sciendo
10.2478/bjdm-2019-0017

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Evaluation of Toothbrush Contamination

SUMMARY

Background/Aim: *Toothbrushes are one of the main means of cleaning teeth and maintaining oral hygiene, but toothbrushes are also potential reservoir of microorganisms, including pathogens. The aim of this paper was to evaluate the oral health, oral hygiene awareness and assess the degree of contamination of toothbrushes among students attending Secondary Medical School.* ***Material and Methods****: Sixty students (32 boys, 28 girls; mean age ≅ 15,7±2,1) attending highschool were randomly selected for this study. Each student included in the study filled out a questionnaire regarding his/her life habits and oral hygiene. Clinical examinations were initiated in order to determine the DMFT, as well as the CIP, CIT, CIA and CPITN indexes, based of which the assessment of oral health status was performed. One stack of fiber was collected from each toothbrush used by the participantes in the study, and than prepared for further microbiological sampling.* ***Results****: Only 11% of the students had the awareness of potential sources of toothbrushes contamination. The average value of DMFT was 3,2 (%D = 22,5; %M = 4,96; %F = 72,5). A statistically significant difference in comparison of the CPITN index and subjects' response to the professional plague removal in the last 12 months was identified (χ2 = 13,55; p= 0,033). Staphylococcus aureus, Streptococcus mutans, Micrococcus species and Streptococcus salivarius were most commonly present microorganisms. In most cases, G-positive bacilli or cocci were isolated, while the presence of Candidae albicans was identified in four samples.* ***Conclusions****: Raising the awareness of dental hygiene through the oral health education may improve better plaque control and subsequently the oral health. Hands-on training how to maintain the oral hygiene are not expensive and more over they are easy to be organized can be useful in oral heath promotion.*

Key words: Oral Hygiene, Microorganisms, Toothbrush

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ORIGINAL PAPER (OP)
Balk J Dent Med, 2019;93-97

Introduction

Toothbrushes are one of the main means of *cleaning teeth and* maintaining oral hygiene, but they are also potential reservoir of microorganisms, including pathogens. The different design of toothbrushes, the maintenance of their hygiene, the manner of their disposal, and the formation of aerosols from the drainage pipes represent a potential risk of toothbrushes contamination[1]. Only one act of releasing water into the toilet disposes millions of bacteria into the atmosphere[1]. Different ways of toothbrushes disinfection have been proposed, such as using antiseptics, toothbrushs coating, or the use of toothpaste. Furthermore, recommended usage of protective caps for toothbrushes prolongs the drying time, which favors the development of microorganisms.

Svanberg found that toothbrushes after 24h of use were already significantly contaminated with *Streptococcus mutans*[2]. According to Glass and Lara[3] analyzes, microorganisms not only adhere and multiply on used toothbrushes, but they also have the ability to contaminate other objects and transfer to humans. These

microorganisms may be considered as an additional risk factor for the development of both local and systemic diseases. In recent decades, scientists have proven that microorganisms located on toothbrushes have a role in the local bacteriemia, especially in patients with periodontal infections[4].

In the assessment of the oral health, index of the average number of decayed, extracted and filled teeth (DMFT) and Community Periodontal Index of Treatment Needs (CPITN) are most frequently used. The silent caries epidemic in recent years have been almost stopped in the countries of western and northern Europe and the United States. Lack of implementation of systematic school preventive programs, healthcare programs on the promotion of the oral health importance and the oral hygiene, have influenced the fact that dental caries, in the eastern and central Europe, still remains a serious health and social problem.

The aim of this paper was to evaluate the oral health, oral hygiene awareness and assess the degree of toothbrushes contamination among students attending Secondary Medical School.

Material and Methods

Clinical examination

For this study, sixty students attending Secondary Medical School were randomly selected (32 boys, 28 girls; mean age ≅ 15,7±2,1). Each student included in the study filled out a questionnaire regarding his/her life habits and oral hygiene.

After taking anamnestic data from the subjects, clinical examinations were initiated in order to determine the DMFT, as well as the CIP, CIT, CIA and CPITN indexes, based of which the assessment of oral health status was performed. The following formulas were used for necessary calculations: CIP = Number of subjects with DMFT/ Number of examined subjects × 100; CIT = Total DMFT/ Number of present + extracted teeth × 100; CIA = Total DMFT/ Number of examined subjects. The DMFT structure was determined by the following formula: %D = D teeth number/ total DMFT × 100; %M = M teeth number/ total DMFT × 100; %F = F teeth number/ total DMFT × 100 (D- decayed, M- missing, F- filled teeth). The CPITN index was used to assess the health of periodontium. The probe was placed parallel to the longitudinal axis of the teeth, and one representative tooth from each sectant was examined (16, 11, 26, 36, 31, 46). The most severe damage to periodontium was recorded in the chart according to the following points: 0-healthy parodontium; 1-gingiva bleeding after probing; 2-present solid deposits on the teeth or prominent edges of filings and/or prosthetic works; 3-periodontal pockets 4-5 mm deep; 4-periodontal pockets 6 or more mm deep.

Samples

The toothbrushes that were currently used by the participants in the study were collected and prepared for further microbiological sampling (Figure 1). One stack of fiber from the central part of the brush was cut off with sterile scissors and transferred to the transport medium.

Figure 1. The toothbrushes collected from the participants enrolled in the study

All of 60 samples have been enriched in thioglycollate broth (Institute of virology, vacines and sera Torlak, Belgrade, Serbia) for 24h at 37ºC. Overnight culture was streaked on appropriate media for the cultivation of both aerobes (including yeast) and anaerobes. Aerobes were put on Columbia agar with 5% sheep blood (COS, bioMérieux, Marcy-l'Étoile, France) and MacConkey agar (Becton, Dickinson and Company, Sparks, USA) and incubated in aerobic atmosphere overnight. Yeast cultures were inoculated on Saburoud agar (Becton, Dickinson and Company, Sparks, USA) and incubated at 37ºC for 24 hours and then on room temperature for 2-5 days. Anaerobic cultures were plated on Columbia agar with 5% sheep blood (COS, bioMérieux, Marcy-l'Étoile, France) and incubated in a jar under anaerobic conditions using GasPack (GasPak™ EZ Gas Generating Container Systems**,** Becton, Dickinson and Company, Sparks, USA), at 37ºC for 2 to 5 days. Grown bacterial colonies from anaerobic conditions were put on Columbia agar with 5% sheep blood (COS, bioMérieux, Marcy-l'Étoile, France) on 37ºC overnight to determine demand for obligatory anaerobiosis in such bacteria.

For yeast cultures Gram stain and growth on Saboroud agar was used to identify yeast. Gram stain, hemolysis on COS, catalase, oxidase, oxidase (Oxidase Reagent Droppers Becton, Dickinson and Company, Sparks, USA), coagulase (Rabbit plasma, Veterinary Medicine Institute Inc. Zemun, Serbia) tests and BD BBL Crystal™ Identification Systems Gram – Positive ID (Becton, Dickinson and Company, Sparks, USA)

kit were used to identify Gram positive bacteria. Gram negative bacteria identification were based on Gram stain, catalase reaction, oxidase test (Oxidase Reagent Droppers Becton, Dickinson and Company, Sparks, USA), reactions of indole (Institute of virology, vacines and sera Torlak, Belgrade, Serbia), methyl red (Merck, Darmstadt, Germany) Voges-Proskauer test (Merck, Darmstadt, Germany), citrate (Institute of virology, vaccines and sera Torlak, Belgrade, Serbia), triple sugar iron agar slant (TSI, Becton, Dickinson and Company, Sparks, USA) and confirmation by BD BBL Crystal™ Identification Systems Enteric/Nonfermenter ID (Becton, Dickinson and Company, Sparks, USA) kit.

Statistical analysis

Data analysis was assessed using using statistical software IBM SPSS 20, t-test. The level of significance was determined at $p<0.05$ and the data were processed.

Results

Most of the subjects responded that they go to the regular dental examinations, while 7,5% did not visit dentist during past year. When it comes to the frequency of tooth brushing, 75% said that they brush their teeth twice a day, in the morning before breakfast and before bedtime, and 22,5% of teeth brush teeth three times a day. At the time of the study 32,5% of subjects used a toothbrush for less then one month, and only 15% of subjects brushed their teeth for more then three minutes. Only 11% of the students had the awareness of potential sources of toothbrushes contamination.

The most common toothpaste used was Colgate. Oral rinses were rarely used by 97,5% of subjects, while the dentalfloss used only 8% of the subjects daily basis, 40% did not use it, 47% very rarely, and 5% once a week. Based on the questionnaire, it was concluded that 82,5% of the subjects did not have a professional plague removal during previous year, while 17,5% were subjected to the same dental treatment on their own initiative or dentist's recommendation.

The average value of DMFT was 3,2 and its structure expressed in the mean values were %D = 22,5; %M = 4,96; %F = 72,5. When it comes to the CPITN index, 62,5% of the subjects had a healthy periodontium. In 22,5% of subjects, slight bleeding of the gums on probing, while in 15% of cases solid deposits were present. A statistically significant difference in comparison of the CPITN index and subjects' response to the professional plague removal in the last 12 months was identified ($\chi 2$ = 13,55; p= 0,033).

Microbiological analysis revealed the presence of 15 bacterial species (Table 1). *Staphylococcus aureus, Streptococcus mutans* (Figure 2), *Micrococcus species and Streptococcus salivarius* were the most commonly present. In most cases, G-positive bacilli or cocci were isolated, while the presence of *Candidae albicans* (Figure 3) was identified in four samples.

Table 1. Results of microbiological analysis

Bacterium	Gram dye	Need for O_2	No	Percent
Staphylococcus aureus	+	Facultative anaerob	*10*	*25,0*
Streptococcus mutans	+	Facultative anaerob	*7*	*17,5*
Mycrococcus spp.	+	Facultative anaerob	*7*	*17,5*
Streptococcus salivarius	+	Facultative anaerob	*6*	*15,0*
Klebsiella pneumnoniae	-	Facultative anaerob	*6*	*15,0*
Enterococcus faecalis	+	Facultative anaerob	*3*	*7,5*
Enterobacter cloacae	-	Facultative anaerob	*3*	*7,5*
Mycobacterium spp.	+	Facultative anaerob	*3*	*7,5*
Streptococcus pneumoniae	+	Facultative anaerob	*2*	*5,0*
Streptococcus pyogenes	+	Facultative anaerob	*2*	*5,0*
Listeria spp.	+	Facultative anaerob	*2*	*5,0*
Lactobacillus spp.	+	Facultative anaerob	*1*	*2,5*
Streptococcus epydermidis	+	Facultative anaerob	*1*	*2,5*
Streptococcus agalactiae	+	Facultative anaerob	*1*	*2,5*
Pseudomonas spp.	-	Aerob	*1*	2,5

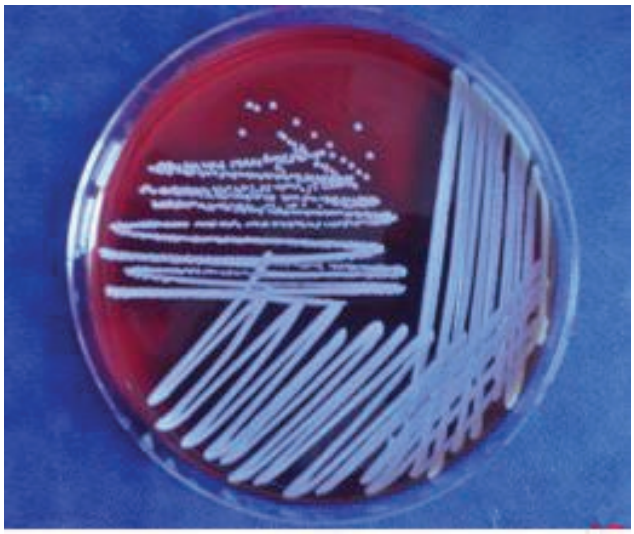

Figure 2. The isolation of Streptococcus mutans

Figure 3. The isolation of Candida albicans

Discussion

Considering the fact that the gingival trauma, bleeding and local bacteremia usually occur during teeth brushing, the awareness of toothbrushes contamination and their maintenance is of great importance. Despite of millions sold toothbrushes each year, there is still a very low awareness about their contamination[5]. Dental brushes can be contaminated by direct or indirect contact. Indirect contact can occur via fomites, such as spoons, toys, or other contaminated toothbrushes[3,4,5].

Glass and Lara have suggested that toothbrushes may be significant in the transmission of pathogenic microorganisms through gingival lesions when referring to patients undergoing organ transplantation or immunocompromised patients[3]. Glass has determined that HSV-1 can remain vital on a dry dental brush for at least 48h, and in wet conditions for one day. Under the usual conditions of disposal, the dental brush may be a source or vector of transmission or reinfection.

One of the most predominant bacteria isolated from the collected toothbrushes was *Streptococcus mutans,* considered as main cariogenic bacteria[6]. Svanberg also found that toothbrushes and toothpaste tubes were contaminated with *Streptococcus mutans* only 15 min after teethbrushing[2]. Malmberg *et al.* showed that old toothbrushes, 2h after their use without toothpaste, were mostly contaminated with aerobic species[7], which is not in the accordance with the findings of this study. These results can be most likely explained by the way toothbrushes were stored. However, the facultative anaerobic bacteria detected in this study may indicate their ability to colonize supragingival plaque or to act as a possible reservoir of bacteria after periodontal therapy[8].

Furthermore, periodontopathogens like *Pseudomonas spp.* were also found in one collected sample in this invastigation. Similar bacetria were also detected in the study of Efstratiou *et al.*[8]. Contrary to the findings of Muller *et al*.[9] who showed the common presence of *Actinobacillus actinomycetemcomitans* sampeling the patients with the juvenile periodontitis, 24h after the toothbrushes were used[9].

Enterobacteria which spread through aerosols formed from wc mugs or emanating from bathroom and other wet areas or through contaminated fingers and skin can also contaminant toothbrushes[10]. The findings of our study suport these results, as *Enterococcus spp.* have been isolated in six samples, raising the need for the appropriate toothbrushes disposal.

Resent study showed that *Candida albicans,* also detected in this investigation, may have a role in cariogenic processes, due its acidic nature and ability to produce collagenolytic proteinases[11]. Additionally, there was no correlation between *Candida albicas* presence in the saliva and toothbrushes, which is an indication of their indirect contamination[12].

Having in mind these facts, many investigators have been focused on finding the most suitable methods of toothbrushes disinfection. Different ways of toothbrushes disinfection have been proposed, such as using antiseptics, toothbrushs coating, or the use of toothpaste.

Although there are no relevant data regarding the oral health in the Republic of Serbia of general population[13] and adolescents as well, results of this study showed that DMFT index is high, as expected for developing countries. The low level of oral health is related to the lack of awareness of the oral hygiene importance and its impact on general health, as well documented in our study, even the participantes were the students of medical school. The lack of awareness of oral health importance was also seen among adolescents is some European counties[14, 15]. On the other hand.

Conclusions

Raising the awareness of dental hygiene through the oral health education may improve better plaque control and subsequently the oral health. Hands-on training how to maintain the oral hygiene are not expensive and more over they are easy to be organized can be useful in oral health promotion and raising the awareness of importance of oral health.

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Conflict of Interests: Nothing to declare.
Financial Disclosure Statement: Nothing to declare.
Human Rights Statement: All the procedures on humans were conducted in accordance with the Helsinki Declaration of 1975, as revised 2000. Consent was obtained from the patient/s and approved for the current study by national ethical committee.
Animal Rights Statement: None required.

Received on September 2, 2018.
Revised on October 20, 2018.
Accepted on November 10, 2018.

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