

## **Effectiveness of different cleanser solutions on the microbial contamination of toothbrushes.**

### **فاعلية المحاليل المطهرة على التلوث الميكروبي لفرش الأسنان**

Dhifaf Mohammed Saleh

Center of Market & Consumer Protection

University of Baghdad

#### **الخلاصة :**

تعتبر عملية تفريش الاسنان الطريقة الشائعة لتنظيف الاسنان وتجويف الفم وحماية الأسنان من التسوس واللثة من الامراض, من جانب اخر تتعرض فرشاة الاسنان للتلوث نتيجة الاحياء المجهرية ليس من الوسط الفموي فقط وانما من المحيط التي ستحفظ فيه فرشاة الاسنان. يهدف البحث الى تقييم فاعلية عدة محاليل منظفة لفرشاة الاسنان بعد استعمالها للقضاء على التلوث الميكروبي وهذه المحاليل (الكلور هكسدين, بيروكسيد الهيدروجين, محلول ملحي) واعتماد الماء كمحلول قياسي. اجريت التجربة على 32 متطوعاً اخضعوا قبل اجراء التجربة للفحص التام للتأكد من صحة وسلامة الفم والاسنان. حيث وزعت فرش ومعاجين اسنان ذات نوعية واحدة لكل متطوع وتم ارشادهم لاتباع نظام يومي خاص لاستعمال المحاليل المطهرة بعد استعمال فرشاة الاسنان. بعد انتهاء التجربة والتي استمرت اسبوعاً واحداً جمعت عينات فرشاة الاسنان واخضعت للاختبار الميكروبي. اظهرت النتائج انخفاض نمو الاحياء المجهرية بأستعمال محلولي الكلور هكسدين (2%) وبيروكسيد الهيدروجين (3%) بنسبة تصل الى 87.5% و 75% على التوالي مقارنة مع المحلول الملحي بنسبة (3%) فقد اظهر مفعولاً اقل لتطهير الاحياء المجهرية بنسبة 25%.

#### **Abstract:**

Toothbrushes may become heavily contaminated with microorganisms; these microorganisms may originate not only from the oral cavity but also from the environment where the toothbrushes are stored. The purpose of this study was to evaluate the effectiveness of different antimicrobial cleanser solution such as chlorhexidine, hydrogen peroxide and salt solution with tap water as a control group. 32 individual were supplied with the same type and brand of new toothbrush and tubes of toothpaste, and asked them to follow their usual oral hygiene practice. After one week the toothbrushes were collected for assaying the microbial contamination. The result was concluded that the effectiveness of disinfectant solution of chlorhexidine and hydrogen peroxide was reduced the microbial growth as much as 87.5% and 75% respectively comparing with the salt solution which was 25%.

#### **Introduction:**

Tooth brushing is the most common method of maintaing oral hygiene routine, tooth brushing helps clean accumulated dental plaque on the tooth surface and keep it healthy[1]. Toothbrushes may become heavily contaminated with microorganisms. These microorganisms may originate not only from the oral cavity but also from the environment where the toothbrushes are stored [2,3,4], contaminated toothbrushes may act as reservoirs for microorganisms originating from the environment depending upon storage condition, the toothbrush can therefore serve as a reservoir for the reintroduction of potential pathogens, such as mutans streptococci[5].

Many families normally store their toothbrushes in a common container in the bathroom, ignorant of the fact that micro-organisms from storage environments can also be introduced to the toothbrush. The moist and humid condition such as in bathroom may facilitate bacterial growth and cross contamination especially those encountered via aerosols from toilet flushing or from contaminated fingers and skin commensals and pseudomonads emanating from bathroom and other wet area[6].

This contamination implicates in the possibility of re-infection of a patient by toothbrushes

harboring pathogenic microorganisms. In (1920) Cobb was the first investigator who reports the recurrence of mouth infection that extended to the throat. When the patient was advised to soak his toothbrush in alcohol before and after using it, the patient recovered from the disease.

Glass and Lare [8] observed a correlation between contaminated brushes and the presence of diseases. Later, Glass and Shapiro[9] concluded that regardless of the nature of the disease, patients could achieve elimination of the symptoms and disease by just changing the toothbrush. Malmberg, *et al.*[2] reported heavy growth of enteric, yeasts and molds in toothbrushes used by children. Coli forms were also found in toothbrushes and their origin presumably is the toilet. [3,4,10]

Procedures for decontamination of toothbrushes would prevent the risks of reinfection or infection by other pathogenic microorganisms from the environment. Soaking the toothbrush in alcohol was one of the first recommended procedures for toothbrush disinfection in 1920. Later, in 1929, Kauffman [11] listed some methods for sanitation and drying of toothbrushes such as sunlight and table salt to absorb their moisture and to keep the brush inside a closed container with a preparation containing formaldehyde gas for its disinfection. Other methods included the use of ultraviolet light[12], immersion in a disinfecting solution[13,14], spraying of antimicrobial solutions on the bristles[15,16], use of a microwave oven and washing of the toothbrush in a dishwasher[6, 13].

### **Material and methods:**

Three mouth rinses containing different active compounds (2% Chlorhexidine (CHX), 3% Hydrogen peroxide and 3% Salt solution). All toothbrushes and toothpaste used in the study were purchased from a local shops. For the standardization purposes the same brand of both toothbrush and toothpaste were used throughout the study. The experiment was carried out for one week, 30 volunteers were given four new toothbrushes labeled as T1 (toothbrush rinsed with CHX), T2 (toothbrush rinsed with HP), T3 (toothbrush rinsed with Salt solution) and T4 (toothbrush rinsed with tap water). volunteers were to follow a normal oral hygiene routine by tooth brushing three times daily (Table1) with each time using a different toothbrush, each group was advised with the following instructions.

The percentage of contaminated toothbrush is measured according to this formula :

$$A = \frac{B * 100}{C}$$

A= Percentage (%) of contaminated toothbrush.

B=Number of contaminated toothbrush.

C=Total no. of toothbrush for each group.

#### **Group I:**

Volunteers were instructed to rinse their tooth brushes after brushing in running tap water for 20 seconds, and then soak their brushes in glass containing (CHX) for 20 minutes. The brushes were removed after 20 minutes and the solution was discarded and washing the container with tap water. Brushes were kept in containers that the head of the brush faces outward and left for drying in the bathroom. The amount of solution was taken in a container such that it covers the head of the brush every day new solution was used.

#### **Group II & III:**

In group II and III same procedure was followed, the disinfectant solution used to soak the brushes were 3% hydrogen peroxide in group II and 3% Salt solution in group III.

#### **Group IV:**

Volunteers were instructed to rinse their teeth brushes after brushing in running tap water for 20 seconds and left for drying in the bathroom in the container by keeping head of the brush facing outwards.

Table 1: Toothbrush rinsing schedule for seven days using disinfectant Solutions.

Days Time	1	2	3	4	5	6	7
Moring	T1	T4	T3	T2	T1	T4	T3
After lunch	T2	T1	T4	T3	T2	T1	T1
Before bed time	T3	T2	T1	T4	T3	T2	T2

T1= CHX    T2= HP    T3= Salt solution    T4= tap water

After one week the tooth brushes were collected and transported in separate sterile test tubes with a cotton plug for microbiological analysis. The handle of the toothbrush was disinfected with spirit. Each tooth brush was kept in a test tube containing peptone solution in which only the head of the brush was immersed. On opening of test tube the handle of tooth brush was covered with autoclaved cotton pellets and incubated for 5 hours in the incubator. Ten fold dilutions were made and 10µl was spread on a blood agar plate. The inoculated plates were incubated at 37 C° for 24 hours. Following incubation, the total colony forming units as well as counts of each individual colony types were recorded from all plate. The viable count was calculated from the average colony count/plate. Pure cultures of the isolated colonies were made and each representative colony was Gram-stained and examined for cell morphology and Gram reaction under a light microscope. The isolates were then subjected to bacterial identification procedures using the API Identification System.[17, 18].

### **Results and Discussion:**

Thirty two adult volunteers were divided into 4 groups eight for each group. In group I chlorhexidine mouthwash. Group II Hydrogen peroxide, group III salt solution, were used to disinfect the tooth brushes and group IV was control in which tooth brushes were rinsed with tap water. The total bacterial populations in tooth brush rinsed with CHX and HP showed a drastic reduction in the total bacterial population compared to salt solution and tap water. Tooth brush rinsing with PH ( $1.5 \times 10^6$  CFU ml<sup>-1</sup>) and CHX ( $3.2 \times 10^6$  CFU ml<sup>-1</sup>) has effectively reduced the total bacterial count as much as 87.5% and 75% respectively, compared to tooth brush rinsed with salt solution 25% ( $62.6 \times 10^6$  CFU ml<sup>-1</sup>) and tap water ( $67.5 \times 10^6$  CFU ml<sup>-1</sup>) (Table 2).

The data obtained (Figure 1) clearly shows the effectiveness of antimicrobial agent such as CHX and PH resulted in the reduction of microbes of toothbrushes.

Table 2: Percentage and number of contaminated tooth brushes and types with total colony forming units of microorganisms isolated from rinsed toothbrushes.

Groups	No. (%) of contaminated tooth brush	Bacteria	Total colony forming units (CFU <sup>-</sup> )
Group I (CHX)	2 (25.0)	<i>Klebsiella pneumonia</i> , <i>Bacillus</i>	3.2×10 <sup>6</sup>
Group II (PH)	1(12.5)	<i>Bacillus</i>	1.5×10 <sup>6</sup>
Group III (salt solution)	6 (75.0)	<i>Klebsiella pneumonia</i> , <i>Bacillus</i> , <i>Staph. Epi</i>	62.5×10 <sup>6</sup>
Group IV( tap water)	8 (100.0)	<i>Klebsiella pneumonia</i> , <i>Bacillus</i> , <i>Staph. Epi</i> , <i>Micrococcuspp</i> , <i>Staph.warneri</i> , <i>staph.haemolytic</i>	78.8×10 <sup>6</sup>

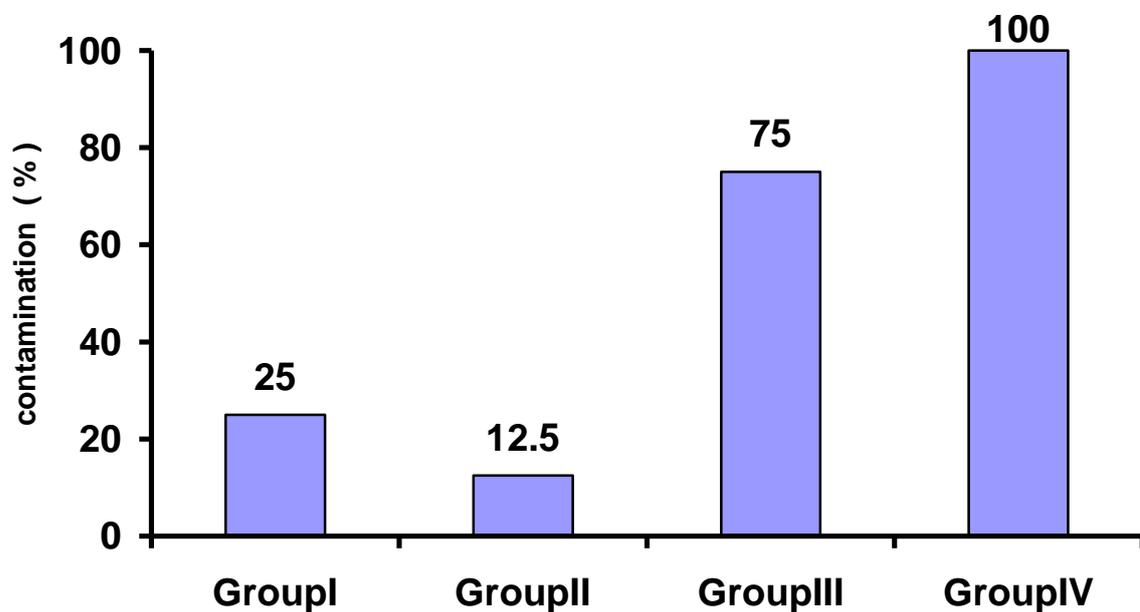


Figure (1): Percentage of contamination

of effectiveness disinfectant solution on microorganisms

There is little public awareness that tooth brushes may become contaminated by microorganisms with use. This contamination had already been well documented [2,3,4 ] and a contaminated tooth brush can be the cause of re-infection of a person with pathogenic bacteria [7,8,9 ] or can be the reservoir for environmental microorganisms [3,4,12 ] methods for toothbrush disinfection have been searched in order to avoid such events.

In this study various mouthwashes were used as disinfectants to know the efficacy in reducing the contamination of toothbrush. Disinfectants used were chlorhexidine, hydrogen peroxide, salt solution. In group I chlorhexidine showed that 25.0% microorganisms growth. Chlorhexidine acts against a wide range of gram positive and gram negative organisms and against fungi at PH5 to 8 ,bacterial spores are prevented from germinating [22], so it is indicated as the first-choice antiseptic in dentistry due to its high antimicrobial activity and effectiveness for presurgical antisepsis [20], while in the group II with hydrogen peroxide, it was observed that there was reduction in microbial growth as 12.5%. This result is agree with the finding of Sogi *et al.* [19], the observation may be attributed to the fact that in addition to hydrogen peroxide antimicrobial action. It also acts as a cleaning agent due to its ready release of nascent oxygen and its effervescence removes the debris from otherwise inaccessible regions. So hydrogen peroxide can be considered to be safe for usage as a disinfectant for toothbrush [20,21]

The brushes soaked in group III salt solution was increase in contamination of tooth brushes as 75%, this result maybe due to the bacteria in the tap water had over powered the salt. The salt could not kill all the bacteria in the tap water. [13].

The observation in group IV was also increase in contamination of tooth brushes in the control group as 100.0%, which implies that rinsing with water and air drying leads to the tooth brush contamination soon after the brushing. As chloride has been added to the water supplied for household consumption, thus the presence of this additional ion may have suppressive effects on microorganisms but it somehow promoted the growth of oral bacteria it implies that rinsing with water and air-drying is an incomplete procedure in cleaning the toothbrush.[18]

Storage conditions of toothbrushes are an important factor for bacterial survival. bacterial contamination can be reduced by washing toothbrushes after use and drying in aerated conditions , a wet environment increases bacterial growth and cross contamination. Therefore, as time increases between one tooth brushing and another, more microorganisms development can occur in the toothbrushes stored in a wet/moisture environment.[23,25] also the number of microorganisms in the tooth brushes kept in aerated conditions was lower than in toothbrushes stored in plastic bags.[15]

The American dental association recommends a routine change of toothbrushes every 3 months.[15] Patients who are sick should change their toothbrushes at the beginning of an illness, when they first feel better, and when they are completely well. Chemotherapy or immune-suppressed patients should change their toothbrushes every three days, and persons submitted to major surgery should change their toothbrushes every day. Many patients, however, reported psychological, economic, and environmental barriers to changing their toothbrushes so frequently. Establishing an easy and effective method for disinfecting a toothbrush would be an important and economical way to prevent the continuation of re-infection of oral diseases.[14]

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