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The Microbial Contamination of Toothbrushes and Their Disinfection by Antimicrobial Solutions

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ABSTRACT

Aims: To evaluate the bacterial survival rate on toothbrushes after brushing and the efficacy of their decontamination by immersing them in different antimicrobial solutions when toothbrushes were not in use. **Materials and Methods:** Twenty healthy dental students participated in this study, they were all supplied with a new toothbrush of the same brand and type together with identical tubes of fluoridated toothpastes and were asked to brush twice daily for a period of 4 weeks during which they were asked to follow their oral hygiene practices. The students were divided into 3 groups, the first group kept their toothbrushes in a ventilated area exposed to air after brushing; the second group kept their toothbrushes soaked in 1% Sodium hypochlorite solution (1 part bleach, 4 parts of distilled water); the third group immersed their toothbrushes in 0.2% chlorhexidine gluconate solution. After one month the toothbrushes were collected, decapitated and examined in the laboratory by making bacteriological cultures to identify the aerobic and anaerobic microorganisms in each toothbrush, also the total live bacterial counts per toothbrush were obtained. **Results:** The toothbrushes that were not immersed in any antimicrobial solution were heavily contaminated and the immersing of toothbrushes in 0. 2 % chlorhexidine gluconate solution was a very effective method in reducing the mean number of aerobic and anaerobic microorganisms. **Conclusions:** Toothbrushes can become contaminated after approximately 1 month of use, it is therefore recommended for individuals to use solutions like 0.2 % chlorhexidine gluconate which proved to be an effective antimicrobial agent to reduce toothbrush contamination.

Key words: Toothbrush, microorganism, chlorhexidine, sodium hypochlorite.

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INTRODUCTION

There is evidence that toothbrushes in regular use can be heavily contaminated with microorganisms [1–3], depending upon storage conditions, the toothbrush can serve as a reservoir for reintroduction of potential pathogens such as Streptococcus mutans [4]. Microorganisms from storage environments can also be introduced, which include enteric bacteria via aerosol near toilet flushing or from contaminated fingers and skin commensals and Pseudomonas originating from the bathroom and other wet areas [5].

Soaking the toothbrush in alcohol was one of the first recommended procedures for toothbrush disinfection in 1920 [6]. Later in 1929 Kauffmann [7] listed some methods for sanitation and drying of toothbrushes such as sunlight and table salt to absorb their moisture and to keep the brush in a closed container with a preparation containing formaldehyde for its disinfection, other methods included

the use of ultra violet light [8], immersion in a disinfecting solution [9,10], and spraying of antimicrobial solution on bristles [11–13].

The purpose of this study was to evaluate the bacterial survival rate on toothbrushes after brushing and the efficacy of their decontamination by immersing them in different antimicrobial solutions such as 1% Sodium hypochlorite and 0.2% Chlorhexidine gluconate solution in regard to bacterial contamination.

MATERIALS AND METHODS

Twenty dental students in their final year of study were given a new toothbrush of the same brand and type (Nice, manufactured by Nice House of Plastics, Iraq), along with identical tubes of fluoridated toothpaste Formula manufactured by PT Ultra Prima Abadi, Indonesia. they were all requested to follow their normal oral hygiene practices for 4 weeks period and not to take any antimicrobial drug during this period ,the students were randomly divided into three groups, the first group (six students) were asked to keep their toothbrushes in a ventilated area (not immersed in any solution); the second group (seven students) were asked to keep their brushes soaked in 1% sodium hypochlorite (1 part bleach and 4 parts of distilled water) after brushing; while the third group (seven students) kept their toothbrushes soaked in 0.2% chlorhexidine gluconate, at the end of which each toothbrush was collected in a sterile paper bag and processed within 18 hours of its last use.

Each toothbrush was decapitated using a sterilized end cutting nippers and the head transferred to a tube containing 10 ml of sterile phosphate– buffered saline (P.B.S) [14], the contents were then subjected to vigorous mixing for 60 seconds (Hook and Tucker instruments LTD /England), ultrasonication for 30 seconds by using an ultrasonic device (England), followed by further vortex mixing for 15 seconds [15]. Ten fold dilutions in (P.B.S) were then prepared for each toothbrush head and 0.1% of the appropriate dilutions was spread on duplicate of blood agar plates with a sterilized spreader [16]. One plate was incubated anaerobically by using anaerobic jar with gas pack (Oxoid, England), a H_2 and CO_2 generator envelope, which was activated simply by adding 10 ml of distilled water; then the jar was closed properly and incubated at 37 °C for 48 hours [17,18], while the other plate was incubated aerobically at 37°C for 48 hours. Total counts were then obtained [19].

For identification of microorganisms, an inoculating loop was used to transfer a drop of each (P.B.S) contents on to duplicate of blood agar plates, Sabouraud dextrose agar plate and MacConkey agar plate, which were then spreader and incubated. The first blood agar plate was incubated anaerobically for 48– 72 hrs, the second blood agar plate and MacConky agar plate were incubated aerobically, while Sabouraud dextrose agar was incubated at room temperature for one week, then the aerobic and anaerobic culture plates were examined and checked under light microscope. The morphology of different types of colonies were smeared to study the isolated types and then sub cultured to get isolated colonies and make biochemical tests on each microorganisms which were included, all organisms isolated were identified at least to genus level [20]. Statistical analysis was performed using ANOVA and Duncan's multiple range test, the results were statistically significant when $P \leq 0.05$.

RESULTS

The types of aerobic and anaerobic microorganisms isolated from the toothbrushes and incubated on the various medias are displayed in Table (1). The number of aerobic and anaerobic microorganisms isolated on blood agar from each toothbrush, counts are expressed as $\log_{10}$ colony forming unit per toothbrush, ranged between log 4.924 – 0 CFU in the aerobic microorganisms and 4.65–0 CFU in the anaerobic microorganisms depending upon the type of antimicrobial agent the toothbrushes were stored in, are shown in Table (2).

Table (1): Types of microorganisms found on the used toothbrushes.

Toothbrush number		Aerobic microorganisms	Anaerobic microrganisms
1	**Kept in air**	Staph. epidermidis , Lactobacillus sp. , Candida albicans	Bacteroides sp.
2		Moraxella catarrhalis, Staph. Epidermidis, α hemolytic streptococcus	Peptococcus sp.
3		Staph. epidermidis, Candida albicans , Bacillus subtilis	Peptostreptococcus sp.
4		Esch . coli , α hemolytic streptococci , Corynebacterium sp. (diphtheroids)	Peptococcus sp.
5		Staph. epidermidis , α hemolytic streptococci	Peptococcus sp.
6		Proteus sp. , Klebsiella sp. , Staph. epidermidis	Peptostreptococcus sp.
7	**NaOCl**	α hemolytic streptococci , Corynebacterium sp. (diphtheroid s)	Peptostreptococcus sp.
8		Staph. epidermidis ,	Peptostrept coccus sp.
9		Staph. epidermidis , α hemolytic streptococci Corynebacterium sp. (diphtheroid s)	Peptostreptococcus sp.
10		Candida albicans	No growth
11		Candida albicans , α hemolytic streptococci .	No growth
12		Staph. epidermidis , Pseudomonas sp.	No growth
13		Staph. epidermidis , Pseudomonas sp. Corynebacterium sp. (diphtheroids)	Veillonella sp. ,
14	**CHx**	Staph. epidermidis	Peptostreptococcus sp.
15		Corynbacterium sp. (diphtheroids)	Peptostreptococcus sp.
16		No growth	No growth
17		No growth	No growth
18		No growth	No growth
19		α hemolytic streptococci	Peptostreptococcus sp.
20		No growth	No growth

Table (2): Numbers of aerobic and anaerobic microorganisms isolated on blood agar from used toothbrushes , counts are expressed as log $_{10}$ colony forming unit (CFU) per toothbrush.

Toothbrush number	Aerobic microorganisms	Anaerobic microrganisms
Kept in air		
(1)	4.924	4.000
(2)	4.812	3.977
(3)	4.851	4.650
(4)	4.255	3.870
(5)	3.462	3.540
(6)	4.785	3.790
NaOCl		
(7)	3.477	1.690
(8)	3.041	1.845
(9)	3.690	2.040
(10)	3.602	0
(11)	3.361	0
(12)	3.301	0
(13	3.806	2.301
CHx (14)	2.040	2.000
(15)	2.860	1.840
(16)	0	0
(17)	0	0
(18)	0	0
(19)	2.410	1.954
(20)	0	0

NaOCl = Sodium hypochlorite, CHx = Chlorhexidine gluconate.

There was a highly significant difference in the total number of aerobic and anaerobic microorganisms between the three groups of toothbrushes as shown in Table (3), there was a statistically significant difference in the mean number of aerobic microorganisms depending on the type of solution the toothbrushes were soaked in with the least number of microorganisms in the toothbrushes that were soaked in the chlorhexidine solution with a mean of 1.044 CFU per toothbrush, followed by sodium hypochlorite 3.468 CFU per toothbrush, and the highest number was found in those which were kept in the air and not immersed in any solution with a mean of 4.515 CFU per toothbrush. On the other hand results showed that although the mean number of anaerobic microorganisms was less in the chlorhexidine group (0.699 CFU per toothbrush) compared with the sodium hypochlorite group (1.125 CFU per toothbrush), there was no significant difference between them and most of the microorganisms were found in the toothbrushes that were not immersed in any type of antimicrobial solution (3.971 CFU per toothbrush).

Table (3) Analysis of variance and Duncan's Multiple Range Test for the aerobic and anaerobic microorganisms in the three different groups of the toothbrushes according to their storage medium.

Groups of solutions	No. of tooth brush	Mean ± (SD)	
		Aerobic m.o	Anaerobic m.o
Kept in air (not immersed in any solution)	6	4.515 ± (0.568) ^{A}	3.971 ± (0.372) ^{A}
NaOCl	7	3.468 ± (0.258) ^{B}	1.125 ± (1.069) ^{B}
CHX	7	1.044 ± (1.324) ^{C}	0.699 ± (919) ^{B}

For aerobic m.o: F–value = 28.47; *p* value < 0.001. For anaerobic m.o: F–value = 89.92; *p* value < 0.001. NaOCl = Sodium hypochlorite, CHx = Chlorhexidine gluconate. Means with different letters are statistically significant at P ≤ 0.05 %.

DISCUSSION

There is increasing evidence that decay and periodontal disease are both contagious diseases that can be transmitted from one person to another, and in this day of organ transplants and alteration of the immune system [21] it is important to consider the toothbrush as a source of potential pathogens, due to microscopic cuts in gums and tongues caused by sharp toothbrush bristles that may act as portals of entry for bacteria, viruses and fungi that can rapidly bread on our toothbrushes .

The results obtained in this study showed that there were many types of microorganisms that were isolated from the toothbrushes, especially those which were not immersed in any antimicrobial solution as shown in Table (1), this is in agreement with other studies [1,2,15,22]. Staphylococci which were one of the mostly found microorganisms on many toothbrushes are non fastidious organisms that grow well on a range of selective media, their presence may be related to the fact that most of the individuals used their fingers during post brushing rinsing of their toothbrushes. Corynebacteria could have originated from the skin or the mouth, Streptococci almost certainly originated from plaque trapped in toothbrush bristles and Candida could have oral origins. The origin of Pseudomonas and Coliform could be environmental [5, 15], other types of microorganisms like Moraxella catarrhalis (which was formely classified in the genus Neisseriae catarrhalis), Bacteroids, Veillonella, and Lactobacilli could have also originated from the mouth [23].

The largest number of microorganisms were found on the toothbrushes that were kept in air Table (2), this group represents the most common hygienic measure that is undertaken by the majority of individuals with their toothbrushes, which is only rinsing in tap water without immersing in any antimicrobial solution, the same results were seen in previous studies [10,13].

As shown in Table (3), there was a significant reduction in the mean number of total aerobic and anaerobic microorganisms with in the three different groups that the toothbrushes were immersed in, that means that chlorhexidine and sodium hypochlorite significantly reduced the total numbers of microorganisms counts and in case of the aerobic microorganisms chlorhexidine appeared to have a superior inhibitory effect on microorganism growth. In case of anaerobic growth although chlorhexidine gluconate had a lower mean

number of microorganisms compared with sodium hypochloride, there was no significant difference between the two antimicrobial agents.

In previous studies chlorhexidine gluconate has been considered as a gold standard in its use as a potent antimicrobial agent in various uses [24 –28], although in another study [9] it was found that soaking the toothbrush in phenolic compounds (Listerine) for 20 minutes was sufficient to eliminate bacterial contamination.

There is a new product found in the markets of many developed countries called the toothbrush sanitizer or germ terminator [8] that uses an ultra violet bulb or steam combined by a proprietary automatic drying process to kill 99.99 % of the microorganisms present on toothbrushes, also another study [28] found that the design of the toothbrush in terms of filament anchoring had an effect on the retention of microorganisms on the toothbrush, in the absence of such products in our markets the method used to minimize contamination is by soaking the toothbrush in an antimicrobial solution, rinsing the bristles thoroughly after each use, and storing in an upright position which will help drain the water and dry the brush faster. If the brush was not soaked in an antimicrobial solution do not keep it in a closed container because a moist environment is more conducive to the growth of microorganism than open air, also try to put toothbrush away from sink or toilet to prevent air borne contamination and finally if more than one brush is stored in the same holder, keep the brushes separated to prevent cross contamination.

CONCLUSIONS

Toothbrushes can become contaminated after approximately 1 month of use which may play a role in systemic or local diseases, it is therefore recommended for individuals to use solutions like 0.2 % chlorhexidine gluconate which proved to be an effective antimicrobial agent to reduce toothbrush contamination, in addition toothbrushes should be changed at least every 1–3 months and after any illness and more frequently in children.

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