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Article

In vitro efficacy of five pediatric toothpastes in the inhibition of *Streptococcus mutans* ATCC 25175.

Eficacia *in vitro* de cinco pastas dentales pediátricas en la inhibición de *Streptococcus mutans* ATCC 25175.

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Abstract: The present *in vitro* study compared the inhibitory action of five pediatric toothpastes against *Streptococcus mutans* ATCC 25175. **Materials and Methods:** Cross-sectional, comparative and experimental study. The microorganism *Streptococcus mutans* ATCC 25175 was inoculated onto solid culture medium of Müeller-Hinton supplemented with blood, then the plates were inoculated with five pediatric toothpastes: Oral B Stages, Colgate Smiles, Aquafresh My Little Teeth, Dentito and Denture Kids. Samples were incubated at 37°C for 48 hours. Subsequently the inhibition halos were measured; the experiment was repeated 20 times for each sample. Statistical analysis was performed with ANOVA complemented with Tukey's test. **Results:** Oral B Stages yielded a mean inhibitory halo of 23.2mm, Colgate Smiles an average of 21.7mm, Aquafresh My Little Teeth of 13.6mm, Dentito of 18.5mm, and Denture Kids a mean of 23.0mm. When performing the ANOVA test, it was found that there was a significant difference in the inhibitory action of pediatric toothpastes (p<0.005) and when using Tukey's multiple comparison test, Oral B and Denture Kids presented similar inhibitory action. **Conclusion:** All toothpastes presented inhibitory action against *Streptococcus mutans* ATCC 25175. A significant difference between their effectiveness was observed. Oral B Stages showed the greatest degree of inhibition.

Keywords: Toothpaste; Streptococcus mutans; culture media; child; cross-sectional studies; analysis of variance.

Resumen: El presente estudio *in vitro* comparo la eficacia inhibitoria de cinco pastas dentales pediátricas frente a la bacteria *Streptococcus mutans* ATCC25175. **Material y Métodos:** El estudio fue transversal, comparativo y experimental. Se inoculó *Streptococcus mutans* ATCC 25175 en un medio de cultivo Müeller-Hinton complementado con sangre, luego a las placas cultivadas se le inocularon cincopastas dentales pediátricas: Oral B Stages, Colgate Smiles, Aquafresh My LittleTeeth, Dentito y Denture Kids. Se incubó a 37°C por 48 horas y posteriormente semidió los halos de inhibición, se replicó el experimento 20 veces para cada uno. El análisis estadístico se realizó con el test de ANVA complementado con el test de Tukey. **Resultado:** Oral B Stages generó una media inhibitoria de 23,2mm, ColgateSmiles una media de 21,7mm, Aquafresh My Little Teeh de 13,6mm, Dentitode 18,5mm y finalmente Denture Kids una media de 23,0mm. Al realizar laprueba ANOVA se encontró que hay diferencia significativa en la acción inhibitoriade las pastas dentales pediátricas (p<0.005) y al emplear la prueba Tukey (comparación de múltiples) la pasta Oral B y Denture Kids presentaron acción inhibidora similar. **Conclusión:** Todas las pastas presentaron acción inhibitoria sobre *Streptococcus mutans* ATCC 25175 existiendo diferencia significativa entre la efectividad de estas, con la pasta Oral B Stages demuestrando mayor acción inhibitoria.

Palabra Clave: *Pastas de dientes; Streptococcus mutans; medios de cultivo; niño; estudios transversales; análisis de variación.*

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INTRODUCTION.

There is currently a growing interest in improving the oral health of children from an early age. The World Health Organization (WHO) defines oral health as the absence of caries, periodontal disease, orofacial pain, loss of teeth and other diseases or disorders that hamper the ability to chew, talk and smile in humans, damaging their psychosocial well-being. Caries is the most prevalent oral disease in preschool and school age children, in contrast to periodontal or pulp diseases. According to the data provided by the WHO, 60 to 90% of children worldwide suffer from caries.[1] According to the ministries of health of Latin American countries, the prevalence of caries is high in countries such as Brazil with 80%,[2] Colombia with 52.2%,[3] Chile with 70.4%,[4] and Argentina with 87.4%.[5] According to an epidemiological study conducted by the Peruvian Ministry of Health (MINSA) from 2012 to 2014, the prevalence of caries in children under 12 years of age was 85.6%.[6]

Caries at early ages is a significant aggravating factor in the oral health of children, since they will be more likely to develop caries in mixed and permanent dentition.[7] The oral health of children depends on the constant supervision of parents or guardians.[8] There is sometimes difficulty because parents are not aware of correct brushing techniques and which toothpastes or mouthwashes are suitable for their children.[9]

The main risk factors for caries are the high prevalence of *Streptococcus mutans* and lactobacilli in the oral cavity, teeth with weaken enamel susceptible to an acidic medium and with poor mineralization capacity, a cariogenic diet based on sugar consumption, and poor oral hygiene associated with cognitive, economic and social factors.[10]

The most prevalent microorganism in incipient caries and enamel lesions is *Streptococcus mutans*. It plays a very important role at the early stages of the disease but not in its progression.[11] *Streptococcus mutans* is native to humans and becomes pathogenic when there is a change in the oral environment, that is, high consumption of refined sugars (such as sucrose).[12]

The use of toothpastes for the oral hygiene of children has increased in recent times and their composition has changed. Currently, many types of toothpaste contain antimicrobial agents with the aim of preventing and controlling oral diseases associated with common microorganisms of the oral cavity.[13]

The presence of caries in children affects their early childhood development and those with active caries have psychosocial behavioral problems since they have higher rates of lack of sleep, attention deficit, depression, anxiety, aggressiveness and hyperactivity.[14]

There is a high prevalence of childhood caries in Peru, related to multiple factors such as high sugar diet, acid-producing bacteria, poor oral hygiene, and socioeconomic status. Oral health procedures should be supervised by parents and they should also favor those toothpastes that contain antimicrobial agents such as xylitol, fluoride or others. Little is known about the inhibitory action against *Streptococcus mutans* of pediatric toothpastes available in the Peruvian market. As this microorganism is related to the presence of dental caries, being able to identify the components of toothpastes and their properties that can help children deal with dental caries. The aim of the present study is to determine the in vitro efficacy of five pediatric toothpastes in the inhibition of *Streptococcus mutans* ATCC 25175.

MATERIALS AND METHODS.

Cross-sectional, comparative and experimental study, carried out in the Medical Microbiology Laboratories of the School of Human Medicine at Universidad Nacional de Trujillo - La Libertad, Perú. All biosafety and laboratory standards were met. Five pediatric toothpastes available in the Peruvian market were used: Aquafresh My Little Teeth15, Colgate Smiles,[16] Dentito,[17] Denture Kids,[18] and Oral B Stages.[19] They were placed in a sterile tuberculin syringe (Figure 1) in order to be inoculated in the form of discs onto the plates where strains of *Streptococcus mutans* ATCC 25275 had been previously inoculated.

The strain of *Streptococcus mutans* ATCC 25175 was cultured in thioglycolate broth, then incubated for 48 hours at 37°C. Two days later the growth of the strain was confirmed when a strong turbidity was observed in the test tubes.

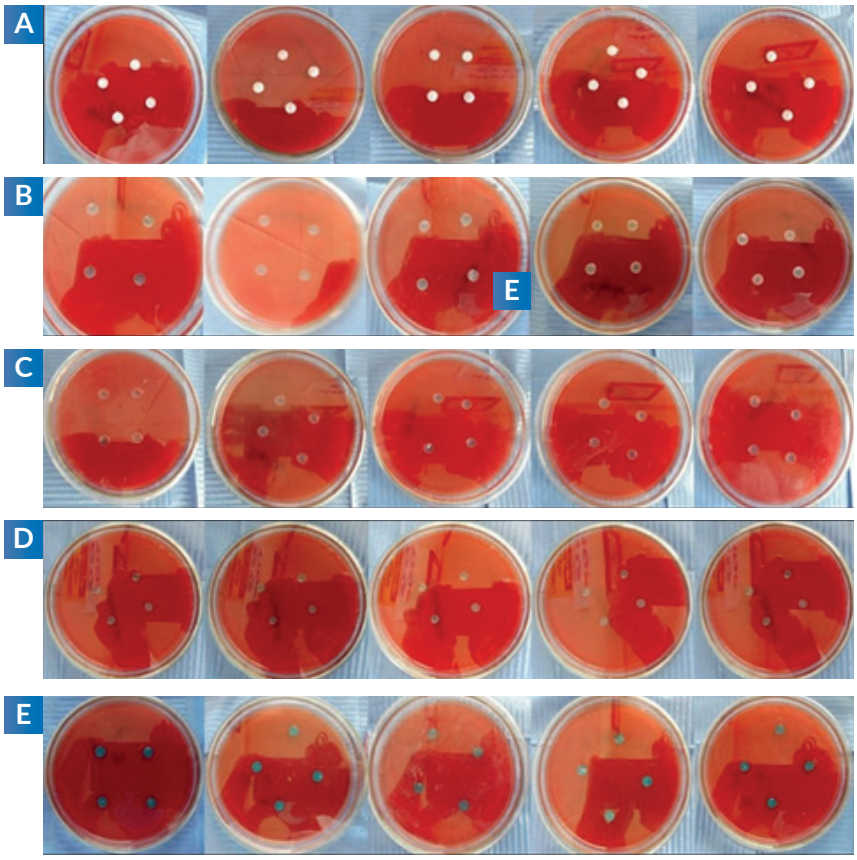
Thirty Petri dishes of Mueller Hinton medium enriched with 5% rabbit blood were prepared. Inoculation of the plates was performed, and then the inoculated plates were perforated with a microbiological punch making four holes, 6mm in diameter, in each plate. Then five control plates were made by filling two holes with 0.1ml of each toothpaste using of tuberculin syringes, and in the two other holes the negative control (distilled water) and positive control (0.12% chlorhexidine) were inoculated. Then, 25 repeat plates were made by inoculating directly in the four holes 0.1ml of toothpaste

(Figure 2). Finally, each plate was labeled with the name of the corresponding toothpaste and the repeat number. Plates were incubated in a micro anaerobic environment at a temperature of 37°C for 48 hours. After 48 hours of incubating the plates, the inhibition halos generated by each pediatric toothpaste were measured (Figure 3). A 0.01mm precision digital Vernier caliper was used to obtain the diameter of the halos.

Figure 1. Pediatric toothpastes in aliquoted into tuberculin syringes.

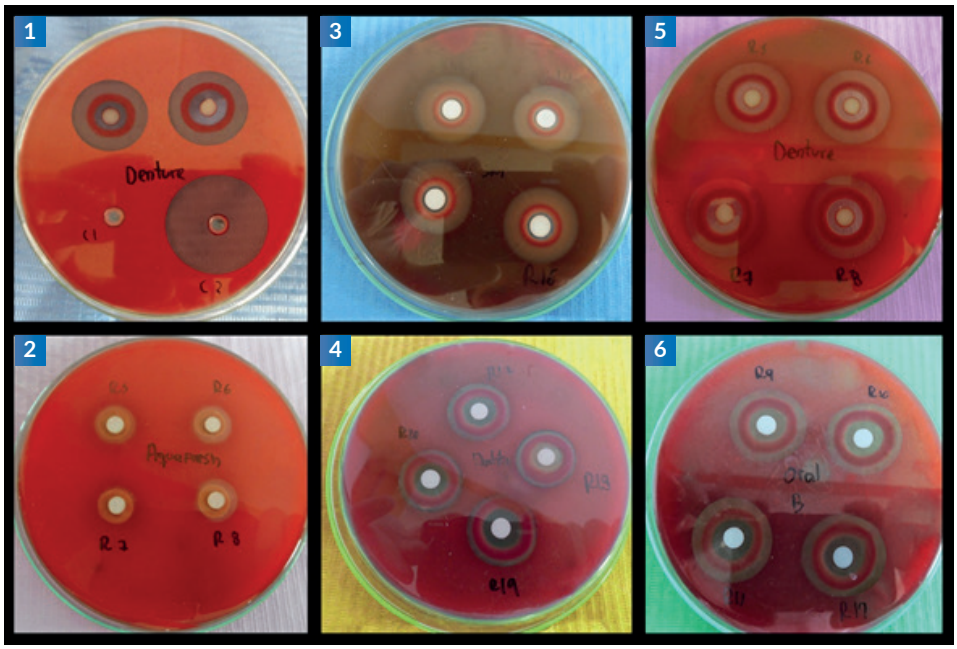
A: Aquafresh, My Little Teeth **B:** Colgate Smiles. **C:** Dentito. **D:** Denture Kids. **E:** Oral B Stages.

Figure 2. Repeat plates of the five pediatric toothpastes.



Repeat plates of toothpastes: **A:** Aquafresh, My Little Teeth **B:** Colgate Smiles. **C:** Dentito. **D:** Denture Kids. **E:** Oral B Stages.

Figure 3. Halos of bacterial inhibition of the five pediatric toothpastes.



1: Halos of inhibition of control plate Denture (C1: Distilled water, C2: chlorhexidine). **2:** Halos of inhibition of Aquafresh My Little Teeth. **3:** Halos of inhibition of Colgate Smiles. **4:** Halos of inhibition of Dentito. **5:** Halos of inhibition of Denture Kids. **6:** Halos of inhibition Oral B Stages.

RESULTS.

The inhibitory effect of each pediatric toothpaste was determined quantitatively by the value of the means of the inhibition halos that each toothpaste generated on the culture plates of *Streptococcus mutans* ATCC 25175.

The inhibition halos of Oral B Stages were the largest, with an average of 23.2mm (Table 1). The negative control (distilled water) resulted in no inhibition halo, while the positive control (chlorhexidine) resulted in a mean halo of 29.0mm.

The analysis of variances or ANOVA (Table 2) reveals a significant difference in the inhibitory action of each of the pediatric toothpastes against *Streptococcus mutans* ATCC 25175. After the ANOVA test, a multiple comparison test was performed, in this case, the Tukey's HSD test (Table 3).

It showed 94.1% similarity in the inhibitory action of Oral B Stages and Denture Kids.

Table 1. Measures of inhibition halos for *Streptococcus mutans* ATCC 25175 of the five pediatric toothpastes.

	N	Mean (mm)	S.D (mm)	Lowest (mm)	Highest (mm)
Aquafresh My Little Teeth	20	13.6	1.0	11.8	15.7
Colgate Smiles	20	21.7	1.1	18.9	22.9
Dentito	20	18.5	1.6	16.3	21.7
Denture Kids	20	23.0	0.7	21.8	24.3
Oral B Stages	20	23.2	0.9	22.1	24.9

S.D: Standard deviation.

Table 2. Analysis of Variance between groups of pediatric toothpastes.

	Sum of squares	df	Mean sum of squares	F-Statistic	*p*-value
Between groups	10223.18	5	1703.86	1715.45	0.000
Within groups	132.10	133	.99		
Total	10355.28	139			

p-value: $p<0.05$. **df:** degrees of freedom. **F:** F-statistic

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Table 3 . Pediatric toothpaste comparison test.

Tukey's HSDa					
Type of toothpaste	N	Subset for alpha = 0.05			
		1	2	3	4
Aquafresh My Little Teeth	20	13.554			
Dentito	20	18.508			
Colgate Smiles	20	21.726			
Denture Kids	20	22.986			
Oral B Stages	20	23.231			
p-value		1.000	1.000	1.000	.941

The means for the groups are displayed in the homogeneous subsets. [a]: It uses the sample size of the harmonic mean=20.000.

DISCUSSION.

Dental caries in children is a serious and a recurrent oral health problem. This condition begins at very early ages of life and has a tendency to become a chronic problem even in adulthood.[20] This disease is associated with early colonization with cariogenic microorganisms such as *Streptococcus mutans*.[3]

One of the oral hygiene measures to control these microorganisms is the use of toothpaste, as it contains antimicrobial agents such as fluoride and others.[21] The present study found that Aquafresh My Little Teeth is the toothpaste with the lowest inhibitory effect, possibly because it does not contain extra antimicrobial substances such as xylitol, containing only fluoride in the form of sodium fluoride.

The addition of xylitol in fluoridated dentifrices is currently being promoted for the prevention and control of dental caries, and its use is supported by the American Academy of Pediatric Dentistry.[22]

The Peruvian toothpaste Dentito has a slightly higher inhibitory power compared to Aquafresh My Little Teeth. This may be because it contains sodium benzoate, which is a salt used as a preservative that has antiseptic, antifungal and bacteriostatic properties.[23] Colgate toothpaste presented a greater inhibitory action than the previous two, maybe due to the inclusion of cinnamaldehyde and eugenol, which are used to give this toothpaste the smell and taste of cinnamon and cloves. This result is consistent with the study by Erazo *et al.*,[24] who found that these aromatic substances are able to inhibit *Streptococcus mutans* Denture Kids is a toothpaste with an increased presence and use in the market in recent years.

This toothpaste generated the second most significant inhibitory action in this experiment. It is the only toothpaste that contains xylitol (10%), an artificial sweetener used in toothpastes as a bactericidal agent against *Streptococcus mutans*[25] strains. It also has methyl and propyl paraben that is used as preservatives for their bacteriostatic properties against Gram positive bacteria and as an antifungal agent.[26]

Xylitol is a substance of natural origin that prevents the appearance of caries, helps to decrease the amount of bacterial plaque, and improves halitosis and the quality of oral soft tissues. In addition this toothpaste contains calcium lactate which, when combined with xylitol, provides calcium to the developing teeth.[18] Finally, Oral B Stages toothpaste showed the greatest inhibitory action. This may be due this toothpaste containing other components such as benzyl alcohol, which is an antimicrobial preservative effective against Gram positive bacteria and some fungi.[27]

It also contains thymol, which is a flavoring substance derived from thyme, which has antimicrobial and fungicidal properties and is used in some dental rinses and toothpastes.[28] *Streptococcus mutans* is a facultative anaerobic microorganism and its virulence is related to its ability to metabolize large amounts of sugars.

It also produces lactic acid and lives in an acidic environment conditioned by it. It has a high capacity of adherence to enamel walls due to the synthesis of glucans and fructans from glucosyltransferase. *Streptococcus mutans* has a spherical, ovoid round shape (cocci), grows in chains or in pairs, and does not present mobility nor form spores.[29]

It should be noted that the statistical analysis found that Denture Kids has an inhibitory action 94.1% similar to Oral B Stages. They are the only toothpastes that

presented statistically comparable inhibitory action in the experiment. They could be recommended to parents for the oral health care of their children, although achieving good oral health does not only depend on choosing the right toothpaste but also on other relevant factors.

The differences shown between toothpastes regarding their inhibitory efficacy against *Streptococcus mutans* could be related to the addition of extra components that could have synergy with fluoride, the antibacterial and anticariogenic active ingredient present in all toothpastes, in the form of sodium or sodium monofluorophosphate.

In Peru, most *in vitro* studies related to the inhibition of *Streptococcus mutans* are carried out with homemade toothpastes, which are not yet commercialized, so it becomes relevant to study the products available in the market. A common question by parents is "Which toothpaste is the best?", and the answer must be based on scientific evidence. For many parents it probably does not matter which brands are used for oral hygiene, as all they perceive all toothpastes to be good as long as the brushing technique and the frequency of the brushing are adequate.

However, it is known that tooth decay is a multifactorial disease and more efficient measures are needed to help prevent the onset of this disease. Therefore it is important to choose the toothpastes that best inhibit pathogens such as *Streptococcus mutans* or those whose inhibitory effects last longer in the mouth.

The present study does not define which toothpaste is the best due to the natural limitations of an *in vitro* study. For drawing such conclusion, it is necessary to study other factors such as their effectiveness preventing biofilm formation, how they counteract acid salivary pH, or the antimicrobial inhibitory action against other cariogenic microorganisms.

CONCLUSION.

The *in vitro* antimicrobial inhibitory action that pediatric toothpastes have against *Streptococcus mutans* ATCC 25175 has been demonstrated. Oral B Stages, Colgate Smiles and Denture Kids are the toothpastes that inhibited this microorganism most effectively.

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