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**Research Article** 

# Microbiological Evaluation of Toothbrushes Contamination while their using Period and Storage - a

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#### **INTRODUCTION**

Toothbrush, toothpaste and floss are an integral part of everyday life. Due to convenient packaging, toothpaste and floss do not provide conditions, suitable for microorganism's accumulation on their surface, which is not true for toothbrush. It gradually assembles plaque together with all contents. S. Taji and A. Rogers [1] revealed that in period of 3 weeks manual toothbrush accumulate critical level of microorganisms on its working surface, which is direct indication for its replacement.

Composition of microorganisms on working surface of toothbrush is quite individual and depends on variety of factors. Frazelle MR, Munro CL [2] in their study showed that infectious diseases significantly increase microbial contamination; therefore it is necessary to replace the brush frequently.

Baluda MI et al. [3] revealed that exposition of 0.05% chlorhexidine solution can bring to complete absence of microorganisms on brush surface.

In 2000, Nelson Filho and Gisele Faria in their study studied the microbial contamination of toothbrushes and methods of their disinfection. As antiseptic solutions, they used a 0.12% chlorhexidine gluconate solution and a 1% sodium hypochlorite solution. After their use, toothbrushes were soaked in these solutions for 20 hours. The results of microbiological studies showed a complete absence of microbial coordination [4].

Another factor of brush contamination is storage method. According to recommendations of ADA (American Dental Association-2011), toothbrush should be stored vertically in a cup or in a wall holder, brushes should not touch each other and any other surfaces in the bathroom (walls, mirrors, etc.) and should be located at least 1 meter from the pan [5].

Fine results of toothbrush antiseptic processing motivated us to perform our own study. The aim of the study was to analyze microbial contamination of toothbrushes surface depending on it's storage method and using period.

#### MATERIAL AND METHODS

We studied 28 toothbrushes with synthetic bristles from one batch of the same manufacturer. They were divided into 4 groups:

The first set was not used for teeth cleaning and served as a control set.

Second set was given to a group of visually healthy students together with 0.05% chlorhexidine solution. Group of students were told to keep daily twice teeth cleaning procedure with following rinsing in water. After rinsing, students need to process working surface of toothbrush with chlorhexidine solution without washing it off until next use.

Third set used by a group of students also process twice teeth cleaning per day, but after each cleaning they washed the brush with water and store it in a glass with working surface put upward.

Forth set of new toothbrushes was put in the individual bathroom according to the recommendations of ADA (2011).

Microbial contamination of the first set was analyzed immediately

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after unpacking, 2-4 sets - after 2 weeks. Each toothbrush was dipped in sterile sugar broth for 1 minute, without touching the plastic base of the brush field. Then a standard loop was sown on blood agar using the J. Gold method (1992) to quantify CFU / ml (CFU is a colonyforming unit)

Visually divide the Petri dish into 3 sectors. We apply a standard loop with the culture on the surface of agar in sector 1. The loop is sterilized in a flame and allowed to cool. It is then looped over the surface of the medium in sector 1, and then immediately zigzag it into the surface of the medium in sector 2. The loop is heated in the flame and allowed to cool. And the last step is a loop on the surface of the medium in sector 2, and then it touches the surface of the medium in sector 3.

The cultivation of microorganisms was carried out under aerobic and micro aerophilic conditions at a temperature of  $37^{\circ}$ C for 24 hours. The number of grown colonies was calculated by the formula N = 2nk, where k is a factor equal to  $10^2$ ,  $10^4$ ,  $10^6$ , respectively, for sectors 1,2,3; n - the number of colonies of microorganisms in the last sector, where there was an increase. Results are presented in decimal logarithms.

#### RESULTS

#### The analysis revealed following results

The analysis revealed that lg of infection with aerobic and optionally anaerobic microorganisms of the working field of toothbrushes of group 1 is 2,  $74 \pm 0$ , 12 CFU, which indicates the presence of microorganisms on the bristles from the stage of placement of brushes in the original packaging.

When comparing the contamination of toothbrushes of group 2, microbial contamination was lg CFU = 0, 91 ± 0, 33, and the contamination of bristles in toothbrushes of group 3 was 4, 71 ± 0, 64 CFU. The data clearly show the reduction of contamination of the working field of toothbrushes as a result of their treatment with a 0.05% chlorhexidine solution 5 times (p < 0.05).

When analyzing the microbial contamination of the working field of toothbrushes, 4 groups revealed that the value lg CFU is equal to 6,  $73 \pm 0$ , 67. The result shows a high degree of protection of microorganisms.

#### CONCLUSION

Toothbrushes in their retail packaging are already contaminated with a sufficient number of aerobic and micro aerophilic microorganisms. Using a 0, 05% solution of chlorhexidine seems to be efficient to eliminate microorganisms from toothbrush surface.

Despite applying all recommendations for toothbrush storage, practically it is impossible to meet fine contamination rate on toothbrush.



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