Bacterial Contamination of the Toothbrushes

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Abstract

Toothbrushes are an environment for transfer of microbes, their retention and growth. The aim of the study was to evaluate the bacterial contamination of toothbrushes during everyday use in the periodontal healthy student population and to record the way of maintaining the toothbrush and the time and the reason for their replacement with new ones. The research included 20 students of both sexes, with a healthy periodontium, who filled out a special questionnaire and got a new toothbrush for everyday use in one month period. After the test period, toothbrushes were transported in sterile conditions at the Institute of Microbiology of the Medical Faculty in Skopje and further processed. The total number of bacteria in each plate was determined and larger colonies identified by the method of Gram and other biochemical tests. The microbiological findings showed a high contamination of the used toothbrushes at 100% of the analyzed samples, with a domination of coliform bacilli (Escherichia coli-40%, Klebsiella-25%, Enterobacter cloacae-5%, Serratia-15%) and Pseudomonas aeruginosa -15%. Toothbrushes became highly contaminated after everyday use and can be carriers of microorganisms, increasing the risk of diseases caused by oral biofilm in healthy people.

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Introduction

The most common way to maintain the complex of proper oral hygiene is the tooth brushing; its main goal is to remove the dental plaque which is responsible for a number of oral diseases: tooth decay, periodontitis, as well as halitosis.

The first and most efficient tool for removing the oral biofilm and the soft debris out of the mouth, especially from the tooth and tongue surfaces, is the toothbrush[1]. Not only the proper choice, but the care and maintaining of the toothbrush are important for the good oral hygiene and health as well, because although the toothbrush is not the ideal environment for microorganisms' growth, it is capable to obtain life of the microbes[2].

Toothbrushes are sterile after their manufacturing[3] and they get contaminated

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immediately after the first tooth brushing[4,5]; the microbial colonization riches higher level with every further use of the toothbrush[6,7]. The biofilms that develop in the toothbrushes after their use, may contain different bacterial species'[8,9], viruses[10] and fungi[11,12] that are present in the mouth, as well as some from the environment[13], the boxes for their keeping, contaminated fingers and skin commensals[11,14,15].

The conditions by which the toothbrushes are kept are of a big importance for the bacterial survival: toothbrushes which are kept in air conditions have less bacteria than those which are kept closed, and the bacterial growth is 70% higher in wet and protected environment[9].

The wet environment in the bathroom, where toothbrushes are usually kept, may facilitate the bacterial growth and the crossed contamination, especially that one which happen through the aerosols produced during the water passing in lavatories, with enteric types and pseudomonas from the toilets and sanitary drainage[16].

Toothbrushes are containers for microorganisms and thus they are a reason for greater risk of bacterial transmission and a possibility for biofilm-associated diseases.

Back in early 20's of the 20th century, Cobb reported that the toothbrush can cause recurrent infections in the mouth[17]. A number of factors, including the long microbial surviving in the toothbrushes – from 2 days to one week[18], the inadequate keeping, the toothbrush use without decontamination – which leads to autoinoculation and the untimely changing of the toothbrush with new ones, may result in repeated entry of potential pathogens and crossed infection in the oral cavity[19], especially in children, elderly people, those with concomitant somatic disease[20], patients with high risk i.e. immunocompromised ones, those with transplanted organs or oncologic patients[21].

Actualizing the problem of toothbrushes contamination, the choice of proper tools and methods for their disinfection and the patients' education are important issues which should bring into the focus of dentists in everyday practice, because of the need of prevention the potential influence to the oral and systemic health.

Because toothbrushes are characterized as an environment for microbial transport, retention and growth, and the highly contaminated toothbrushes may be a reason for repeated reinfection, these aims of the study were set:

- To evaluate the bacterial contamination of the toothbrushes during a daily use (after 1 and 2 months) in student population with healthy periodontium
- To note the way of keeping of the toothbrushes and the time of their changing with new ones

Methods

40 students from the Faculty of Dentistry in Skopje were included in this study; they were from both sexes and with healthy periodontium, without a gingival inflammation. They also met the next criteria: absence of systemic disease, absence of gingival or periodontal diseases, not receiving antibiotic treatment 3 months prior the study, and during the test period to apply the standard oral hygienic habits. The examinees filled specially prepared questionnaire (to get information about the way of keeping, maintaining and changing of the toothbrush) and signed a written consent to participate in the study. All the examinees who met the criteria to get into the study signed the consent for participation, by which they approved that their data may be used for scientific and research purposes. A specially designed questionnaire was prepared, in which the individual data and clinical parameters for each respondent were filled.

All the examinees got a new manual toothbrush (Curaprox ultra soft CS5460 – Curaden Swiss) to maintain the oral hygiene in the standard way (obligatory tooth brushing in the morning and in the evening). All respondents were recommended to use herbal or other toothpaste without antimicrobial components in the test period (Colgate herbal, Kolynos). After one month they were asked to bring the toothbrushes, which were collected in sterile conditions and were sent for microbiological analysis, not longer than 18 hours after the last tooth brushing.

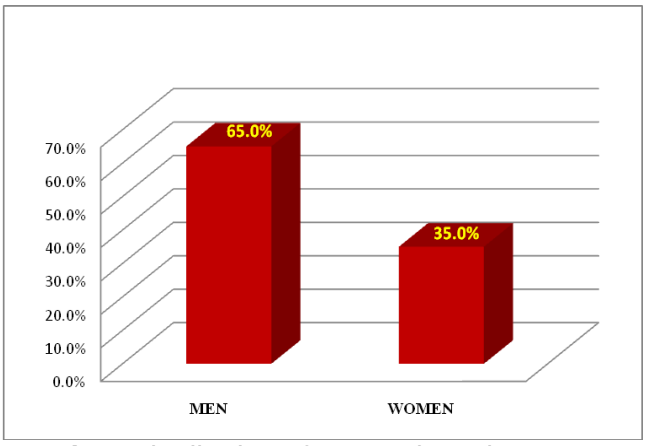
The toothbrush analysis was performed in the Institute for Microbiology and Parasitology, Faculty of Medicine in Skopje. All the toothbrush heads were removed by using sterile gloves and forceps. Every toothbrush head was put in sterile container with 10 ml brain-heart agar (Oxoid) and incubated in 37°C for 24 hours, after which they

were placed in different bases (blood agar, Schaedler agar, McConkey, as well as base for fungal determination (CALB). The bases for aerobic cultivation were incubated in thermostat for 24 hours, after what the grown colonies in the blood agar and McConkey were compared; for differentiation of the species, additional biochemical analysis (IMCV, DNA esculin) was performed.

Anaerobic plates were incubated in anaerobic pot in a thermostat, and after 48 hours the colonies were analyzed on the basis of microscopic slides with Gram stain; they were compared to the aerobic plates and by the susceptibility to novobiocin. The bases for fungal isolation were incubated for 3 days and then analyzed. The total number of microorganisms was determined for each plate separately; the dominant colonies were identified for each plate separately, as well. The percentages of presence of the analyzed data were determined and shown.

Results

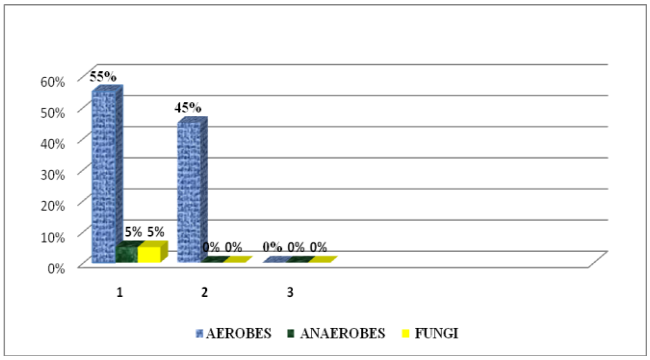
The mean age of the examinees was 21.4±1.0; the males were dominant in the study (65%), with females representing 35% of the respondents (Graph 1).



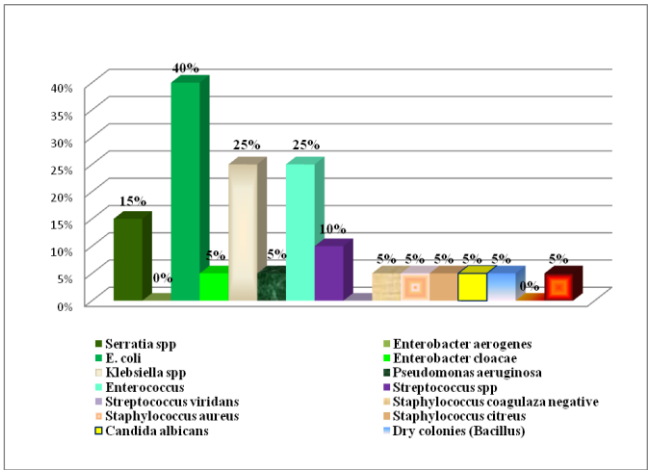
Graph 1. Distribution of respondents by sex

In 55% of the analyzed toothbrushes only one bacterial type was identified; in 45.0% there were two aerobic bacterial species'; in 5% the funga Candida albicans was determined. Graph 2 shows that in 5% only one type of anaerobic bacteria was identified.

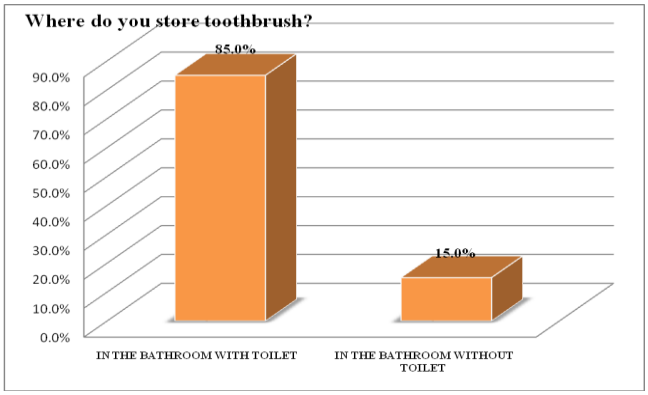
The results from the bacterial analysis (Graph 3) show a high bacterial contamination after the first month of daily toothbrush use in 100% of the samples, in which coliform bacilli were the dominant ones: Escherichia coli – 40% (Figure 1), Klebsiella – 25%, Enterobacter cloacae – 5%, Serratia – 15%, as well as Pseudomonas aeruginosa – 15%. On basis of the results from the questionnaire, from the Graph 4 can be noted that 85% of the examinees keep the toothbrush in a bathroom with toilet, while only 15% in a bathroom without a toilet.



Graph 2. The number of bacterial species' in the sample

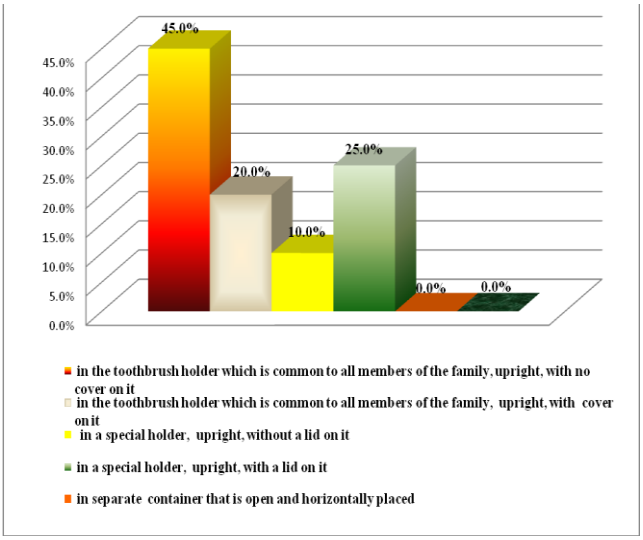


Graph 3. Distribution of respondents according to microbiological findings

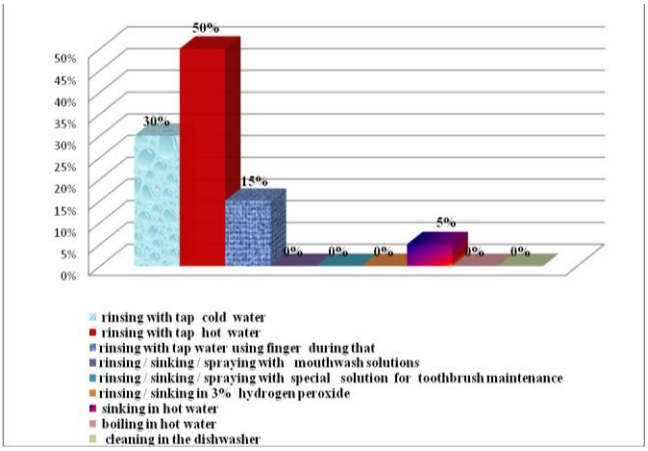


Graph 4. Distribution of respondents according to the answer to the first survey question

The toothbrushes were mostly kept in a toothbrush holder which is mutual for all family members; the toothbrush was vertically placed without a cap in 45%, while 25% of the respondents answered that they keep the toothbrush in a special holder with a cap (Graph 5). The most common ways for cleaning the toothbrushes after their usage were: rinsing the toothbrush with hot water (50%), cold water (30%) or rinsing with cold water while using a finger for thoroughly cleaning the toothbrush (15%)(Graph 6).

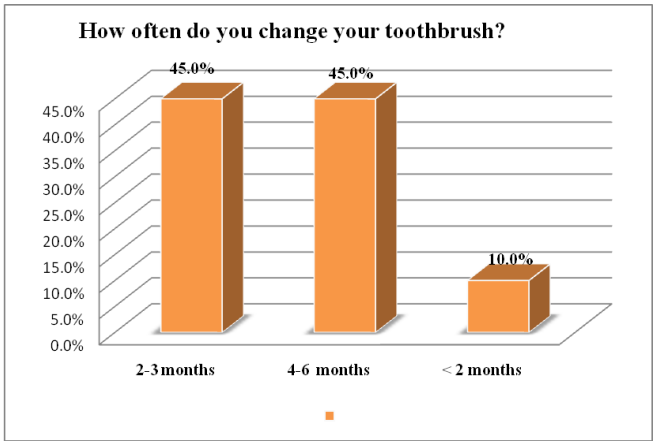


Graph 5. Distribution of respondents according to the answer to the second survey question: How do you keep the toothbrush?

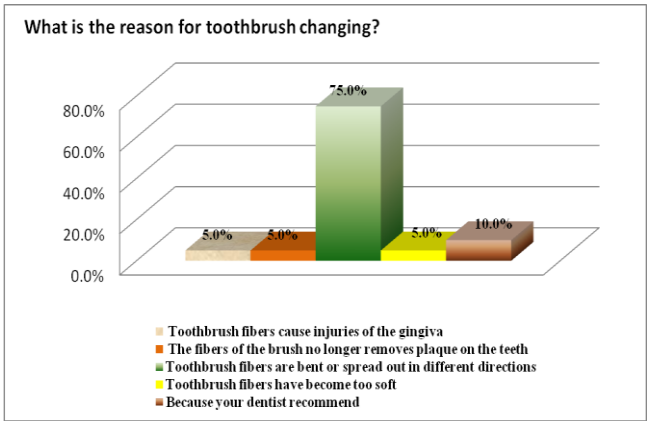


Graph 6. Distribution of respondents according to the answer to the third survey question: How do you clean the toothbrush after its use?

By the answers of the examinees, it can be noted that 45% of them change the toothbrush not longer than 2-3 months after its usage (Graph 7) with main reason in 75% being the deforming of the toothbrush filaments (buckled or spread in all directions), as shown in Graph 8.



Graph 7. Distribution of respondents according to the answer to the fourth survey question: How often do you replace the toothbrush with a new one?



Graph 8. Distribution of respondents according to the answer to the fifth survey question: What is the reason for you to replace the toothbrush with a new one?

Discussion

According to the literature reports, the toothbrush contamination in healthy individuals happens early after the first use, and it gets higher with its further usage[2,3], with our findings confirming that. Oral cavity is a place with the highest concentration of different microbial populations (more than bacterial 700 species', from which 400 are found in the periodontal pockets); it is especially colonized by Staphylococcus sp., Streptococcus sp., Neisseria sp., Bacteroides sp., Actinomyces sp.,

Treponema sp., Mycoplasma sp.[22]. These microorganisms can settle the toothbrushes, as well[21], including the organisms which are not normally associated with the oral flora, like the family of Enterobacteriaceae.

The presence of streptococci on the analyzed toothbrushes (10%) shows that they are a great mediator for bacterial transfer, because it is most likely that those bacteria originate from the residual plaque on the toothbrush filaments. In the same time, the alpha hemolytic streptococci which have a low periodontal pathogen potential, can prevent the colonization of the more pathogenic species', as well as viridans streptococci which can have a useful role because of the growth inhibition of the periodontal pathogens[23,24]. In this study, the examinees used a proper method of tooth brushing and they were free of tooth decay; therefore, the absence of mutans streptococci, which are part of the oral flora that are cariogenic agents, is not surprising.

Although Staphylococcus aureus belongs to the oral microflora (found in 5% of the analyzed toothbrushes), more attention should be paid due to the possibility for it to cause not only a number of oral diseases, but opportunistic infections as well; therefore, its presence is very important in individuals with impaired health. In this study, the domination of the members of the Enterobacteriaceae family (Serratia spp., E. coli, Klebsiella spp., Enterobacter cloacae, Enterobacter aerogenes) and Pseudomonas aeruginosa is evident. The contamination with enterobacteria and pseudomonads is due to incorrect keeping of the toothbrushes, usually out of cupboards or above the sinks, where the aerosols from the toilet can easily rich them[15]. Pseudomonads partly origin from the fountain water[25]. It is posited that Staphylococcus aureus and Escherichia coli survive for 6 days despite there is a minimal lowering of their number, while Pseudomonas aeruginosa and Klebsiella survive for 72 hours, but they were not isolated 6 days after the toothbrush use[26,27].

Thus, the repeated usage of the toothbrushes in a longer period of time is one of the greatest factors that promote the bacterial survival. After every-day use of the toothbrushes in a period of one month, in 55% of the tested samples only one bacterial species was determined, while in 45% there was contamination with two aerobic bacterial species'. With time, conditions for growth of anaerobes occur gradually, which is proven by the finding of one anaerobic species on the toothbrushes in 5%.

Microorganisms with a possible periodontal pathogenic importance are Peptostreptococcus, Eubacterium species, betahemolytic streptococci, staphylococci, enterococci, pseudomonadas and other enteric species'. The microbiological finding suggests domination of the members of the Enterobacteriaceae family (Serratia spp, E. coli. Klebsiella spp, Enterobacter cloacae, Enterobacter aerogenes), as well as Pseudomonas aeruginosa, one month after every-day use of the toothbrushes (Graph 3), hence more attention on the issue of their contamination should be paid.

Those organisms that can cause superinfections, like multiple drugs resistant Enterococcus faecalis and enteric gram negative species', pseudomonads and Candida albicans, may also colonize the periodontal areas, especially in immunocompromised and older individuals, as well as in patients with previous extended or excessive antibiotic treatment[23].

Toothbrushes that are kept in wet conditions, like the bathrooms and non-covered toilets are a origin of fecal bacteria and microbes that are being spread in the air through the aerosols[4]. This is in accordance with the fact that 85% of the examinees keep the toothbrushes right in a bathroom with a toilet (Graph 4), as well as with the domination of these bacterial species' (Graph 3).

Studies show that the water drops spread in a non-visible cloud 2 – 2.5 meters outwards and upwards, hence the areas in the bathroom that are not close to the toilet are being contaminated though. Wet conditions in the bathrooms may facilitate bacterial growth and crossed contamination when toothbrushes are kept in a bathroom, in a mutual holder without a head cap, either through a direct contact (in 45% of the examinees, as shown in Graph 5), contaminated fingers and skin commensals (in 15% of the examinees that use their fingers during tooth brushing, as shown in Graph 6), or due to the usage of a mutual toothpaste[28,29]. It has to be noted that in the toothbrushes which are kept in closed boxes, as well as in those who are exposed to contaminated surfaces or are covered with a cap, there are bigger chances for surviving or increasing of the number of bacteria[9].

The toothbrush environment is also influenced by its design, in the mean of the filaments (number, position, color, grouping, fixation), as well as by the design of its holder. Caudry et al.[30] found that bacteria are strongly adhered to the toothbrush filaments and the retention of moisture, epithelial and oral debris in the filament bundles raise the bacterial survival[9]. In our study, only one toothbrush brand and type was used, in order to exclude the toothbrush design as a factor that can influence the results. The most used method for toothbrush cleaning was rinsing it with water (50% with hot and 50% with cold water). The usage of the same toothbrush in an extended period of time makes it a reservoir of microorganisms despite the fact that it is used to lower the present flora in the dental plaque, so the contaminating microorganisms may be imported in the mouth again.

The time period of using the toothbrush may also be connected with its contamination, hence not only the proper cleaning will reduce the microbe volume, but the replacing of the used toothbrush with a new one is a condition for better oral health in individuals, as well. This can be noted in our study, where 10% of the examinees replace the toothbrush in a period not longer than two months, while 45% replace it after 2-3 months using (Graph 7). It is assumed that this is because of the properly informed student population about the oral health habits.

Bacteria that adhere, accumulate and survive on the toothbrushes may be transferred to the individuals and may cause a disease[30,31]. Taking into consideration that often some injuries happen during the tooth brushing and that a part of the population uses aggressive methods of tooth brushing that cause permanent microtraumas to the oral mucosa, it is clear that there is a risk these traumas to be a potential entrance to the microorganisms.

Glass and Shapiro[32] pointed that contaminated toothbrushes may have a role in developing of local and systemic diseases. The possibility these toothbrushes to be associated with the influence to some systemic conditions, like heart diseases, arthritis, bacteremia and brain stroke has been already documented[33].

Bunetel et al.[11] found that the toothbrushes in patients with oral diseases are easily being contaminated, and the patients with oral inflammatory diseases responded well to the treatment when they replaced the used toothbrushes with new ones often (i.e. replacing the toothbrush every two weeks).

Although researches show that different microorganisms can grow on the toothbrushes after its usage[1,9,16], there is insufficient data about the negative consequences of the bacterial growth on the toothbrushes to the oral and systemic health. However, the pathogenic contamination in the vulnerable population, like critically ill patients, immunosuppressed patients, elderly persons, pregnant women and children may raise the risk of infection and its transfer.

It is obvious that there is insufficient information about toothbrush contamination as a factor that can influence the oral health; the data that the main reason for replacing of the used toothbrush was the deformity of the filaments in 75% of the examinees (Graph 8) shows that. It is worrying that only 10% of the respondents in the study answered that the recommendation of the dentist is the main reason for toothbrush replacement; it shows that only a little attention is paid to this segment of the oral hygiene.

Our expectations are that the dentists will become more active in including the advices and recommendations for maintaining proper toothbrush care during giving the instructions for the oral hygiene methods, because of the high bacterial contamination that dynamically increases with the time of use, hence increasing the potential for worsening the patients' health.

Conclusion

In the time of sophisticated therapeutic methods it is important to see the toothbrush as a possible source for potential pathogens that can compromise not only the health of the patient, but the results of the undertaken actions in the contemporary medicine. Toothbrushes may have an important role in transferring microorganisms and increasing the risk of infection, because they can be a reservoir of microorganisms in healthy individuals, in those with oral diseases, as well as in those with impaired general health.

The contemporary dentistry underlines the prevention and control of infection, it is very important the toothbrushes to be properly kept, disinfected and replaced in regular periods of time in health population, but especially in individuals affected with oral or systemic diseases. It is needed more attention to be paid and the dentists to be more involved in order to

aware the patients for the issue of choosing, keeping and maintaining the hygiene of the toothbrushes, as well as their replacement in an optimal time intervals.

Declaration of Interest

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