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Original Article

Evaluation of relationship between toothbrush keeping place and its microbial content

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Abstract

Introduction: Maintaining good oral hygiene is an important factor in health. Toothbrushes are commonly used to maintain oral health and prevent dental disease, but unfortunately how keeping the toothbrush is neglected. The aim of this study was to investigate the relationship between toothbrush keeping method and its microbial content.

Methods: In this cross-sectional study, 60 volunteers were enrolled and divided into 3 groups based on the places of keeping their toothbrushes (bedroom, bathroom and lavatory). The participants were asked to brush once a day for one month using the first toothbrush which had been delivered; then the first toothbrushes were gathered and a second toothbrush was delivered. The participants were asked to brush once a day using the second toothbrush for 3 months. All toothbrushes were sent for culture and evaluation. All toothbrushes were evaluated by a blind microbiologist. Toothbrush bristles were washed in BHI broth medium; then the resulting liquid was cultured in MacConkey's agar for gram-negative bacteria and in blood agar and chocolate agar for gram-positive bacteria. Colony counts of Streptococcus mutans, Candida albicans, Pseudomonas, Klebsiella, S. aureus, and E. coli were determined and multiplied by one thousand. Data were analyzed by SPSS version 18 and using Kruskal-Wallis test.

Results: At the end of the study the results showed statistically significant differences in microbial load between the groups (p=0.014). Toothbrushes that were kept in bathroom had highest microbial load.

Conclusions: Toothbrushes kept in the bathroom had the greatest microbial contamination after three months. According to the results of this study, bathroom is the worst place and bedroom is the best place for keeping toothbrushes.

Keywords: Hygiene,Toothbrushing,Candida albicans, Streptococcus mutans, Pseudomonas, Klebsiella

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بررسی ارتباط محل نگهداری مسواک و محتوای میکروبی آن

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چکیده

مقدمه: نگهداری مناسب بهداشت دهان یک فاکتور مهم در سلامتی است. امروزه مسواک ها به طور شایع برای نگهداری بهداشت دهان و جلوگیری از بیماری های دندانی استفاده می شود. اما متأسفانه نحوه نگهداری از مسواک مورد غفلت قرار می‌گیرد. هدف مطالعه حاضر بررسی ارتباط نحوه نگهداری مسواک و محتوای میکروبی آن می باشد.

مواد و روش ها: در این مطالعه مقطعی پس از انتخاب ۶۰ نفر داوطلب سالم بر اساس معیارهای ورود و خروج، داوطلبین بر اساس پرسش از آنها درباره نحوه معمول نگهداری مسواک (اتاق خواب، حمام و دستشویی) به سه گروه ۲۰ نفری تقسیم شدند. از داوطلبین هر گروه خواسته شد که از مسواک های تحویل شده جهت مسواک زدن یک بار در روز استفاده نمایند و مسواک پس از یک ماه توسط محقق جمع آوری گردید، سپس مسواک دوم در اختیار داوطلبین قرار گرفت و سه ماه بعد جمع آوری گردید. سپس مسواک ها جهت کشت و بررسی به آزمایشگاه ارسال شد. بریستل های مسواک در یک میلیمتر از محیط کشت Bhi broth شسته شده و مایع حاصل شده را در دو محیط کشت Mac conkey برای باکتری های گرم منفی و blood agar و شکلات آگار برای باکتری های گرم مثبت کشت داده شد. سپس تعداد کلونی های میکروارگانیسم ها از لحاظ وجود استرپتوکک موتان، کاندیدا آلبیکنس، سودوموناس، کلبسیلا، استافیلوکک طلایی Ecoli شمرده شده و سپس در هزار ضرب گردید. داده ها در نرم افزار SPSS 18 و با استفاده از تست کروسکال والیس آنالیز گردید.

یافته ها: نتایج نشان داد در ماه اول و سوم بین گروههای مختلف از نظر تعداد میکروارگانیسم ها ارتباط آماری معنی دار وجود دارد$p\text{-value}=0.014$ مسواکهایی که در حمام نگهداری می شدند بیشترین بار میکروبی را داشتند.

نتیجه گیری: مسواک های نگهداری شده در حمام، پس از ۳ ماه بیشترین میزان آلودگی میکربی را دارا بودند. طبق یافته های این مطالعه بدترین محل نگهداری حمام و مناسب ترین محل جهت نگهداری مسواک ها اتاق خواب می باشد.

واژگان کلیدی: بهداشت، مسواک زدن، کاندیدا آلبیکنس، استرپتوکوکوس موتانس، سودوموناس، کلبسیلا

Introduction

Oral health is a part of the general health and influences it directly and indirectly. Therefore, maintenance of good oral hygiene is an important factor in the general health.[1] Today, toothbrushes are commonly used for cleaning the oral cavity and preventing dental diseases. Unfortunately, toothbrush care methods are often ignored.[2] Limited studies have evaluated the toothbrush microbial content and these studies have shown the growth of different microorganisms such as Streptococcus, Staphylococcus, and Lactobacillus on toothbrushes lead to infections in the oral cavity. These microorganisms can produce

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caries, gingivitis or infectious endocarditic. These problems can affect the general and oral health.[3]

However, no studies have been investigated the Pathogenesis of these microorganisms. This study was designed to assess the relationship between toothbrush keeping method and its microbial content and the possibility of pathogenesis of organisms which were cultured in the toothbrushes.

Methods

Sixty healthy (without known disease) volunteers were selected in this cross-sectional study. The Ethics Committee of Zahedan University of Medical Sciences approved the study protocol. Exclusion criteria included known systemic disease, use of medicine, pregnancy, hospitalized people in six months ago, use of oral rinses, any oral lesions, smoking or use of any form of tobacco and people with periodontal diseases (pocket depth>3 mm), severe caries (DMFT>4) and people under 18 or over 60.

The three groups were matched for age and sex. Volunteers were included in the study based on Poisson method (In this manner the samples were selected from all individuals who came to the center of this study over time).[4]

Volunteers were divided into 3 groups based on the places where they kept their toothbrushes:

Group 1: people who kept their toothbrushes in the lavatory

Group 2: people who kept their toothbrushes in the bathroom

Group 3: people who kept their toothbrushes in the bedroom

After signing a consent form, a soft cross-action Oral B toothbrush (Procter & Gamble Company, New bridge, Co Kildare, Ireland) was delivered to each volunteer. The volunteers were asked to use their toothbrushes once a day for one month; we reminded them periodically for the keeping place, after this period the toothbrushes were collected and another toothbrush was delivered to each subject. The subjects were asked to use the new toothbrushes once a day for three months; we reminded them periodically for the keeping place. After 3 months, the second toothbrushes were collected. All toothbrushes were transferred to a laboratory in sterile bags and were evaluated by a blind microbiologist. Toothbrush bristles were washed in BHI (Brain Hard Infusion) broth medium; then the resulting liquid was cultured in MacConkey's agar for gram-negative bacteria and in blood agar and chocolate agar for gram-positive bacteria.

Then the colonies underwent Gram staining. Additional tests including oxidase and catalase and specific tests including mannitol salt agar, Sabouraud dextrose agar, coagulase and Simmons citrate TSI (Triple Sugar Iron aguar) and SIM (SH2, Indol, Motivation) were used.

Colony counts of Streptococcus mutans, Candida albicans, Pseudomonas, Klebsiella, *S. aureus*, and *E. coli* were determined and multiplied by one thousand. Data were analyzed by SPSS version 18 software. Kruskal-Wallis test was used for comparison of groups. Statistical significance was defined at $p<0.05$.

Results

In this study, 60 healthy volunteers were participated. The mean age of the participants was 25 (20-35) in all groups. In each group, 50% of the participants were women and 50% were men. The average microorganisms counts and counts of contaminated toothbrushes at the end of the first month and the end of the third month are presented in table 1. Kruskal-Wallis test revealed statistically significant differences between the groups ($p=0.014$ and $p=0.046$ respectively).

At both intervals, the greatest microorganisms counts were observed in group 2 and the least ones were observed in group 3. At the end of third month, 81 percent of toothbrushes were averagely contaminated and the most contamination was belonged to the toothbrushes that were kept in bathroom.

The incidence of microorganisms that reached pathogenic levels at the end of first month is shown in table 2.

At the end of first month, Staphylococcus aureus, E. coli and Klebsiella reached pathogenic levels in group 2. There were no pathogenic levels of microorganisms in other groups.

Table 3 presents the incidence of microorganisms that reached pathogenic levels at the end of the third month. The maximum microorganisms that reached pathogenic levels were observed in group 2 and the least ones were in group 3.

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Table1. The microorganisms counts at the end of the first month and third month

	First month				Third month			
	No	Mean	Min	Max	No	Mean	Min	Max
Bedroom	13	2×10^2	10	10^3	15	3×10^4	10	10^5
lavatory	13	2.5×10^4	10	10^5	16	5×10^4	10	10^5
Bathroom	16	4.5×10^4	10	10^6	18	7×10^4	10	10^5
p-value	p=0.014				p=0.046			

Table2. The incidence of microorganisms that reached pathogenic levels at the end of the first month

	Bedroom		lavatory		Bathroom	
	Mean	IPM*	Mean	IPM	Mean	IPM
Candida	10	0	10^3	0	5.5×10^2	0
Staphylococcus aureus	4×10^2	0	7.5×10^3	0	7.5×10^4	15%
Klebsiella	4×10^2	0	10	0	6×10^4	15%
E. coli	2.5×10^4	0	10^3	0	5×10^4	5%
Anterobacter	0	0	10	0	0	0
Staphylococcus epidermis	4×10	0	10	0	10	0
Lactobacillus	0	0	10^2	0	0	0

***Incidence of pathogenic micro-organisms**

Table3. The incidence of microorganisms that reached pathogenic levels at the end of third month

	Bedroom		lavatory		Bathroom	
	Mean	IPM	Mean	IPM	Mean	IPM
Candida	10^3	0	5.5×10^4	0	10^5	10%
Staphylococcus aureus	5×10^4	5%	10^5	30%	10^5	35%
Klebsiella	3×10^3	0	10^3	0	7×10^4	40%
E. coli	2.5×10^4	5%	6.7×10^4	10%	5×10^4	10%
Anterobacter cloacae	10^3	0	10^4	0	5×10^4	10%
Haphnia	10	0	0	0		0
Stinobacter	10^2	0	0	0		0
Staphylococcus epidermis	10	0	5×10^4	5%	10	0
Streptococcus mutans	4×10	0	6×10	0	7×10	0

Discussion

The results of this study revealed that the place of keeping toothbrushes and the duration of their uses play important roles in their contamination and these findings were consistent with the results of Karibasappa.[1] It seems to regard that the bath temperature and humidity can cause the growth of microorganisms. Glass in 1998 studied patients with inflammatory oral diseases and reported that 34% of patients were completely cured when they changed their toothbrushes.[5] 1978-survey results indicated that brushing with a contaminated toothbrush could transfer new microbes into the oral cavity and alter the balance of microbial flora.[6]

In various studies, a variety of microorganisms including Streptococcus, Staphylococcus, Candida,

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Corynebacterium, Pseudomonas, Porphyromonas gingival is, Streptococcus mutans, Lactobacillus and Klebsiella were cultured on toothbrushes after using them and some of them were not part of the normal flora of the mouth. However, only one study assessed the location of toothbrush and its microbial content. Microbial content of the toothbrushes, which were kept in the bathroom adjacent to the lavatory, was higher.[1, 7-9]

Taji et al. in 1998 gave unused and sterile toothbrushes to 10 volunteers and showed that after 3 weeks all these toothbrushes were contaminated with Streptococcus, Staphylococcus, Candida, Corynebacterium and Pseudomonas.[7] In 2000, Bunetel evaluated microbial load of three different types of toothbrushes after 24 hours and isolated Porphyromonas gingivalis, Streptococcus mutans and Candida albicans from all the toothbrushes.[8]

Karibasappa et al. in 2011 showed that all toothbrushes kept in the bathroom adjacent to the lavatory were contaminated with Streptococcus mutans, Staphylococcus aureus, Lactobacillus and Klebsiella after 3 months and their results were similar to the results of the present study.[1]

Ferreira in 2012 investigated 40 toothbrushes in people aged 3-58 years. E. coli, Klebsiella, Streptococcus pyogenes and coagulase-negative Staphylococcus were found in toothbrushes[9] (table 4).

Table4. Types of microorganisms in different studies

study	year	Type of microorganisms
Taji	1997	Streptococcus, Staphylococcus, Candida, Corynebacterium and Pseudomonas
Bunetel	2000	Porphyromonas gingivalis, Streptococcus mutans and Candida albicans
Karibas appa	2011	Streptococcus mutans, Staphylococcus aureus, Lactobacillus and Klebsiella
Ferreira	2012	E. coli, Klebsiella, Streptococcus pyogenes and coagulase-negative Staphylococcus

In many studies, different species have been reported different microbial flora in people. Sogi et al. investigated the incidence of microbial contamination of toothbrushes at different intervals and demonstrated that toothbrushes had the maximum contamination at the end of 28_{th} day and had the least contamination after one day. It was shown that time had an important factor for the incidence of toothbrush contamination which was consistent with the results of this study.[10]

Although other studies did not evaluate the role of toothbrushes place where they are kept, they demonstrated that the toothbrushes had microbial contamination. These studies were not investigated the pathogenic levels of microorganisms therefore it was not possible to compare the results of the present study with those of other studies

Conclusions

The results of this study showed that keeping toothbrushes in the bathroom for 3 months resulted in the highest incidences of microorganisms pathogenic levels. According to the results of this study, the bathroom is the worst place and the bedroom is the best place for keeping toothbrushes. Since some microorganisms reach pathogenesis levels after 3 months in the bedroom, changing toothbrushes before this time is recommended.

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Conflict of interest: We declare that there is no conflict of interest.

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