

ANALYSIS OF THE ANTIMICROBIAL ACTIVITY OF CHILDREN'S DENTIFRICES OF THE BRAZILIAN MARKET

Análise da ação antimicrobiana de cremes dentais infantis do mercado brasileiro

Análisis de la acción antimicrobiana de cremas dentales infantiles del mercado brasileño

Original Article

ABSTRACT

Objective: To evaluate the antimicrobial activity of children's toothpastes of the Brazilian market. **Methods:** Experimental study conducted in a university of Fortaleza, CE, Brazil, in 2014, using 12 Petri dishes with culture media Mueller Hinton for *Streptococcus mutans*, and Sabouraud Agar for *Candida albicans*, where wells containing equal amounts of toothpaste (Tandy®, Colgate Barbie®, Oral B Stages®, Bitufo Ben 10®, Even Mônica®, Boni Looney Tunes® with fluoride, Malvatrikids Baby®, Condor Tigor®, Even Baby®, Bitufo Cocoricó® and Sanifill Fase 1® without fluoride) were seeded and incubated with *Streptococcus mutans* in anaerobic jars, both in bacteriological incubator at 37°C, for 48h. After this period, it was examined whether or not there was formation of inhibition zones, which represent the antimicrobial activity. **Results:** The results of fluoride and non-fluoride toothpastes showed discrete differences in inhibition zones measures compared to each other, but the ones with fluoride presented antimicrobial activity against the evaluated strains. Malvatrikids Baby®, Even Baby® and Sanifill Fase 1® showed no activity against *Streptococcus mutans*, and the last did not have effect against *Candida* either. **Conclusion:** All fluoride toothpastes evaluated and two of those without fluoride (Condor Tigor® e Bitufo Cocoricó®) showed antimicrobial activity against the evaluated strains.

Descriptors: Dentifrices; Products with Antimicrobial Action; *Candida*; *Streptococcus mutans*.

RESUMO

Objetivo: Avaliar a ação antimicrobiana de cremes dentais infantis do mercado brasileiro. **Métodos:** Estudo laboratorial realizado em uma universidade de Fortaleza/CE, em 2014, utilizou 12 placas de Petri com meios de cultura Mueller Hinton para *Streptococcus mutans* e Agar Sabouraud para *Candida albicans*, nas quais poços contendo quantidades iguais de cremes dentais (Tandy®, Colgate Barbie®, Oral B Stages®, Bitufo Ben 10®, Even Mônica®, Boni Looney Tunes® com flúor, Malvatrikids Baby®, Condor Tigor®, Even Baby®, Bitufo Cocoricó® e Sanifill Fase 1® sem flúor) foram semeados e incubados com *Streptococcus mutans* em jarra de anaerobiose, ambos em estufa bacteriológica a 37°C, por 48 horas. Após esse período, analisou-se a formação ou não de halos de inibição, que representa a ação antimicrobiana. **Resultados:** Os resultados obtidos dos cremes dentais fluoretados e não fluoretados apresentaram diferenças discretas de medidas de halos de inibição entre si, mas os fluoretados mostraram atividade antimicrobiana contra as cepas avaliadas. Malvatrikids Baby®, Even Baby® e Sanifill Fase 1® não mostraram atividade contra *Streptococcus mutans* e somente o Sanifill Fase 1® não apresentou contra *Candida albicans*. **Conclusão:** Todos os cremes dentais fluoretados avaliados e dois sem flúor (Condor Tigor® e Bitufo Cocoricó®) apresentaram atividade antimicrobiana contra as cepas avaliadas.

Descritores: Dentifricios; Produtos com Ação Antimicrobiana; *Candida*; *Streptococcus mutans*.

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RESUMEN

Objetivo: *Evaluar la acción antimicrobiana de las cremas dentales infantiles del mercado brasileño. Métodos:* Estudio de laboratorio realizado en una universidad de Fortaleza/CE, en 2014 que utilizó 12 placas de Petri con medios de cultivo Mueller Hinton para el *Streptococcus mutans* y Agar Sabouraud para la *Candida albicans* las cuales pozos con cantidades iguales de cremas dentales (Tandy®, Colgate Barbie®, Oral B Stages®, Bitufo Ben 10®, Even Mônica®, Boni Looney Tunes® con flúor, Malvatríkids Baby®, Condor Tígor®, Even Baby®, Bitufo Cocoricó® y Sanifill Fase 1® sin flúor) fueron incubados con *Streptococcus mutans* en una jarra anaeróbica, ambos en una estufa bacteriológica de 37°C durante 48 horas. Después de ese período se analizó la formación o no de halos de inhibición que representa la acción antimicrobiana. **Resultados:** Los resultados obtenidos con las cremas dentales con y sin flúor presentaron diferencias discretas de las medidas de halos de inhibición entre ellos pero las con flúor mostraron actividad antimicrobiana contra las cepas evaluadas. Malvatríkids Baby®, Even Baby® y Sanifill Fase 1® no mostraron actividad contra el *Streptococcus mutans*, y solamente el Sanifill Fase 1® no la presentó contra la *Candida albicans*. **Conclusión:** Todas las cremas dentales con flúor evaluadas y dos sin flúor (Condor Tígor® y Bitufo Cocoricó®) presentaron actividad antimicrobiana contra las cepas evaluadas.

Descriptorios: *Dentífricos; Productos con Acción Antimicrobiana; Candida; Streptococcus mutans.*

INTRODUCTION

Tooth decay is regarded as one of the most prevalent diseases in the world and a public health problem. A disease of infectious nature and characterized by mineral loss in the tooth structure, it has a close relation with the presence of dental biofilm, the main responsible for major tooth losses⁽¹⁻³⁾. In Brazil, a decrease in tooth decay was observed with the aid of various factors, including the decentralization of public services, with inclusion of the oral health team in the Family Health Strategy⁽⁴⁾, the fluoridation of the public water supply, and the regular use of toothpaste with fluoride in its composition⁽⁵⁾.

The accumulation of bacteria and other microorganisms forming a community on the tooth surface can be previously avoided or later treated, with brushing as the main mechanical prevention mechanism, preventing bacterial activity, and thus avoiding enamel demineralization^(3,6).

As a way to decrease dental caries prevalence and high risk⁽⁷⁾, chemical agents that act on the biofilm are indicated, interfering with bacterial adhesion to the tooth surface. The main medium with chemical agent that aids in the removal of biofilm for home use, recommended by dental professionals, is tooth brushing with toothpaste or

dentifrice⁽⁶⁾, which is any preparation used in oral hygiene. It can contain abrasive, detergent, binder, flavoring, and preventive medicines against decay in its composition, and be found as a liquid, paste, gel or powder⁽⁸⁾.

Children's toothpaste has been modified throughout history⁽⁷⁾ for better use and effectiveness. In the past, only those with high fluoride content were introduced in the market, playing an important role in combating caries⁽⁹⁾, for they act in the remineralization of the dental enamel surface and inhibit demineralization, maintaining the balance between the mineralized tooth structures⁽¹⁰⁾.

However, when fluoride ingestion occurs⁽¹¹⁾, there is a risk that the patient presents acute toxicity if large amounts are ingested at one time, manifesting itself clinically in abdominal pain up to cardiac arrest, or chronic toxicity, namely fluorosis, an undesirable effect that can cause stains on tooth enamel when it is present in a large amount during dental formation⁽¹¹⁾.

Therefore, aiming at decreasing the risk of fluorosis due to accidental ingestion of dentifrice containing high fluoride content, dentifrices with a small amount of this drug have entered the market and, soon after, those without any fluoride, indicated for babies. These have the fluorine substance replaced by therapeutic agents, such as chamomile, enzymes, xylitol, among others⁽¹²⁾. The promise was to control tooth decay, reduce oral microorganisms, and work as a method of motivation for oral health of infants and children⁽¹²⁾.

From the 1980s on, with considerable increase in the availability of fluoride toothpastes, tooth decay has declined in developed countries, and after ten years, such decline was observed in Brazil^(3,13), showing that the use of fluoride at high frequency and low concentration, by means of tooth brushing with toothpastes containing the recommended concentration, from 1000 to 1500 ppm, is effective in controlling tooth decay, and indispensable for health promotion^(2,3,9).

The Brazilian market has been taken by different types of toothpastes aimed at children: the ones that have fluoride in optimal amount for the control of dental decay, those containing small amounts of fluoride, and also those with a fluoride-free composition⁽¹⁴⁾. Therefore, it has been recently pointed out the need to have the current Brazilian legislation modified as regards the toothpaste market⁽¹⁵⁾, since there is a difficulty in selecting and using them, as the parents or guardians are not always aware of which decay risk their children are subject to, nor have they knowledge of the potential presented by these different types of toothpastes in controlling dental biofilm, nor do they know if those can pose a risk to their children's health due to the substitution of drugs⁽¹⁶⁾.

Given the shortage of laboratory evaluations with scientific evidence of efficacy against microbial growth of children's toothpastes available in the domestic market, containing several active ingredients in their formulations⁽¹⁵⁻¹⁷⁾, the aim of this study was to evaluate the antimicrobial activity of children's toothpastes of the Brazilian market.

METHODS

Laboratory and microbiological study performed at a private university in the capital of Ceará state, Brazil. Eleven children's toothpastes were selected for this research, of different brands/manufacturers and easily found in Brazil, bought at drugstores and supermarkets in March 2014. They

Chart I - Description of the investigated children's toothpastes (dentifrices), according to the manufacturer and components. Fortaleza, CE, 2014.

	Dentifrices	Manufacturer	Components
A	Tandy® - strawberry flavor	Colgate-Palmolive Industrial Ltda. – Brazil	Sorbitol, water, hydrated silica, sodium lauryl sulphate, PEG-12, cellulose gum, flavoring, sodium saccharin, colorant; sodium fluoride (1100 ppm).
B	Colgate Smiles Barbie®	Mission Hills para Colgate-Palmolive – Mexico	Sorbitol, water, hydrated silica, sodium lauryl sulphate, PEG-12, cellulose gum, flavoring, sodium saccharin, titanium dioxide, red colorant, dipentene, cinnamaldehyde, eugenol; sodium fluoride (1100 ppm).
C	Oral B Stages Pooh®	Procter & Gamble Manufatura – Mexico	Sorbitol, water, hydrated silica, sodium lauryl sulphate, cellulose gum, flavoring, sodium saccharin, carbomer, disodium pyrophosphate, sodium hydroxide, colorant; sodium fluoride (500 ppm).
D	Bitufo Ben 10®	Cosmed Indústria de Cosméticos e Medicamentos – Brazil	Sorbitol, water, hydrated silica, sodium lauryl sulfate, calcium carbonate, carboxymethyl cellulose, polyethylene glycol, flavoring, xylitol, sodium silicate, sodium saccharin, methylparaben, propylparaben, green pigment, d-limonene, sodium monofluorophosphate.
E	Even - Turma da Mônica®	Indústrias Reunidas Raymundo da Fonte - Brazil	Sorbitol, water, silica, glycerin, sodium lauryl sulfate, sodium saccharin, carboxymethyl cellulose, polyethylene glycol, potassium sorbate, colorant, flavoring, sodium monofluorophosphate (1100 ppm).
F	Boni - Looney Tunes®	Laboratório Boniquet do Brasil – Brazil	Sorbitol, water, hydrated silica, sodium saccharin, methylparaben, propylparaben, cellulose gum, PEG-8, flavoring, calcium glycerophosphate, titanium dioxide, sodium fluoride (1100 ppm).
G	Malvatrikids Baby®	Laboratório Daudt Oliveira – Brazil	Sorbitol, water, sucralose, xylitol, cellulose gum, glycerin, mallow (malva silvestris) extract, silica, sodium benzoate, flavoring, sodium lauroylsarcosinate.
H	Condor - Tigor Baby®	Dental Prev Indústria e comércio - Brazil	Sorbitol, water, silica, sodium lauryl sulphate, sodium saccharin, sodium benzoate, carboxymethyl cellulose, flavoring.
I	Even Baby®	Indústrias Reunidas Raymundo da Fonte S/A - Brazil	Sorbitol, glycerin, sodium saccharin, silica, xylitol, carboxymethyl cellulose, flavoring, polyethylene glycol, potassium sorbate, sodium lauryl sulfate, colorant, water.
J	Bitufo Cocoricó®	Cosmed Indústria de Cosméticos e Medicamentos – Brazil	Sorbitol, water, hydrated silica, sodium saccharin, glycerin, sodium lauryl sulfate, alcohol, polyethylene glycol 400, triclosan, xylitol, carboxymethyl cellulose, flavoring, xanthan gum, titanium dioxide, calcium disodium EDTA, methylparaben, propylparaben.
L	Sanifill Fase 1®	Cosmed Indústria de Cosméticos e Medicamentos – Brazil	Sorbitol, water, hydrated silica, chamomile extract, flavoring, xanthan gum, sodium benzoate, sodium saccharin, yellow colorant, blue colorant.

were all acquired in the same month to prevent interference from the storage time on the shelves in the quality of the products.

The description of the materials tested in the current study, as well as their manufacturer and components, are listed in Chart I. All materials were tested before expiration.

The experimental phase took place in the Microbiology Laboratory, with participation of properly trained examiners, using personal protective equipment (PPE). Additionally, prior to the use, the laboratory bench had its surface decontaminated by applying 70% rubbing alcohol for three times.

For the microbiological growth inhibition assay, the agar well diffusion method⁽⁶⁾ was employed, which occurred by checking the formation of growth inhibition halos around the wells, using a 2% chlorhexidine gel (Farma-Essence Brasil[®]) as the positive control and no material as a negative control.

The most prevalent strains of microorganisms in children's oral cavity, which are responsible for the tooth decay process, are *Streptococcus mutans* and *Candida albicans*^(18,19). Therefore, these strains were chosen for use in this study, aiming at a closer similarity to the actual clinical situation.

The study was performed in 12 Petri dishes prepared with Mueller Hinton Agar culture medium for *Streptococcus mutans* (n=6) and Sabouraud Agar for *Candida albicans* (n=6). Both strains were provided by the Microbiology Laboratory of the University of Fortaleza. Performed in triplicate, the microorganisms were previously replicated and then plated onto plates containing their specific media in agar, prior to inserting the children's toothpastes.

In Petri dishes containing the aforementioned media, wells measuring 4 mm in diameter and 5 mm deep were constructed, with the aid of a plastic ferrule disinfected with 70% ethanol, and then filled with 0.13 grams of each of the children's toothpaste investigated, according to Chart I, with the aid of a Centrix[®] syringe (DFL, Indústria e Comércio S.A., Brazil). The plates were then incubated with *Streptococcus mutans* in an anaerobic jar at 37°C, allowed in a bacteriological incubator for 48 hours, awaiting bacterial growth. The plates with *Candida albicans* were placed in the bacteriological incubator, waiting up to 48 hours for the collection of results⁽²⁰⁾.

After the specified time, microbial growth was verified in the Petri dishes, and through inhibition of halo formation, it was observed whether there was growth inhibition, thus confirming the antimicrobial activity of the product. To emphasize this finding, the halos formed were measured with the aid of a millimeter ruler by two calibrated

examiners, who entered the data into tables and obtained the arithmetic mean for the set of values.

RESULTS

In Petri dishes with *Streptococcus mutans*, it was observed, after 48 hours, the formation of inhibition zones, that is, antimicrobial action was evidenced, in the following children's toothpastes: A, B, C, D, E, F, H, J (Chart 1), and in control with 2% chlorhexidine gel.

Table I displays the results obtained by both examiners for the measurements of the inhibition halos (in millimeters - mm) in the children's fluoride toothpastes, and Table II presents the results of children's toothpastes without fluoride, when evaluated with *Streptococcus mutans*.

Table I - Measurement of inhibition halos (mm*) in *Streptococcus mutans*, in fluoride children's toothpaste (dentifrice). Fortaleza, CE, 2014.

Dentifrice	Examinator 1	Examinator 2	Mean
A	9	9.6	9.3
B	9	10	9.5
C	10.6	11.3	10.9
D	8	8.3	8.1
E	8.3	8.3	8.3
F	9.6	10.3	9.9

*mm: millimeters

Table II - Measurement of inhibition halos (mm*) in *Streptococcus mutans*, in non-fluoride children's toothpaste (dentifrice). Fortaleza, CE, 2014.

Dentifrice	Examinator 1	Examinator 2	Mean
G	0	0	0
H	8.3	7.6	7.9
I	0	0	0
J	8.6	8.6	8.6
L	0	0	0

*mm: millimeters

In Petri dishes containing *Candida albicans*, inhibition halos were formed in the following dentifrices: A, B, C, D, E, F, G, H, I, J, and in control with 2% chlorhexidine gel. Tables III and IV show the measurements of the halos formed in children's fluoride and non-fluoride toothpastes, when tested against *Candida albicans*.

The dentifrice L (Sanifill Fase 1[®]) showed no inhibition zone formation, evidencing that there was neither antifungal activity against *Candida albicans*, nor antibacterial activity against *Streptococcus mutans* (Tables II and IV).

Empty wells, used as a negative control, showed no inhibition halos for the tested strains.

Table III - Measurement of inhibition halos (mm*) in *Candida albicans*, in fluoride children's toothpaste (dentifrice). Fortaleza, CE, 2014.

Dentifrice	Examinator 1	Examinator 2	Mean
	7.3	8	7.6
B	5.3	5.6	5.4
C	9	10	9.5
D	8	7	7.5
E	8.3	7.6	7.9
F	8.6	9	8.8

*mm: millimeters

Table IV - Measurement of inhibition halos (mm*) in *Candida albicans*, in non-fluoride children's toothpaste (dentifrice). Fortaleza, CE, 2014.

Dentifrice	Examinator 1	Examinator 2	Mean
G	6.6	6.6	6.6
H	6.6	7	6.8
I	7.6	7.6	7.6
J	8.3	9	8.6
L	0	0	0

*mm: millimeters

It stands out that control with 2% chlorhexidine gel presented mean inhibition halo of 7 mm in plates with *Streptococcus mutans*, and mean inhibition halo of 13.1 mm in plates with *Candida albicans*.

DISCUSSION

Despite the vast importance of evaluating the antimicrobial activity in children's toothpastes, a universal methodology for this process has not been established yet⁽¹⁷⁾. In conducting this research, samples of undiluted, fluoride and non-fluoride toothpastes were chosen for use, in order to evaluate the antimicrobial activity simulating the actual oral condition, as stated by a study performed in 2011⁽²⁰⁾, which advocates putting the toothpaste on the brush without water, in opposition to other study that used diluted toothpastes⁽¹²⁾.

The results of the present study showed that the children's fluoride toothpastes Tandy strawberry flavor[®], Colgate Smiles Barbie[®], Oral B Stages[®], Bitufo Ben 10[®], Even Turma da Mônica[®], and Boni Looney Tunes[®] obtained inhibition halos ranging from 8.1 mm to 10.9 mm against *Streptococcus mutans* strains, compared to the 2% chlorhexidine gel used as a positive control, with average halo of 7 mm. The same toothpastes obtained results from 5.4 mm to 9.5 mm against *Candida albicans* strains, also compared to 2% chlorhexidine gel with inhibition halo of 13.1 mm.

Other studies that evaluated the toothpaste Tandy[®] demonstrated an excellent inhibitory rate against *Streptococcus mutans* strains^(10,12,13,17,20,21). One investigation also found that the dentifrice Bitufo Ben 10[®] presented antimicrobial activity against *Streptococcus mutans*⁽²¹⁾.

Among the fluoride toothpastes tested in this study, the one with the lowest concentration of fluoride reported by the manufacturer is Oral B Stages Pooh[®], containing 500 ppm, whereas the others have 1100 ppm, according to information by the manufacturers. A larger amount of fluoride could result in higher antimicrobial activity and greater efficacy in caries control⁽⁹⁾.

The current study also showed results for non-fluoride toothpastes against the clinical strains of *Candida albicans*, in which Malvatrikids Baby[®], Condor Tigor Baby[®] and Even Baby[®] obtained results with similar halos of inhibition, with 6.6 to 7.6 mm, on average. The dentifrice Bitufo Cocoricó[®] showed the best result, with halo of 8.6 mm, whereas toothpaste Sanifill Fase 1[®] had the worst performance, without formation of inhibition halo, that is, it showed no antimicrobial activity against the strains evaluated in this study. Non-fluoride toothpastes Condor Tigor Baby[®] and Bitufo Cocoricó[®], tested with clinical strains of *Streptococcus mutans*, obtained similar results, with mean value ranging from 7.9 mm to 8.6 mm, in the evaluation of this research.

It is noteworthy that no scientific evidence was found in the recent literature on the same children's toothpastes/dentifrices, thus hampering the discussion of the current research findings, by drawing a comparison between the brands in antimicrobial assays. Other non-fluoride toothpastes such as Weleda[®] and Baby Oral Gel[®], on the other hand, showed no formation of inhibition halos⁽²⁰⁾ in a study performed with the Agar well diffusion method.

Additionally, some components within the formulation of non-fluoride toothpastes may have antimicrobial activity, such as EDTA (ethylenediaminetetraacetic acid), triclosan, mallow, sweeteners, colorants and parabens. It is noteworthy that such components can pose a risk to children's health⁽¹⁶⁾.

The non-fluoride toothpaste Bitufo Cocoricó[®] contains in its formula triclosan, an agent with potential antimicrobial activity⁽²²⁾, and EDTA, for which the antifungal activity in disinfecting root canals⁽²³⁾ during endodontic treatment has been proven; it should not be used on the tooth surface or mucosa during brushing and cleaning, because of its acidity and likelihood to cause injuries.

Mallow, on the other hand, a component of Malvatrikids Baby[®], does not have antimicrobial activity against the strains of *Candida albicans*⁽²⁴⁾, in agreement with the findings of the current research.

A recent publication emphasizes the use of fluoride toothpastes in small amounts for children, for prevention of tooth decay and fluorosis⁽²⁵⁾. Thus, an optimal formulation of toothpastes aimed at children should provide health, and not bring harmful components, and should as well maximize the availability of fluoride, minimize abrasiveness, and provide suitable flavors, so that it could provide a pleasant toothbrushing experience to children of various ages, aiming at good oral habits in the future, and good oral health for life⁽²⁶⁾.

It suggests the need for studies that evaluate the antimicrobial activity of children's toothpastes, even laboratory studies such as this one, and dissemination of findings to the health care field, to assist in signalling to parents and professionals the best kind of toothpaste/dentifrice for the promotion of child health, given the existence of such a vast amount of them in the market, confusing the user.

CONCLUSION

All of the children's fluoride toothpastes evaluated and two of those without fluoride (Condor Tigor® e Bitufo Cocoricó®) showed antimicrobial activity against the evaluated strains.

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