In vitro studies with methodology of culture in Petri dishes are still used to perform antibiograms and to discover new microorganisms (23,24). These techniques, in many places, are not replaced by more advanced diagnostic in microbiology (24). The results in this study should be carefully interpreted because they cannot deal the dental biofilm complexity, a limitation in any current technique, and why they do not deal any issue about the quality control of the products. However, it seems that there is the need for further clarification with other *in vitro* studies and also in health practice.

Because the microorganisms used in this study are colonizers of the oral and oro-pharynix cavity, often related to nosocomial infections (11,13), it is imperative the use of others bucal antiseptics as alternative. Even the results demonstrated relevant differences in the antimicrobial effect of the bucal antiseptics, with and without alcohol, perceive the need of other *in vitro* and *in vivo* investigations to confirm the results presented in this study.

Conclusion

The alcohol-free mouthwashes did not present anti-microbial action similar to alcohol ones, in relation to tested microorganisms in this study.

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