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Research Paper: Evaluation of microbial contamination of tooth brush and its related factors

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ABSTRACT

Introduction: Toothbrushes can have a significant role in the transmission of diseases. This study aimed to assess the type and amount of toothbrush microbial contamination and the associated factors.

Materials and Methods: In this comparative cross-sectional descriptive study, 36 students were randomly selected. A modified bass brushing technique was prescribed and participants were given a toothbrush and toothpaste in a sterilized package. After five days, the brushes were gathered and the bristles were stored in Nutrient Broth. Specimens were inseminated in blood Agar, chocolate agar, and Mac conkey medias. Biochemical and microbiological tests were performed and bacterias were identified. Contaminations with <10,000 colonies were considered as low contaminated, between 10,000 to 30,000 colonies were considered as medium contaminated, and 30,000< colonies were considered as highly contaminated. Data were analyzed using SPSS 16 by applying Kruskal-Wallis and Fisher tests at a significance level of 0.05.

Results: 16(44.4%) toothbrushes belonged to females and 20 (55.6%) to males. The average age of participants was 23.70±2.43. All the toothbrushes were contaminated. Streptococcus Mutans had the highest and Streptococcus Sanguis had the lowest levels. A significant relation was observed between the amount of toothbrush contamination and toothbrush storage place, time of flossing, usage of mouthwash, brushing the palate and tongue, drying the toothbrush, washing with warm water, and having a cap.

Conclusion: Sufficient care should be taken in the storage, washing, and drying of the toothbrush; using mouthwash; brushing the tongue, and not using toothbrush caps to prevent toothbrush contamination.

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Introduction

The oral cavity is free of microorganisms at birth. (1-2) Overtime several microorganisms settle in the oral cavity. (3) Streptococcus mutans (S.Mutans), a gram-positive facultative anaerobic bacteria, normally appears in the mouth after the first year of life, and causes dental caries. (4-6) Streptococcus mitis (S. Mitis), a selective gram-positive catalase-negative anaerobic cocci, is one of the first and most prevalent bacteria found in the oral cavity of newborns and helps the biofilm formation. (4,7-9) Streptococcus Sanguis (S. Sanguis), a gram-positive facultative anaerobic bacteria, is colonized after the teeth grow in the oral cavity and acute periodontitis is associated with a lack of this bacteria. (10-13)

Oral hygiene practice especially using toothbrushes becomes important as these microorganisms exist in the oral cavity. (14-17) But toothbrushes themselves can be contaminated by the mouth and environment, and provide a condition for microbes to survive. (12,18-19)

Several studies found that toothbrushes can significantly transmit diseases from one area to another and increase the risk of infection. (17,20) Pesevska et al. (16) stated that 100% of toothbrushes are infected with different types of bacteria. Heidarzadi et al. (17) reported that 40% of used toothbrushes were infected by Staphylococcus, Neisseria, Diphtheria, Actinomycetes, and Enterobacteriaceae. Karibasappa et al. (19) observed S. Mutans, Candida, Pseudomonas, Kalbisla, and Staphylococcus aureus in used toothbrushes.

Numerous studies have assessed the microbial contamination of toothbrushes and their impact on oral health. Contaminated toothbrushes play an important role in the development of different diseases. The purpose of this study was to investigate the type and rate of TMC and its related factors.

Materials and Methods

In this cross-sectional study, 36 dental students were selected. The inclusion criteria were physical ability to brush the teeth, and exclusion criteria were antibiotic use in the last three months, extensive dental caries, high risk of caries, severe periodontal disease, Clinical Attachment Loss (CAL)>5mm, extensive dental prostheses, orthodontic treatments, and systemic diseases.

After obtaining ethical approval from the Research Ethics Committee of Guilan University of Medical Sciences and written consent from the selected individuals, demographic characteristics, location, and storage conditions of toothbrushes and flossing were recorded.

Before the study, the modified bass toothbrushing technique was taught to the participants. A toothbrush (oral-b, extra soft, Advantage Sensitive, Ireland) and a toothpaste (pooneh, Clean and fresh, simple, Iran) containing fluoride with no antibacterial substances were given to the participants.

Before starting the study, 10 unused toothbrushes were selected as a positive control group for the validity of the research and sent to the laboratory for microbial evaluation to ensure that the toothbrushes are not contaminated before use. Participants were asked to use toothbrushes for 5 consecutive days and at least once a day.

During the study, participants were prescribed to floss, use the mouthwash, dry the toothbrushes, and clean their tongue as before; but they should not have used an antibacterial mouthwash to wash their toothbrushes. Toothbrushes should have been kept away from contact with other family members but in the same environment as before. Also, if their previous toothbrush had a cap, they were asked to use the cap for the toothbrush. After this time, each toothbrush was received in a sterile plastic container.

Using a sterile and molten razor blade, the toothbrush head was removed from the handle. The toothbrush head was placed in a tube containing 10ml of broth neutrino culture medium (to increase the growing power of possible bacteria) and was transferred to the microbiology laboratory.

Nutrient broths were incubated at 37°C for 24 hours to replicate possible aerobic microorganisms in toothbrushes. Then, the contents were stirred vigorously for 30-45s to separate the microorganisms from the toothbrush. The toothbrush section was then removed from the tube under sterile conditions and shaken vigorously again for complete homogenization for 10-15s. They were inoculated to isolate gram-negative bacteria and incubated at 37°C for 24-48 hours. Then, the bacteria that grew in the McConkey environment were reported as contaminants without any further testing. Bacteria grown in blood agar and chocolate agar were subjected to hot staining. A catalase test was performed under a microscope for samples that tested positive in the hot test and were reported as contaminated if they were catalase positive. Catalase-negative cases were cultured in the specific culture medium of Mitis. To identify the types of streptococci, bacteria grown in Mitis culture medium were subjected to

antibiotic susceptibility tests and other biochemical and microbiological tests. Cultures with a colony number of less than 10.000 were considered as low contamination, between 10.000-30.000 were recorded as moderate contamination and 30.000< were reported as high contamination. (20-21)

The data were analyzed by Kruskal-Wallis and Fisher tests at the significant level of 0.05 using SPSS version 16.

Results

The relations of the type and level of TMC with the toothbrush storing place were significant. (P<0.001 and P=0.002, respectively) (Table1) The toothbrushes stored in W.C. had higher levels of microbial contamination. Toothbrushes stored out of W.C. and the bathroom had the lowest levels of microbial contamination.

A significant relation was observed between the type of TMC and flossing. (P=0.044) (Table1) The relation between the level of TMC and flossing was not significant. (P=0.553) The results showed a significant relationship between the type of TMC and the frequency of flossing. (P=0.002) On the other hand, the relationship between the level of TMC and the frequency of flossing was not significant. (P=0.371)

There was a significant relationship between the type of TMC and the flossing time. (P<0.001) (Table1) Also, the relationship between the level of TMC and the flossing time was

Table 1: The relationship of the type of TMC and toothbrush storing place, flossing, frequency of flossing, flossing time, using mouthwash and brushing the tongue and palate.

Variables		Most common microbia	P-value
The toothbrush storing place	stored in the W.C. and bathroom	S. Mutans	P<0.001
	stored out of W.C. and bathroom	S. Mitis and S. Vestibular	
Flossing	With flossing	S. Mutans and S. Mitis	P=0.044
	Not flossing	S. Mutans	
Frequency of flossing	once/week	S. Mutans and S. Mitis	P=0.002
	every few days	S. Mutans and S. Mitis	
	once/day	S. Vestibular and S. Mitis	
	more than once/day	S. Vestibular and S. Mitis	
Flossing time	Flossed before brushing	S. Vestibular	P<0.001
	Flossed while brushing	S. Mitis	
	flossed after brushing	S. Mutans	
Using mouthwash	not using mouthwash	S. Mutans	P=0.001
	using mouthwash	S. Mitis	
Brushing the tongue and palate	not brushing the tongue and palate	S. Mutans	(P=0.022)
	brushing the tongue and palate	S. Mitis and S. Sanguis	

significant. (P=0.032) So that the toothbrushes of participants who flossed before and after toothbrushing had higher levels of TMC than those who flossed during the toothbrushing.

There was a significant relationship between the type of TMC and using mouthwash. (P=0.001) (Table1) Also, there was a significant relationship between the level of TMC and using mouthwash. (P=0.001) So, the toothbrushes of participants who used mouthwash had the lowest levels of TMC, and participants who did not use mouthwash had the highest level of TMC.

There was a significant relationship between the type of TMC and brushing the tongue and palate. (P=0.022) (Table1) Also, there was a significant relationship between the level of TMC with brushing on the tongue and palate. (P<0.001) Toothbrush People who did not brush their tongues and palate were highly contaminated.

According to the results, there was no significant relationship between the type of TMC and the frequency of toothbrushing/day. (P=0.364) There was a significant relationship between the level of TMC and the frequency of toothbrushing/day. (P=0.005) People who brushed once/day had the highest levels of TMC.

There was a significant relationship between the type of TMC and drying the toothbrush after using it. (P=0.001) Toothbrushes in people who did not dry them mainly had S. Mutans while in those who dried their toothbrush S. Mutans was the lowes. There was no significant relationship between the type of TMC and the method of drying the toothbrush after use. (P=0.584)

There was a significant relationship between the level of TMC with the drying of the toothbrush after using it. (P=0.001) There was no significant relationship between the level of TMC and the method of drying the toothbrush after use. (P=0.202)

A significant relationship was observed between the type of TMC and using hot water to wash the toothbrush. (P=0.002) Toothbrushes in people who used hot water to wash their toothbrushes mostly had S. Mitis and S. Vestibular while in those who did not use hot water mainly S. Mutans was seen.

There was a significant relationship between the level of TMC and using hot water to wash the toothbrush. (P<0.001) Toothbrushes in people who did not use hot had a higher rate of TMC. While people who used hot water had lower levels of TMC.

There was a significant relationship between the type of TMC and having a toothbrush cap. (P=0.003) Toothbrushes with no cap mainly had S. Mitis and those with a cap mainly had S. Mutants. While there was no significant relationship between TMC and having a toothbrush cap.(P=0.068)

Discussion

S. Mutans (most frequent), S. Mitis, S. Vestibular, and S. Sanguis (least frequent) were found on toothbrushes. Following studies reported the same findings; Nascimento et al. (15) and Sato et al. (22) respectively reported that 92.7% and 80% of the toothbrushes were contaminated with S. Mutans. Svanberg et al. found that S. Mutans was more frequent than other microorganisms. (9)

No relation was found between age and gender with the type of TMC. Nourbakhsh et al. (21) reported the same findings, while Heidarzadi et al. (17) reported that women were more commonly contaminated with coccies. In the study of Heidarzadi et al. (17) the female participants were more than males.

In this study, toothbrushes kept in W.C. were more infected than toothbrushes kept in other places. Kalati et al. (23) stated that the bathroom was the worst and the sleeping room was the best place to store the toothbrushes. Pesevska et al. (16) and Glass et al. (14) also reported the same findings. Lee et al. stated that toothbrushes should be stored in the sunlight and away from moisture.

In this study, toothbrushes that were changed after 2-3 months were mainly contaminated with S. Mitis and S. Vestibular, respectively,

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and toothbrushes that were changed after 4 and 5 months the more frequent microorganism was S. Mutans. Different studies assessed the appropriate time for changing the toothbrushes. Ferreira et al. (6) and Pesevska et al. (16) reported that long-term use of toothbrushes increases the microorganism accumulation. Karibasappa et al. (19) reported that 95% of the toothbrushes are contaminated, among them toothbrushes which were used for 1 month were less contaminated than those used for 3 months. In the current study, the rate of microorganisms was not related to the time of changing the toothbrushes which was not similar to the result of Nourbakhsh et al. (21) This result may be due to the difference in sample sizes of the current study and the study of Nourbakhsh et al. (21)

The results revealed that the rate of microorganisms was higher in the toothbrushes which were covered. This result was in accordance with the studies of Dayoub et al. (24), Pesevska et al. (16), and Heidarzadi et al. (17) who claimed that covering the toothbrushes leads to a higher rate of TMC and increases the bacterial survival. Similarly, Mehta et al. (20) stated that covering the toothbrushes maintains the moist and increases the number of microorganisms.

The rate of microorganisms was higher in the toothbrushes of those who brushed their teeth only once/day and was less in the toothbrushes of those who brushed their teeth more than once/day. The results of Heidarzadi et al. (17) and Nourbakhsh et al. (21) were not consistent with the current studies. The divergence between the studies may be due to different study populations. Unlike other studies, the population of this study was dental students who were aware of correct toothbrushing techniques. It seems that if the teeth are brushed more frequently with a correct technique, the rate of dental plaque is decreased therefore, the rate of TMC is reduced.

The toothbrushes of those who flossed after brushing were mostly contaminated with S. Mutans. These findings were not significant which is similar to the study of Heidarzadi et al. (17) The results also revealed that the rate of TMC was higher in the toothbrushes of those who flossed before and after the brushing and was less in the toothbrushes of those who flossed during the brushing. Flossing removes the dental plaque and decreases the oral microbial. Flossing during brushing maintains the fluoride of toothpaste in the mouth and facilitates the removal of dental plaque with toothbrushes.

Heidarzadi et al. (17) reported that the use of mouthwash decreases the TMC. Nascimento et al. (15) claimed that only toothbrushes which were merged in CHX were not contaminated. Mehta et al. (20) revealed that the use of CHX removes 100% of S. Mutans. Suido et al. (25) assessed the effect of CHX on the contamination of inter-dental brushes and the results showed that 2-week use of CHX decreases TMC. Similarly, in the current study, the toothbrushes of those who did not use CHX had the highest level of TMC. The use of CHX decreases the level of oral microbial and affects the TMC.

In the current study, similar to the findings of Nourbakhsh et al. (21) toothbrushes that were not dried had higher levels of TMC and were mainly contaminated with S. Mutans. No study has evaluated the relation between TMC and the method of drying the toothbrushes.

Conclusion

According to the results of the present study and other studies, it is clear that toothbrushes are highly contaminated by a large number of microorganisms after use, and it is possible to transmit these microorganisms through toothbrushes between people and between different areas of the mouth. For this reason, sufficient care should be taken in the place of storage, washing, and drying, not using toothbrush caps, and using mouthwash and brushing the tongue to avoid further contamination of the toothbrush and its transmission to others.

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