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Antimicrobial Activity of Children's Toothpaste on the Bacteria Causing Dental Caries

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ABSTRACT

Background and Aim: Early childhood caries is one of the most common chronic diseases in children, affecting both oral and general health. Oral microorganisms are the most important causative agents associated with dental caries in children. The aim of this study was to compare the antimicrobial activity of common Iranian and non-Iranian children's toothpaste on the growth of four standard bacteria strains, including *Streptococcus mutans*, *Streptococcus sanguinis*, *Lactobacillus acidophilus*, and *Enterococcus faecalis*.

Materials and Methods: In this study, six types of the most common Iranian and non-Iranian children toothpaste produced by different companies were prepared. Different concentrations of toothpaste were prepared according to the Clinical and Laboratory Standards Institute (CLSI) standard. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of Iranian and non-Iranian children's toothpaste were measured by the microbroth dilution method at ten different concentrations.

Results: For the *S. mutans* bacteria, the lowest MIC was found in Misswake, Vi-One, and 2080 toothpaste. In the case of *S. sanguinis* and *L. acidophilus* bacteria, the lowest MIC was related to Frice toothpaste, and for *E. faecalis* bacteria, the lowest MICs were found for Misswake and 2080 toothpaste. Mann-Whitney U test also revealed that the inhibitory and bactericidal activities of Iranian children's toothpaste on the studied bacteria were not significantly different from those of non-Iranian children's toothpaste.

Conclusion: In general, the antimicrobial activity of Iranian children's toothpaste was higher than non-that of Iranian samples. In addition, the MIC of 2080 and Frice toothpaste in the four bacteria examined was lower than in other used toothpaste. To prevent early tooth decay in children use of these two kinds of toothpaste is recommended.

Keywords: Bacteria, Children Toothpaste, Early Childhood Caries, *Enterococcus,* Lactobacillus, *Streptococcus*

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1. Introduction

Early childhood caries (ECC) has become the most common oral disease in children over the past few decades, impacting children's health, quality of life, and even psychological status **(1)**. The American Dental Association identified ECC as the occurrence of one or more decays (non-cavitated or cavitated lesions), loss of teeth due to caries or filled tooth surfaces in any deciduous tooth from birth to 71 months in preschool age **(2)**. Although ECC prevalence varies across populations, being more prevalent in children living in socially disadvantaged communities **(3, 4)**. Many factors, including genetic, dietary, and environmental affect ECC **(1, 5)**. Some studies have shown that sugar consumption, breastfeeding period, brushing frequency, fluoride use, body mass index of children, and even the smoking history of parents are all potential risk factors for ECC **(1)**.

Many oral bacteria, such as *Streptococcus mutans* (*S. mutans*), are implicated in the etiology of ECC **(6)**. These bacteria have been involved in the initiation and progression of ECC as the most significant bacteria. They show various virulent traits making the plaque or biofilm cariogenic **(7, 8)**. Since biofilms are caused by plaque-related microorganisms, they appear to be localized. The best preventive measure known to be necessary for eliminating plaque biofilm is brushing with an appropriate toothpaste **(7, 9)**. As a result, toothpaste provides chemical adjuncts with an ideal vehicle. To create a direct inhibitory effect on plaque formation, a wide range of chemicals, primarily antimicrobial agents, have been added to toothpaste **(10, 11)**.

Triclosan in toothpaste has extensive antimicrobial activity against gram-negative and gram-positive bacteria plus anaerobic bacteria associated with dental plaque, thus lowering the number of oral bacteria and reducing plaque formation **(12, 13)**. Sodium lauryl sulfate is another ingredient in toothpaste that is claimed to play an effective role in preventing microbial plaque formation **(14)**. Various studies have been performed on the effect of toothpaste on oral bacteria. Research in Iran compared the antimicrobial activity of two Iranian kinds of toothpaste (Pooneh and Nasim) against Crest Regular. It confirmed no significant difference between the two kinds of toothpaste in antimicrobial activity **(15)**.

In comparison, another study showed negligible antimicrobial activity of Bath and Pooneh III toothpaste (Iranian- kinds of toothpaste) compared to crest cavity toothpaste **(16)**. Carvalho *et al*. tested the antimicrobial activity of six children's toothpaste in Brazil. Three fluoride-free kinds of toothpaste were inspected, including cashew-based, mango-based, and fluoride-free. With these experimental children's toothpaste, they compared two commercially fluoride-free and fluoridated toothpaste. Their investigation found that the fluoride-free toothpaste had antimicrobial activity against *S. mutans* and *Lactobacillus acidophilus* (*A. acidophilus*) **(17)**. Composite toothpaste containing 2% arginine and fluoride had significantly better antimicrobial activity against *S. mutans* and *Streptococcus sanguis* (*S. sanguis*) than toothpaste without arginine fluoride **(18)**. Another study revealed that toothpaste containing triclosan had better antimicrobial activity against *S. mutans* and *E. coli* compared to toothpaste without triclosan **(19)**.

There is little information available on the antimicrobial activity of Iranian children's toothpaste. There is no study either comparing the antimicrobial activity of Iranian children's toothpaste with non-Iranian children's toothpaste. Based on these considerations, we conducted an in vitro study to assess the efficacy of antimicrobial activity of six common Iranian and non-Iranian children's toothpaste against bacteria commonly found in the oral cavity and causing dental caries.

2. Materials and Methods

An experimental study was performed on the antimicrobial activity of six types of most common Iranian and non-Iranian children's toothpaste on four oral microflora that can potentially cause dental caries. This study was performed in 2020 in the microbiology laboratory of Kashan University of Medical Sciences. Six types of children's toothpaste were purchased from the local market of Kashan, Iran. In this study, Pooneh, Misswake, Frice, and Vi-one were Iranian children's toothpaste, while 2080 (Korea) and Colgate (United States) were the non-Iranian children's toothpaste.

According to CLSI standards protocol, various dilutions of toothpastes were prepared via microdilution broth method for determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Mueller-Hinton broth was used as a diluent to prepare different concentrations of toothpaste. Ten different dilutions of toothpaste were prepared as follows: 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.62 µg/mL, 7.81 µg/mL, 3.9 µg/mL, 1.95 µg/mL, 0.97 µg/mL, 0.48 µg/mL.

S. mutans (PTCC 1683), *S. sanguinis* (PTCC1449), *L. acidophilus* (PTCC1643) and *E. faecalis* (PTCC 1778) were obtained from the Persian Type Culture Collection (PTCC). The microorganisms were transferred into a tube containing Brain Heart Infusion (BHI) broth.

Once the tubes had been incubated for 24 hours at 37° C, bacterial colonies were subcultured in blood agar and incubated for 18 to 24 hours. To prepare the bacterial suspension, a bacterial colony was collected from the blood agar with a sterile loop and transferred to a tube containing normal saline.

After uniformity of the bacterial colony in normal saline, the bacterial suspension turbidity was optimized by comparing the turbidity to the 0.5 McFarland standard. The 0.5 McFarland Standard is equivalent to 1.5×10^8 CFU / mL **(20, 21)**.

To determine the MIC via microdilution broth method according to CLSI standards, the final dilution of the bacterial suspension should be 5 $\times 10^5$ CFU/mL, which is very important to consider **(22)**.

Then, 100 λ from Muller-Hinton broth was transferred in microplates with 96 wells. Next, 100 µL of ten different dilutions of kinds of toothpaste previously described was added into wells. Then, 5 µl of hemolyzed horse blood (LHB) (5%) was added to the wells. Thereafter, 10 µL of the bacterial suspension with a final dilution of 5 $\times 10^5$ CFU / mL was inoculated into the wells.

Muller-Hinton broth containing hemolyzed horse blood and bacterial suspension without toothpaste was used as negative control and positive control, respectively.

The antimicrobial activity for each toothpaste was repeated in triplicate on each bacterium. Then, microplates were placed inside a jar with the candle, the candle was lightened, and the lid of the jar was tightened; this is necessary for the growth of the standard strain used in this study, as these bacteria are capnophiles, or in other words, they need CO_2 to grow. The jar containing microplates was incubated at 35±2°C for 18 to 20 hours. The lowest concentration of kinds of toothpaste in which no growth was observed was considered the MIC **(22)**. Note that the grown bacteria can use LHB and the environment inside the well, thereby changing color from red to green.

After the MIC determination, to determine the MBC or lowest concentration of toothpaste required to kill the microorganisms, 10 µL aliquot from each of the microplate wells clear (which bacteria did not grow in it), was subcultured on blood agar medium. The culture medium was incubated for 18-20 hours at 37°C. Then, we examined the culture medium for bacterial growth.

SPSS software version 19 was used for statistical analysis using paired sample t-test, Mann-Whitney U test, One-way analysis of variance (ANOVA), and Kruskal-Wallis test, as appropriate, with the alpha value set at 0.05.

3. Results

In this study, we evaluated MIC and MBC of six types of toothpaste on four types of bacteria (*E. faecalis, L. acidophilus, S. sanguinis*, and *S. mutans*). Table 1 reports the MIC and MBC of all kinds of toothpaste on four bacteria.

As seen from Table 1, in *S. mutans* strain, MIC and MBC of Vi-one, Misswake, and 2080 kinds of toothpaste was 15.62 µg/mL, which is lower than that of the other three kinds of toothpaste. Considering *S. sanguinis* and *L. acidophillus*, the inhibitory and bactericidal activities of Frice toothpaste were greater than those of other kinds of toothpaste. In *E. faecalis* strain, MIC and MBC of Misswake and 2080 kinds of toothpaste was 15.62 µg/mL, which was lower than other kinds of toothpaste.

The results of the Kruskal-Wallis test also showed that although the MIC and MBC of Misswake and 2080 children's toothpaste on the studied bacteria were greater than those of other kinds of toothpaste, this difference was not significant (P>0.05). In addition, the results of the Mann-Whitney U test showed that MIC and MBC of Iranian children's toothpaste on the studied bacteria were not significantly different from those of the non-Iranian children's toothpaste (P> 0.05).

Table 1. MIC and MBC values of six types of children's toothpaste on four bacteria strains.

Children's toothpaste		*S. mutans*		*S. sanguinis*		*L. acidophillus*		*E. faecalis*	
		MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL
Misswake		15.62	15.62	31.25	31.25	250	250	15.62	15.62
Frice	Iranian	31.25	31.25	15.62	15.62	31.25	31.25	62.5	62.5
Vi-on	Iranian	15.62	15.62	62.5	62.5	125	125	250	250
Pooneh	Iranian	62.5	62.5	125	125	125	125	62.5	31.25
2080	non-Iranian	15.62	15.62	31.25	31.25	62.5	62.5	15.62	15.62
Colgate	non-Iranian	62.5	62.5	62.5	125	250	250>	125	250

4. Discussion

In this study, we investigated the antimicrobial activity of Iranian and non-Iranian children's toothpaste via the microbroth dilution method at ten different concentrations on four bacterial standard strains causing dental caries. Limited studies have compared Iranian and non-Iranian toothpastes, especially in the case of children's toothpaste. Most studies have not performed accurate laboratory comparisons of these kinds of toothpaste in terms of MIC and MBC.

Ghapanchi *et al*. measured non-Iranian kinds of toothpaste in terms of their antimicrobial activity on *S. mutans*. In this study, no difference was found between 2080 and Colgate toothpaste. That study had not examined the MBC and MIC **(23)**. However, our study evaluated MIC and MBC of 2080 children's toothpaste, which was found to be far lower than Colgate children's toothpaste values. It can be stated that 2080 children's toothpaste has better inhibitory and bactericidal activities on *S. mutans*. In another study by Kooshki *et al.* in 2018, an Iranian toothpaste was compared to a non-Iranian toothpaste (Crest). Finally, no significant difference was observed between the antimicrobial effects of these two kinds of toothpaste **(24)**. In this study, Iranian children's toothpaste had more effect on *S. mutans* and *L. acidophilus* than non-Iranian toothpaste, but this difference was insignificant **(24)**. However, in our study, the effect of Iranian children’s toothpaste on *S. mutans* and *L. acidophilus* was greater than the activity of non-Iranian children’s toothpaste. MIC and MBC of Iranian children's toothpaste were lower than those of non-Iranian children’s toothpaste on *S. mutans* and L. *acidophilus*. In another study by Sadeghi *et al*., they examined 12 types of Iranian toothpastes in vitro and their antimicrobial activities were compared with a non-Iranian toothpaste (Crest). In that study, it was observed that among Iranian kinds of toothpaste, Pooneh and Bass had higher antimicrobial activities on *S. mutans* and *S. sanguis* than the non-Iranian kinds of toothpaste **(16)**. However, in our study, non-Iranian children's toothpaste compared with Iranian children’s toothpaste showed better antibacterial activity on *S. sanguis*.

On the other hand, Iranian children’s toothpaste showed better antibacterial activities on *S. mutans* as compared with non-Iranian children's toothpaste. These results suggest that the effect of each children toothpaste on different bacteria is variable. This difference is due to specific structures and bacterial growth conditions.

However, the results do not concur with those of Mofid *et al*. study. In their in vivo study, Mofid *et al*. examined the effect of non-Iranian and Iranian kinds of toothpaste. They found the highest antimicrobial activity and inhibition of plaque formation were higher in non-Iranian kinds of toothpaste compared with Iranian kinds of toothpaste (Nasim and Pooneh), while our work was in vitro. Not those bacteria react differently to environmental conditions. In vivo conditions are different due to the multiplicity of bacteria compared to in vitro conditions, where bacteria are examined in isolation **(9, 25)**. This showed that both Iranian (Daroogar) and non-Iranian (Colgate) toothpaste were effective in inhibiting plaque formation. Still, the rate of plaque formation inhibition in Iranian toothpaste was slightly higher than in non-Iranian toothpaste **(26)**. These results are in line with our study.

Although Mofid *et al*. and Rashidian *et al*. study as well as other studies, have examined the antimicrobial activity based on plaque formation inhibition between non-Iranian and Iranian toothpaste, the MIC and MBC of toothpaste on bacteria have not been studied. Thus, a comparison of the present study with the mentioned study cannot be logical because of differences in the project subjects **(25, 26)**.

Fluoride is one of the most important compounds used in toothpaste to prevent tooth decay. Higher concentrations of fluoride in toothpaste present antimicrobial activity **(27, 28)**. According to a study by Wong *et al.*, only if the amount of fluoride in toothpaste reaches 1000 PPM or more can it significantly reduce the rate of tooth decay in children. This means that toothpaste with a fluoride content of less than 1000 PPM, would have no significant effect on reducing tooth decay **(29)**. Among the children’s toothpaste used in our study, the highest amount of fluoride was related to Frice children's toothpaste (Iranian children's toothpaste) with 1000 PPM fluoride. According to the results of our study, Frice children's toothpaste had more effects on *S. sanguis* and *L. acidophilus*. According to the mentioned results, it seems that the concentration of this substance is directly related to the inhibition of these two bacteria, but it has had no significant effect on *S. mutans* and *E. faecalis*. So antimicrobial activity of Iranian children's toothpaste is related to higher amount of fluoride.

The limitations of this study were the lack of evaluation of the antimicrobial activity of more kinds of toothpaste and other microorganisms which can affect ECC due to cost limitations and the difficult culture of all microorganisms that cause ECC in a laboratory.

5. Conclusion

In general, comparing non-Iranian and Iranian children's toothpaste, it was found that the antimicrobial activity of Iranian children's toothpaste on *S. sanguis* and *E. faecalis* was higher than that of non-Iranian children's toothpaste. This shows that Iranian children's toothpaste has a better influence on *S. sanguis* and *E. faecalis*. So, it is better to use Iranian children's toothpaste.

In other words, based on the results and statistical analyses, the antimicrobial activity 2080 and Frice kinds of children's toothpaste on the four studied bacteria strains was better than that of other children's toothpaste. Thus, it can be stated that 2080 and Frice children's toothpaste prevent ECC, and it is recommended to use these two children's toothpaste. Finally, to select between these two children's toothpaste, due to the high amount of fluoride in Frice children's toothpaste (1000 PPM), as a precaution and to prevent the harm of high fluoride in children, the use of children's toothpaste 2080 is preferable and recommended. Finally, in vivo studies are required to confirm the antimicrobial activity of Iranian children's toothpaste on microorganisms that cause ECC under the normal conditions of the oral environment and apply these in human clinical trials.

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Authors' Contribution

AT, AG, & HF designed and performed experiments and analyzed data. NA, MR & MA wrote the manuscript with support from HF, AT, NA, & AG.

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Conflict of Interest

The authors declared no conflict of interests.

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