

Research Article

Comparing Microbial Colonization and Types of Microorganisms between Oral-B and G.U.M Toothbrushes-A Pilot Study

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Abstract

Background: The manual toothbrush is the most commonly used method of oral hygiene and removing microbial plaque. An important issue is that toothbrushes become contaminated in daily use by various microorganisms and have the potential of being colonized by potential pathogens. There are numerous brands of toothbrushes and patients frequently ask dentists or dental hygienists what brand they recommend. This study compared the microbial colonization and types of microorganisms on two brands of toothbrushes commonly used in Iran.

Methods: This pilot study investigated 20 students (10 boys and 10 girls) using soft and medium types of Oral-B and G.U.M toothbrushes. Students were randomly divided into four groups and each group (N=5) used one type of toothbrush for one week. They were instructed to brush twice daily using the Modified Bass method. Following brushing, the brush was rinsed with running tap water for 30 seconds and stored in the open air outside of the bathroom. The used toothbrushes were collected at the end of each week and each group was randomly provided with another type of toothbrush. This procedure was repeated for three additional weeks. The used toothbrushes were transported in sterile test tubes to the laboratory for culturing. Microorganism colonization and morphology were evaluated after 24 and 48 hours. The data were analyzed by SPSS version 18 and the significant level was considered 0.05.

Results: Oral-B and G.U.M toothbrushes were heavily contaminated by various microorganisms but the difference in colonization between them was not statically significant (P=0.272). Also, the microbial colonization was not statically different between soft and medium size of bristles (P=0.378).

Conclusion:

- The results of the present study indicated that both Oral-B and G.U.M toothbrushes with soft and medium bristles are contaminated with bacteria, especially staphylococcus, *E. Coli* and streptococci .
- Contaminated toothbrushes may become a reservoir for potential pathogens.
- Drying the toothbrushes in free air (outside the bathroom), using no cover for the toothbrushes, using tooth paste did not prevent the microbial contamination of toothbrushes in healthy young individuals.
- This pilot study should be followed-up by one that includes a larger number of participants and with a greater in-depth identification of the bacterial colonization.

Keywords: Toothbrush; Microbial colony count; Contamination; *Microbiota*

Introduction

Oral hygiene is of great importance. Poor oral hygiene is not only associated with oral diseases, such as dental caries and periodontal diseases, but is also associated with systemic inflammatory and infectious diseases, such as pneumonia and respiratory tract infections [1], rheumatoid arthritis [2], endocarditis [3], acute myocardial infarction [4], and cancers of oral cavity and other parts of the body [5,6].

The oral cavity is colonized with more than 700 species including *eubacteria*, *archae bacteria*, and fungi. Since birth, the microbiota of the oral cavity changes to a variety of microorganisms upon eruption of teeth [7,8]. Dental caries and periodontal diseases originate from bacteria found in plaque biofilms. The microorganisms of the oral cavity are factors attributed to a higher risk of oral cancers and inflammatory/infectious diseases in patients with poor oral hygiene [9-11].

Although conventional methods used to control dental plaque include tooth brushing and inter dental cleaning aids [12], these have been associated with transient dental bacteremia in several studies [13-15]. However, other studies have not found an associated bacteremia after tooth brushing in healthy subjects [16].

Various studies have isolated microorganisms from used toothbrushes [17]. Multiple factors have been attributed to the microbial contamination of toothbrushes, including the type of toothbrush head (soft, medium, or hard), the place of storing the toothbrush (inside the bathroom) [18], and the handle design (grooved vs. smooth) [19]. Studies have suggested various disinfection methods for toothbrushes that most people cannot or do not use [20,21]. Even the recommendation on changing the toothbrush once every three months is only effective on its cleansing power, not the microbial contamination that has been reported to occur [18].

The two most commonly used brands of toothbrushes in Iran are Oral-B and G.U.M and dentists and are often asked by patients what toothbrush they should use. In this study, we aimed to compare the microbial colonization and types of microorganisms between these two commonly used toothbrushes.

Materials and Methods

Study design

This study was performed on 20 students (10 girls and 10 boys) from Rafsanjan University of Medical Sciences (ages 22-29 years) over four weeks. All participants were randomly selected from students living in dormitories. After describing the study and its objectives, the students were asked if they were willing to participate. The students who agreed to participate were signed a written informed consent. This study was approved by Ethics Committee of Rafsanjan University of Medical Sciences (IR.Rums.REC.1395.71). Participants were free to leave the study if they wished to do so. No costs were imposed on participants as the toothbrushes and toothpaste were given free to participants. Data

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was analyzed anonymously and participants' information was kept confidential.

All participants were examined by a dental student researcher to evaluate their eligibility for inclusion in the study. The inclusion criteria consisted of having good general health, no current dental caries, no gingivitis, no history of periodontal diseases, not having any fixed orthodontics appliances, and no current throat infection. Before delivering the toothbrush, the participants were instructed to brush their teeth twice daily (morning and night) for one minute each time with the Modified Bass technique. The toothbrush was to be rinsed under running tap water for 30 seconds and stored without cover to dry outside the bathroom.

The studied toothbrushes included two manual toothbrushes: G.U.M Activital (Sunstar, Schaumburg, U.S.A.) with soft and medium bristles, and Oral-B Proexpert All in One (Braun, Newbridge, Ireland) with soft and medium bristle. Unopened examples of the toothbrushes were cultured before the study and assessed for microbial presence. The results were all negative. The same type of toothpaste (Nasim tooth paste, Goltash, Isfahan, Iran) was provided to all participants to be used to brush the teeth during the study period.

The eligible participants were randomized by SPSS software into four groups and each group consisted of 5 participants. Four types of toothbrushes (G.U.M soft, G.U.M medium, Oral-B soft, and Oral-B medium) were coded as A, B, C, or D. One type of toothbrush was given to each participant in a group. They were instructed to use that tooth brush for one week as directed. After one week, the researcher returned and collected the used toothbrushes. Each participant was given another type of toothbrush for use the following week. This sequence continued for four weeks until all participants used all four types of toothbrushes.

Laboratory analysis

the used toothbrushes were collected in sterile Pyrex tubes with a plastic lid. At the end of each week, students brushed their teeth for the last time and delivered their toothbrush to the researcher in the Pyrex tube provided the day before collection. The researcher transferred the coded toothbrushes to Ali-ibn-Abi-Taleb Hospital in Rafsanjan, Iran, where the tube lid was removed and the samples were kept at room temperature for six hours. Then, 1 mL sterile physiological serum was added to the tubes by sterile pipet (HBG, Giessene, Germany) and rotated 20times to mix. A total of 0.01 mL of the liquid was taken using a calibrated loop (HBG) with a 4 mm diameter and applied linearly on disposable plates (PadtanTeb, Tehran, Iran), including blood agarcultivars of Merck KgaA (Darmstadt, Germany), eosin methylene blue agar (orMerck EMB, and Subro Dextrose Agar (Merck KgaA) and cultured for identification of Gram-positive bacteria, gram-negative bacteria and fungi.

The coded cultures were placed in an incubator (Gallenkamp, Munchen, Germany) at 37oC for 24 and 48 hours before assessing for

the number and characteristics of the colonies. Any culture broken, cracked, damaged or opened anyway before recording at the appropriate time was excluded from the study.

A trained microbiology technician read the culture media and counted the number of colonies on each plate. This number was multiplied by 100 to report the colonization. The amount of contamination was divided into three groups according to the number of colonies per culture medium: low contamination for colonies < 10,000 Colony Forming Unit (CFU), moderate for 10,000-30,000 CFU, and high for >30,000 CFUs, and no growth was considered negative. The type of microorganisms was determined by morphology of colonies, Gram stain, and specific diagnostic tests. Catalase test was used to differentiate Staphylococci and Streptococci, gram positive cocci, while Enterococcus colonies were detected by bile Esculin Agar (BEA) method. *Escherichia coli*, *Klebsiella*, *Proteus*, and *Enterobacter*, Gram negative bacilli, were differentiated by TSI (Triple Sugar Iron), Simmon citrate, and SIM (Sulfide Indole Motility). *Pseudomonas aeruginosa* was detected by a jasmine-like smell and a positive oxidative test. Was differentiated from yeast by using a gland tube viewed under light microscope. All results were recorded and compared between the groups.

Statistical analysis

The collected data were input into SPSS software version 18 for statistical analysis. Results were presented as mean and Standard Error (SE) for quantitative variables and frequency (percentage) for categorical variables. Frequency and type of colonization were compared using chi-square test or Fisher's exact test and numeric variables (number of colonies) were compared using one way ANOVA. P values of 0.05 or less were considered statistically significant.

Results

Ten men and 10 women participated in this study with mean and SD age of 23.10±1.12 (range of 22-24) years. The results of the cultures were classified into three negative, low, and high contamination levels. The results of the 24 and 48 hours culture were the same in all samples. We have only reported the 24 hours culture for all samples, except two cases with 100 CFU in the first culture that reached 5000 CFU in the second culture, and the other that was 500 in the first culture and reached 1100 in the second culture. However, these changes in values had no significant effect, as both samples were classified in the same category.

The results of 24 hours culture showed the highest mean colony count in Oral-B soft toothbrush and the lowest in Oral-B medium toothbrush, but there was no statistically significant difference between them (P=0.515) (Table 1). One way ANOVA also showed no statistically significant difference in mean colony count according to the bristle type between male and female patients (P=0.297, and 0.677), respectively. The results of colony count based on study week showed the highest mean colony count in the third week and the lowest in the second week, as shown in Table 1.

	Minimum contamination, CFU*	Maximum contamination, CFU*	Mean ± SE	P-value†	
Type of toothbrush	Oral-B soft	0	100,000	54650 ± 11088	0.515
	Oral-B medium	0	150,000	33210± 11533	
	G.U.M soft	0	100,000	36705 ± 10276	
	G.U.M medium	0	100,000	38180 ± 10577	
Study week	First week	0	150,000	43850 ± 11946	0.307
	Second week	0	100,000	26510± 9766	
	Third week	0	100,000	55005 ± 10837	
	Fourth week	0	100,000	37380 ± 10479	

*colony forming unit

† The results of one Way ANOVA test

Table 1: Mean, SE and range of colony count grown in 24 hours based on the type of toothbrush and week(n=20).

The comparison of colony growth in the 24 hours culture in different brands of toothbrushes showed no significant difference between the Oral-B and GUM brands (P=0.426) (Table 2). The results of Fisher's exact test on this comparison showed no significant difference between toothbrush brands in women (P=0.299) or men (P=0.927) and none between women and men (P=0.307).

To investigate the impact of bristle size, we compared the frequency of microbial contamination in the first 24 hours after culture between Oral-B and G.U.M toothbrushes. The results using the chi-square test showed no statistically significant difference (P=0.272) in total or the first week (P=0.735), the second week (P=0.632), the third week (P=0.168), and the fourth week (P=0.332). When comparing the effect of bristle sizes, regardless of brand of toothbrush (chi-square test) there was no statistically significant difference between soft and medium size bristles (P=0.378) in total or the first week (P=0.289), the second week (P=0.632), the third week (P=0.700), and the fourth week (P=0.077).

The number of microbial colonies in the first 24 hours after culturing the toothbrushes in each of the groups A, B, C and D showed no statistically significant difference. Table 2. Frequency and type of microorganisms grown in the first 24 hours after culturing the toothbrushes is shown in Table 3. *Staphylococcus* (37.5%), *E.coli* (21.3%), and *streptococci* (10%) were the most frequent. Multiple microorganism contaminations were observed in 22.5% of the toothbrushes sampled.

The most frequent type of microorganisms according to the type of toothbrush was as follows: Oral-B soft: *E.coli* (37.5%), *staphylococcus* (31.2%), *Klebsiella*, and *candida*.

Oral-B medium: *Staphylococcus* (58.3%), *streptococci* (25%), *E.coli*, and *Klebsiella*.

G.U.M soft: *staphylococcus* (64.7%), *E.coli* (29.4%), *streptococci*, and *Proteus*.

G.U.M medium: *staphylococcus* (46.6%), *E.coli* (26.6%), *Enterococcus*, and gram positive bacilli. Further analysis showed no statistically significant difference in the type of microorganisms among bristles (P=0.256).

Discussion

Oral hygiene is an important factor in general health and routine mechanical cleaning using a toothbrush is the most commonly used method to clean and reduce dental plaque, but evidence suggests that toothbrushes will become contaminated with several types of microorganisms. As the microbiota of the oral cavity varies based on the patients' race [16,22], we studied the microbial contamination of two commonly used toothbrushes in a young and healthy Iranian population.

The results of this study indicated that both studied brands (Oral-B and G.U.M) showed bacterial growth after one week, with no difference between the brands or bristle sizes. These two brands were selected as they are the most commonly used in Iran. This finding has been confirmed by other Iranian studies, although the place of storing the toothbrush (toilet or bathroom, washing and drying method, and use of cover after tooth brushing) had relationship with CFU [23,24]. In the present study, in order to reduce the effect of other factors, we trained

Groups	Contamination levels	Oral-B soft	Oral-B medium	G.U.M soft	G.U.M medium	Total	P-value*
		No (%)	No (%)	No (%)	No (%)		
A	Negative	0	1 (20)	1 (20)	3 (60)	5 (25)	0.288
	Low	2 (40)	1 (20)	3 (60)	0	6 (30)	
	High	3 (60)	3 (60)	1 (20)	2 (40)	9 (45)	
B	Negative	2 (40)	2 (40)	0	0	4 (20)	0.478
	Low	1 (20)	1 (20)	1 (20)	3 (60)	6 (30)	
	High	2 (40)	2 (40)	4 (80)	2 (40)	10 (50)	
C	Negative	2 (40)	1 (20)	1 (20)	2 (40)	6 (30)	0.927
	Low	1 (20)	3 (60)	2 (40)	1 (20)	7 (35)	
	High	2 (40)	1 (20)	2 (40)	2 (40)	7 (35)	
D	Negative	1 (20)	4 (80)	1 (20)	0	6 (30)	0.406
	Low	0	1 (20)	3 (60)	3 (60)	7 (35)	
	High	4 (80)	0	1 (20)	2 (40)	7 (35)	

* The results of Fisher's exact test

Table 2: Comparing the frequency of microbial contamination between toothbrushes according to the groups A-D.

Microorganisms/Toothbrushes	Oral-B soft	Oral-B medium	G.U.M soft	G.U.M medium	Total
	No (%)	No (%)	No (%)	No (%)	
<i>Staphylococcus</i>	5 (31.3)	7 (58.3)	11 (64.7)	7 (46.6)	30 (37.5)
<i>Streptococcus</i>	1 (6.3)	3 (25)	3 (17.6)	1 (6.6)	8 (10)
<i>E.coli</i>	6 (37.5)	2 (16.6)	5 (29.4)	4 (26.6)	17 (21.3)
<i>Klebsiella</i>	2 (12.5)	2 (16.6)	1 (5.8)	0	5 (6.3)
<i>Candida</i>	2 (12.5)	1 (8.3)	1 (5.8)	1 (6.6)	5 (6.3)
<i>Yeast</i>	1 (6.3)	0	0	0	1 (1.3)
<i>Proteus</i>	0	0	2 (11.7)	0	2 (2.5)
<i>Enterobacter</i>	1 (6.3)	1 (8.3)	0	1 (6.6)	3 (3.8)
<i>Enterococcus</i>	0	0	0	2 (13.3)	2 (2.5)
<i>Pseudomonas</i>	1 (6.3)	0	0	1 (6.6)	2 (2.5)
Gram positive bacilli	0	0	1 (5.8)	2 (13.3)	3 (3.8)
Multiple	2 (12.5)	3 (25)	8 (47.1)	5 (33.3)	18 (22.5)

Table 3: Frequency of the type of microorganisms grown in the first 24 hours after culturing on the toothbrushes.

all participants before the study so that they used the same protocol of tooth brushing (time, duration, frequency, etc.) to investigate the net effect of the brands and bristle size. The results indicated no effects for these two factors, which is an important finding, as dentists are frequently asked these questions by patients.

The most clinically important finding of the present study was the high percentage of microbial contamination of toothbrushes, which is in line with previous studies [25,26]. The results of the present study indicate that even in standardized conditions, including using new toothbrushes, keeping toothbrushes without cover outside the bathroom, and brushing teeth twice daily every day each time for one minute thoroughly, did not significantly reduce the microbial contamination of toothbrushes. Also, the use of toothpaste did not reduce the microbial contamination of toothbrushes, which have been previously suggested [27-29].

The microbial study after 24 hours' culture in the present study indicated the greatest frequencies for staphylococcus (37.5%), *E.coli* (21.3%), and *streptococci* (10%). Staphylococcus and *streptococci* have been identified as the most common pathogens responsible for contamination of tooth brushes in previous studies [24,30], which are consistent with the results of the present study. In addition, researchers have found similar microbial contamination among family members and suggested keeping toothbrushes separately to avoid cross-contamination from the toothbrush of other family members [30]. In the present study on students, all toothbrushes were stored separately, but the most common type of microorganisms remained similar. These results suggest a *phylococcus*, *E.coli* and *streptococci* as the most frequent type of microorganisms responsible for contamination of tooth brushes in most of the groups in the present study. This is consistent with previous findings [31]. About one fourth of all samples had multiple contaminations, which has been previously shown in studies [24]. Assuming that the microorganisms growing on the toothbrushes reflect the microbiota of oral cavity, it is important to recognize the potential risks, especially in high-risk groups like patients with immunodeficiency, pregnant women, and children to prevent serious systemic diseases associated with oral cavity microorganisms [32].

The present study was the first to compare these two brands of toothbrushes in an Iranian population. However, one of the major limitations of the present study was the small sample size. This was addressed by the cross-group study design as each participant used all four types of toothbrushes. Furthermore, we limited our assessment of type of microorganisms to the few mentioned in the study and did not evaluate all types of microorganisms, which was due to the high cost of studying rare microorganisms.

Conclusion

- The results of the present study indicated that both Oral-B and G.U.M toothbrushes with soft and medium bristles are contaminated with bacteria, especially staphylococcus, *E. Coli* and *streptococci*.
- Contaminated toothbrushes may become a reservoir for potential pathogens.
- Drying the toothbrushes in free air (outside the bathroom), using no cover for the toothbrushes, using tooth paste did not prevent the microbial contamination of toothbrushes in healthy young individuals.
- This pilot study should be followed-up by one that includes a larger number of participants and with a greater in-depth identification of the bacterial colonization.

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