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Research Article

In vitro Comparative Antimicrobial Efficacy of Mass and Oral B Mouth Washes against *Streptococcus salivarius*, *Streptococcus sobrinus*, and *Streptococcus sanguinis*

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Abstract

Objectives: This study aimed to compare the antimicrobial efficacy of Mass and non-fluoridated Oral B mouthwashes against *Streptococcus salivarius* (*S. salivarius*), *Streptococcus sobrinus* (*S. sobrinus*), and *Streptococcus sanguinis* (*S. sanguinis*).

Methodology: In this *in vitro*, experimental study, *S. salivarius, S. sobrinus*, and *S. sanguinis* were separately cultured on brain heart infusion (BHI) agar. The antimicrobial activity of Mass and Oral B Pro-Expert mouthwashes against the abovementioned microorganisms was evaluated by the agar well-diffusion test; 0.2% chlorhexidine (CHX) served as the positive control, and saline was used as the negative control. The diameter of the growth inhibition zones was measured. The minimum inhibitory concentration (MIC) of each mouthwash against each microorganism was determined by the micro broth dilution technique. The minimum bactericidal concentration (MBC) of the mouthwashes was also determined.

Results: The MIC of both mouthwashes was the same (2048/1) for *S. sobrinus.* The MIC of Mass for *S. sanguinis* (2048/1) and *S. salivarius* (1/512) was lower than that of Oral B (1/1024 and 1/256, respectively). The MBC of both mouthwashes was the same for all three microorganisms (1/256 for *S. sobrinus*, 1/1024 for *S. sanguinis*, and 1/256 for *S. salivarius*).

Conclusion: The antimicrobial efficacy of Mass was similar to that of Oral B Pro-Expert against *S. salivarius, S. sobrinus*, and *S. sanguinis*.

Keywords: *Streptococcus salivarius; Streptococcus sobrinus*; Mouthwashes

Introduction

The oral cavity harbors several microorganisms that comprise the normal oral flora. The normal oral flora has a wide variety of microorganisms with over 500 species [1]. Some of these microorganisms are responsible for development of dental caries, gingivitis, and periodontal disease [2]. Some others, such as *Candida albicans* (*C. albicans*), cause local oral or systemic infections only in cases with compromised immunity or presence of local predisposing factors such as smoking or denture [3].

Dental caries is the most common oral chronic infection, caused by colonization of oral microorganisms such as Streptococcus mutans (*S. mutans*) and *Lactobacillus spp*. [4]. Following colonization, the bacteria metabolize the carbohydrates, produce acids, demineralize the tooth structure, and cause incipient caries on tooth surfaces [3]. Factors such as the nutritional regimen, hygienic behaviors, socioeconomic factors, age, and race are implicated in caries development [5]. Orthodontic appliances can also enhance the accumulation of microbial plaque and bacterial proliferation and subsequent development of caries, enamel demineralization, and development of gingivitis and periodontitis [6].

Demand for orthodontic treatment in different communities is on the rise. Although orthodontic treatment has been introduced as a caries preventive approach, orthodontists are well familiar with oral health-related problems caused by placement of orthodontic appliances in the oral cavity. Fixed orthodontic appliances provide mechanical retention for the microbial plaque and due to their long-term presence in the oral cavity, they often result in enamel demineralization, and white spot lesions [7]. Removable applianc-

es also interfere with oral hygiene practice, and clasps and other components cause food impaction, and accumulation of microbial plaque, and can lead to caries development and periodontal disease [8]. Removable orthodontic appliances, compared with partial or complete dentures, have more sites for bacterial accumulation. Thus, plaque removal from the oral cavity and surface of orthodontic appliances is highly important to prevent recontamination of cleaned tooth surfaces. Inappropriate care and maintenance of appliances can have serious health consequences for the supporting tissue structures [9].

S. mutans and *Streptococcus Sobrinus* (*S. sobrinus*) from the group of mutans streptococci are the main culprits for caries development in humans. They are among the most common microorganisms in the oral flora, which are isolated from the dental plaque [10].

Streptococcus Sanguinis (*S. sanguinis*) is among the initiators of microbial plaque formation. This microorganism is present at the site earlier than other cariogenic bacteria. *S. sanguinis* adheres to tooth surfaces due to having Pil B and PilC proteins on its fimbria and their attachment to salivary alpha amylase. Accordingly, adhesion of other bacteria and biofilm formation on tooth surfaces are enhanced. *S. sanguinis* can remain active by hydrolysis of arginine even in absence of fermentable hydrocarbons [11].

Mouthwashes are often used to temporarily decrease the load of microorganisms involved in dental caries and gingival inflammation, eliminate the bad taste and mouth malodor, and refresh the oral cavity. They are used as an adjunct to mechanical plaque removal measures, i.e., toothbrushing and dental flossing, for removal of supragingival plaque and prevention/treatment of gingivitis. An optimal mouthwash must have antimicrobial activity, should not cause microbial resistance, and should not significantly eliminate the normal microbial flora of the mouth [12].

Iran Najo Pharmaceuticals is the manufacturer of Mass mouthwash and claims that this mouthwash has antimicrobial activity. Accordingly, this study aimed to compare the antimicrobial activity of Mass and Oral B mouthwashes against *S. salivarius*, *S. sobrinus*, and *S. sanguinis*.

Materials and Methods

This *in vitro*, experimental study was conducted on Mass (Iran Najo, Iran), and alcohol-free non-fluoridated Oral-B Pro-Expert (Proter and Gamble, USA) mouthwashes. The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1399.138)

Preparation of brain heart infusion (BHI) agar medium

BHI agar culture medium was prepared according to the manufacturer's instructions [13].

Preparation of BHI broth medium

Preparation of BHI broth was similar to that of BHI agar except that it was cooled and stored at 5°C in a refrigerator until use [13].

Preparation of bacterial suspension

Standard-strain *S. salivarius* (ATCC 9222), *S. sobrinus* (ATCC 27607), and *S. sanguinis* (ATCC 10556) were purchased from the Iranian Industrial Culture Collection Center and were separately streak-cultured on BHI agar and incubated at 37°C for 24 hours. Single colonies obtained from the culture on BHI agar were then transferred to BHI agar and homogenously mixed. The suspension was adjusted to 0.5 McFarland standard, containing 1.5 x 10^8 microorganisms by a spectrophotometer (with optical density of 0.08 to 0.1 at 600 nm) [13].

Assessment of antibacterial activity of the mouthwashes by the agar-well diffusion technique

S. salivarius, *S. sobrinus*, and *S. sanguinis* suspensions with 0.5 McFarland standard concentration were separately diluted 1:100 by using BHI culture medium. The obtained suspensions were lawn-cultured on the plates in sterile condition, under a class II laminar flow hood. A Pasteur pipette was then used to create four wells with 6 mm diameter at equal distances from each other. In this study, 0.2% chlorhexidine (CHX) served as the positive control and added to one well, and saline was used as negative control and added to another well. Mass and Oral-B mouthwashes (50 µL) were added to the third and fourth wells, respectively, each in. Testing was repeated in three plates for each microorganism. The plates were then incubated at 37°C. However, the culture conditions were different for *S. sanguinis*, and it required 5% CO2. Thus, the plates were placed in a candle jar. After the incubation period, the diame-

ter of the growth inhibition zones was measured by a ruler (Figure 1). The mean of three measurements was calculated and recorded. All procedures were performed according to the Clinical and Laboratory Standards Institute (CLSI) [13].

Determination of minimum inhibitory concentration (MIC)

Assessment of MIC of mouthwashes was done by the broth microdilution test. In this method, 100 µL of BHI broth was added to all wells. Next, 100 µL of the mouthwash was added to the first well, and after complete mixing, 100 µL of the contents of this well was transferred to the second well. This process was repeated until the 12th well for serial dilution of the mouthwash. Finally, 100 µL of the contents of the 12th well was discarded. Accordingly, ½, ¼, 1/8, etc. concentrations of each mouthwash were prepared. Serial dilution of each mouthwash was performed in three rows. Next, 10 µL of microbial suspension containing 10^7 CFUs/mL was added to each well. Another row received bacterial suspension without mouthwash to serve as the positive control. One row included the culture medium and mouthwash without the bacteria and served as the negative control. This test was separately performed for each microorganism.

All steps of the procedure were performed according to the CLSI guidelines [13].

Next, the micro-titration plate was incubated at 37°C for 24 hours, and then the wells were evaluated in terms of turbidity (indicative of bacterial proliferation) or clarity (no bacterial growth). To increase accuracy, 20 µL of 0.01% Resazurin dye was added to all wells, and the plates were incubated again at 37°C for 2 hours. Color change would indicate bacterial growth. The last dilution of the mouthwash that was still blue and showed no color change to pink was considered as MIC of the respective mouthwash, which was separately recorded for each microorganism type [13].

Determination of minimum bactericidal concentration (MBC)

Samples were collected from the wells that showed bacterial growth inhibition in the MIC test and cultured on BHI agar. To increase accuracy, we collected samples from all wells for subculture on BHI agar. All phases of the test were conducted in accordance with the CLSI guidelines [13]. The dilution coefficients were recorded for each mouthwash against each microorganism.

Results

Diameter of growth inhibition zones

The negative control caused no growth inhibition zone while the positive control showed growth inhibition zones in all plates.

Growth inhibition zones were noted in assessment of the antibacterial activity of the two mouthwashes against the three types of microorganisms (Table 1). Mass mouthwash created larger growth inhibition zones in all three bacterial cultures compared with Oral-B.

Group	Diameter of growth inhibition zone in plate 1	Diameter of growth inhibition zone in plate 2	Diameter of growth inhibition zone in plate 3	Mean
Saline	0	0	0	0
0.2% CHX	29	29	29	29
Mass	31	27	31	29.6
Oral-B	16	21	22	19.6

Table 1: Diameter of growth inhibition zones of *S. salivarius* around the two mouthwashes and positive and negative controls.

Table 2 presents the diameter of growth inhibition zones of *S. sobrinus* around the two mouthwashes and positive and negative controls. As shown, 2-unit difference was noted in the diameter of growth inhibition zones around the two mouthwashes, and the growth inhibition zone was larger around the Mass mouthwash. Also, the mean diameter of growth inhibition zone around Oral-B and Mass mouthwashes was smaller than that around CHX. CHX (0.2%) caused the largest and Oral-B caused the smallest growth inhibition zone in *S. sobrinus* culture.

Table 3 presents the diameter of growth inhibition zones of *S. sanguinis* around the two mouthwashes and positive and negative controls. As shown, 9-unit difference was noted in the diameter of growth inhibition zones around the two mouthwashes, and the growth inhibition zone was larger around the Mass mouthwash. Also, the mean diameter of growth inhibition zone around Oral-B and Mass mouthwashes was smaller than that around CHX. CHX (0.2%) caused the largest and Oral-B caused the smallest growth inhibition zone in *S. sanguinis* culture.

Group	Diameter of growth inhibition zone in plate 1	Diameter of growth inhibition zone in plate 2	Diameter of growth inhibition zone in plate 3	Mean
Saline	0	0	0	0
0.2% CHX	28	27	27	27.3
Mass	22	20	23	21.6
Oral-B	18	19	22	19.6

Table 2: Diameter of growth inhibition zones of *S. sobrinus* around the two mouthwashes and positive and negative controls.

Group	Diameter of growth inhibition zone in plate 1	Diameter of growth inhibition zone in plate 2	Diameter of growth inhibition zone in plate 3	Mean
Saline	0	0	0	0
0.2% CHX	35	35	36	35.3
Mass	27	27	27	27
Oral-B	23	20	22	21.6

Table 3: Diameter of growth inhibition zones (mm) of *S. sanguinis* around the two mouthwashes and positive and negative controls.

MIC and MBC

Table 4 presents the MIC and MBC values of the two mouthwashes against the three types of microorganisms. The MIC and MBC of Mass and Oral-B mouthwashes against *S. sobrinus* were the same (with dilution coefficients of 1/2048 and 1/256, respectively). Assessment of MIC of the two mouthwashes against *S. sanguinis* revealed that Mass (MIC = 1/2048) had a greater MIC than Oral-B mouthwash. However, the MBC of the two mouthwashes was the same against *S. sanguinis* (MBC = 1/1024). Assessment of MIC of mouthwashes against *S. salivarius* showed that the MIC of Mass against *S. salivarius* (dilution coefficient of 1/512) was greater than that of Oral-B (1/256). However, the MBC of both mouthwashes was the same (1/256) against *S. salivarius*.

Microorganism type	Test	Mouthwash	Dilution coefficient
S. sobrinus	MIC	Mass	2048/1
		Oral-B	2048/1
	MBC	Mass	256/1
		Oral-B	256/1
S. sanguinis	MIC	Mass	2048/1
		Oral-B	1024/1
	MBC	Mass	1024/1
		Oral-B	1024/1
S. salivarius	MIC	Mass	512/1
		Oral-B	256/1
	MBC	Mass	256/1
		Oral-B	256/1

Table 4: MIC and MBC values of the two mouthwashes against the three types of microorganisms.

Discussion

This study compared the antimicrobial activity of Mass and Oral B mouthwashes against *S. salivarius*, *S. sobrinus*, and *S. sanguinis*. The results showed that the MIC of both mouthwashes was the same (2048/1) for *S. sobrinus*. The MIC of Mass for *S. sanguinis* (2048/1) and *S. salivarius* (1/512) was lower than that of Oral B (1/1024 and 1/256, respectively). The MBC of both mouthwashes was the same for all three microorganisms (1/256 for *S. sobrinus*, 1/1024 for *S. sanguinis*), and 1/256 for *S. salivarius*). CHX was used as the positive control in this study, since it is the gold-standard antimicrobial mouthwash [14]. The Mass mouthwash contains mint essence, menthol, and cetylpyridinium chloride, and is devoid of alcohol. Oral-B Pro-Expert alcohol-free mouthwash that was evaluated in this study is composed of methyl paraben, propyl paraben, and cetylpyridinium chloride. According to the present results, the Mass mouthwash appears to have an antibacterial efficacy comparable or even superior to that of Oral-B against *S. salivarius*, *S. sobrinus*, and *S. sanguinis*.

To the best of the authors' knowledge, no similar study is available regarding the effect of the tested mouthwashes on the aforementioned microorganisms to compare our results with, and this study appears to be the first to address this topic.

Salehi., *et al.* [15] evaluated the antimicrobial effects of CHX and Persica mouthwash on *S. mutans* in orthodontic patients and reported that Persica mouthwash was effective in reduction of *S. mutans* count, but its antimicrobial efficacy was inferior to that of CHX.

In the present study, the mean diameter of growth inhibition zone around the Mass mouthwash was larger than that around Oral-B and also CHX in *S. salivarius* culture, which is a promising finding, and can pave the way for further investigations and clinical trials on its efficacy. This was an interesting finding since many studies have reported that CHX has the highest antimicrobial activity compared with other mouthwashes; although assessment of growth inhibition zone diameter is not sufficient for the comparison of antibacterial activity [16,17].

Regarding *S. sobrinus*, CHX caused the largest growth inhibition zone, followed by Mass and Oral-B mouthwashes in the present study.

Comparison of the antibacterial effects of Mass and Oral-B mouthwashes on *S. sanguinis* revealed that the antibacterial activity of the Mass mouthwash against this microorganism was lower than that of CHX but higher than that of Oral-B. This finding was in line with the results of Hashemipour., *et al,* [18] who showed that CHX had the highest antibacterial activity.

The present results revealed that the Mass mouthwash inhibited the proliferation of *S. sanguinis* and *S. salivarius* at a lower concentration than Oral-B mouthwash; however, it was not the case for *S. sobrinus* since both mouthwashes were equally effective against it. Ghapanchi., *et al.* [19] showed that Oral-B mouthwash inhibited the bacteria at higher dilutions. Thus, the present results regarding the Mass mouthwash are promising, and call for further investigations to confirm the present findings. Biria., *et al*. [20] found no significant difference between Oral-B and Mass mouthwashes regarding their antimicrobial activity against *S. mutans* and C. albicans. Their results are in line with the present findings although they evaluated different microorganisms.

This study was the first to compare the antibacterial activity of Mass and Oral-B mouthwashes against *S. sobrinus*, *S. sanguinis*, and *S. salivarius*, which was a strength of this study.

This study had an *in vitro* design, which is different from the clinical setting due to absence of saliva and many other factors. Thus, clinical trials are required to confirm the present results. Also, future studies are required on the antimicrobial activity of the Mass mouthwash against other oral bacteria and fungi.

Conclusion

Mass mouthwash had similar effectiveness against *S. salivarius*, *S. sobrinus*, and *S. sanguinis*. as Oral-B mouthwash. Because of this similarity, Mass mouthwash can be used to decrease the population of main pathogens in the oral cavity.

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