# Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

J. Med. Sci., 6 (1): 59-62
January-March, 2006

# The Potential Use of Chlorhexidine (CHX) and Hexetidine-containing Mouth Rinse in Maintaining Toothbrush Sterility

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The study was carried out with the aim of demonstrating quantitatively the presence of microorganisms adhered to toothbrush bristles and to determine the potential of using antimicrobial agent (such as chlorhexidine gluconate (CHX) and hexetidine (HX)) in commercialized mouth rinses to reduce microbial contamination. The study was carried out by enumerating the total colony counts of bristles-adhered microbes after three weeks of normal oral hygiene followed by rinsing the toothbrushes with CHX, HX, tap water and deionized water independently following a strict planned schedule. Rinsing toothbrush with tap water was included in the study as a control due to the normal way of cleaning toothbrush after use in every home. Whereas, sterilized deionized water do not contain any ions, minerals and is microbes-free. The total colony counts of microbes obtained from the toothbrush rinsed with tap water, deionized water, CHX and HX were $62.6x10^6$ CFU $mL^{-1}$, $74.4x10^6$ CFU $mL^{-1}$, $2.4x10^6$ CFU $mL^{-1}$ and $7.6x10^6$ CFU $mL^{-1}$, respectively. *Staphylococcus aureus*, *Actinomyces naeslundii* and *Clostridium* sp. were isolated from toothbrush rinsed with tap water. *Staphylococcus aureus* and *Peptostreptococcus* sp. were obtained from toothbrush rinsed with deionized water. *Actinomyces* sp. and *Clostridium* sp. were recovered from toothbrush rinsed with CHX and only *Staphylococcus aureus* was obtained from toothbrush rinsed with HX. Although toothbrush rinsed with mouth rinses containing antimicrobial agent such as CHX and HX still harbour microorganisms, but the microbial load has been very much lowered compared to the control toothbrush. Thus, this indicates that toothbrush rinsing with mouth rinse after the normal oral hygiene is very convenient and cost effective to reduce toothbrush contamination.

**Key words:** Tooth brushing, chlorhexidine, hexetidine, mouth rinse

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## INTRODUCTION

Tooth brushing is the most common method of maintaining oral hygiene. Routine tooth brushing helps clean accumulated dental plaque on the tooth surfaces and keep it thin and healthy. In spite of the millions of toothbrushes sold each year around the world, there is however, little public awareness that with use, the toothbrush bristles may become heavily contaminated by microorganisms[1,2]. Many families normally store their toothbrushes in a common container in the bathroom, ignorant of the fact that microorganisms from storage environment can also be introduced to the toothbrush. The moist and humid condition such as in a bathroom may facilitate bacterial growth and cross contamination especially those encountered via aerosols from toilet flushing, contaminated fingers and skin commensals[2]. In other words, a regularly used toothbrush may act as reservoir for microorganisms and when used to reduce the existing normal oral flora in the plaque, the contaminating microorganisms could be reintroduced into the mouth[3,4]. Many ways have been suggested to avoid, or at least reduce microbial contamination in used toothbrushes[5-8]. The most common practice is to wash a toothbrush with running tap water, before and after use. In this study we would like to observe whether rinsing a toothbrush with two commercially available mouthrinses containing chlorhexidine gluconate (CHX) and hexetidine (HX) would help in controlling cross-contamination between these toothbrushes. A parallel study was also carried out using tap and deionized water for comparative purposes.

## MATERIALS AND METHODS

Two mouth rinses containing two different active compounds (0.12%-CHX and 0.1%-HX) were purchased from a local pharmacy. All toothbrushes and toothpaste used in the study were purchased from a local shop. For standardization purposes the same brand of both toothbrush and toothpaste were used throughout the study.

The experiment was carried out for three weeks in year 2004 and each volunteer was given four new toothbrushes, labeled as TA (toothbrush rinsed with deionized water), TB (toothbrush rinsed with tap water), TC (toothbrush rinsed with CHX) and TD (toothbrush rinsed with HX). Volunteers were to follow a normal oral hygiene routine by tooth brushing three times daily with each time using a different toothbrush (TA or TB or TC or TD) and every time after brushing, each toothbrush was rinsed with different kind of solution according to the label for each toothbrush. A strict usage regime was designed and Table 1 considering that all toothbrushes were used fairly in the experiment. Similar routine was continued till end of the third week study period.

At the end of the third week, the microbial contamination of each toothbrush were collected and cultured for growth following the method of Taji and Rogers[2]. Brain heart infusion agar was used as the agar base for blood agar. The head of the toothbrush was immersed in a culture bottle containing sterile distilled water before it was vigorously vortexed for 2-3 min to dislodge all bacteria adhering to its bristles. Sterile techniques were used to ensure sterility in order to avoid contamination especially from the environment. Ten-fold dilutions in sterile distilled water were then prepared and a surface viable count by spreading method were made with 0.1 mL of appropriate dilutions spreaded evenly on to blood agar surface before they were incubated at 37°C for 18 h. Following incubation, the total colony forming units as well as counts of each individual colony types were recorded from all plates. 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 (BioMerieux, France).

## RESULTS AND DISCUSSION

**Microbial population on rinsed-toothbrush:** The outcome results of this experiment on toothbrush contamination control were based on the viable microorganisms expressed in CFU $mL^{-1}$. The results revealed that toothbrushes in normal use are contaminated with microorganisms even after they were rinsed-off by antimicrobial mouth rinses (Table 2).

The total bacterial population in toothbrush rinsed with CHX and HX showed a drastic reduction in the total bacterial population compared to tap water. Toothbrush-rinsing with CHX gluconate ($2.4x10^6$ CFU $mL^{-1}$) or HX ($7.6x10^6$ CFU $mL^{-1}$) has effectively reduced the total bacterial count as much as 96.2 and 87.9%, respectively, compared to toothbrush rinsed with tap water ($62.6x10^6$ CFU $mL^{-1}$). The data obtained (Table 2) clearly shows that exposure to antimicrobial agent such as CHX and HX resulted in the reduction of oral microbes. It is possible that the antimicrobial effect of HX is exerted on the contaminants with traces of the mouth rinse on the

Table 1: Toothbrush rinsing rotation schedule for day 1 to day 7 using deionized water, tap water, CHX and HX

| | Days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Morning | TA | TD | TC | TB | TA | TD | TC |
| After lunch | TB | TA | TD | TC | TB | TA | TA |
| Before bedtime | TC | TB | TA | TD | TC | TB | TB |

TA = deionized water, TB = tap water, TC = CHX, TD = HX

Table 2: The colony forming units of microorganisms isolated from rinsed toothbrush

| | Deionized water | Tap water | CHX | HX |
|---|---|---|---|---|
| Total colony forming units (CFU $mL^{-1}$) | $74.4x10^6$ | $62.6x10^6$ | $2.4x10^6$ | 7.6x106 |
| Colony count of each isolated microorganism (CFU $mL^{-1}$): | | | | |
| *Staphylococcus aureus* | $45.0x10^6$ | $57.6x10^6$ | 0 | $7.6x10^6$ |
| *Peptostreptococcus* sp. | 29.4x106 | 0 | 0 | 0 |
| *Actinomyces naeslunsii* | 0 | $3.2x10^6$ | $1.0x10^6$ | 0 |
| *Clostridium* sp. | 0 | $1.8x10^6$ | $1.4x10^6$ | 0 |

toothbrush. Hence, this explains the drastic drop of the microbial load from the antimicrobial agent-rinsed toothbrush.

However, rinsing with deionized water ($74.4x10^6$ CFU $mL^{-1}$) has shown to have 18.9% increment in the total bacterial population compared to tap water. This was possibly due to deionized water did not contain any additional ions to suppress the inhibition of microorganisms to the toothbrush compared to tap water.

**Microbial contaminants on rinsed toothbrush:** Four bacterial strains were identified in the study, namely, *Staphylococcus aureus*, *Peptostreptococcus* sp., *Actinomyces naeslundii* and *Clostridium* sp. The total counts of each species is shown in Table 2.

*Staphylococcus aureus* was found on all toothbrushes except toothbrush rinsed with CHX and were often numerically dominant. The result is in agreement to the findings of Glass and Lare[3]. This microorganism, which is a common skin inhabitants, is also thought to be obtained from the environment e.g., water, dust, as toothbrushes were stored in the open air.

Tap water has the most numbers of different bacterial species isolated. Three different bacterial sp., *S. aureus*, *A. naeslundii* and *Clostridium* sp. have been identified and the microbial load was dominated by *S. aureus* ($57.6x10^6$ CFU $mL^{-1}$). As chloride and fluoride has been added to the water supplied for household consumption, thus the presence of these additional ions may have suppressive effects on *Peptostreptococcus* sp., but it somehow promoted the growth of oral bacteria *A. naeslundii* ($3.2x10^6$ CFU $mL^{-1}$). *Clostridium* sp. ($1.8x10^6$ CFU $mL^{-1}$) from the toothbrush could possibly be obtained from the tap water or even the pipeline supplying water for the household consumption.

Post-rinsing used toothbrush with mouth rinses have shown to have influence in reducing the bacterial total colony counts and bacterial types present. CHX gluconate proved to be very effective to reduced toothbrush contamination as this antimicrobial agent effectively inhibit the growth of *S. aureus* and *Peptostreptococcus* sp. as shown in table 2. This antimicrobial agent has moderately lowered the number of *A. naeslundii* ($1.0x10^6$ CFU $mL^{-1}$) compared to using tap water ($3.2x10^6$ CFU $mL^{-1}$). However, CHX gluconate was found to have no effect in controlling toothbrush contamination with *Clostridium* species ($1.4x10^6$ CFU $mL^{-1}$) as approximately the same total count was also obtained from toothbrush rinsed with tap water.

In contrast, HX has been shown to be very effective in maintaining toothbrush sterility from *A. naeslundii*, *Clostridium* sp. and *Peptostreptococcus* sp. when none of the species were isolated on the agar plates. The result is in agreement with the finding of Hefti and Huber[8], which indicated that both CHX and HX were effective antimicrobial agents to inhibit plaque bacterial accumulation, however HX has a better effect in reducing the total count of plaque bacteria compared to CHX. On the other hand, *S. aureus* was the only bacterial species isolated and has been possibly encountered from the environment. Although *S. aureus* was not completely killed, but the microbial load has been greatly reduced ($7.6x10^6$ CFU $mL^{-1}$) after rinsing with HX compared to rinsing with tap water ($57.6x10^6$ CFU $mL^{-1}$).

Deionized water is free of ions and microorganisms, which may interfere with bacterial growth. Thus, toothbrush rinsed with deionized water was expected to harbour bacteria from the mouth, representing the number of bacteria carried over from the mouth to the used toothbrush. Besides *Staphylococcus* sp. ($45.0x10^6$ CFU $mL^{-1}$), *Peptostreptococcus* sp. ($29.4x10^6$ CFU $mL^{-1}$) has been shown to be isolated from the toothbrush rinsed with deionized water. This may be due to the contaminants acquired from the environment where the toothbrush has been kept throughout the experiment.

The study has shown that bacteria either from dust or water from the environment, contaminated hands, skin commensals or the oral cavity could easily contaminate toothbrushes. Rinsing toothbrush regularly with mouth rinse-containing CHX or HX every time after being used appeared to be a practical and cost effective procedure to control and eliminate microbes in the home environment as microbes were commonly trapped or adhered to the toothbrush bristles.

**ACKNOWLEDGMENTS**

The authors gratefully acknowledge Khoo, G.S. and S.G.K. Hasnah, for the laboratorial assistance and Mohd-Ariff, Z. for the technical assistance throughout the study undertaken.

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