The Effectiveness Of Controlling Toothbrush Contamination By Chlorhexidine

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Introduction

Tooth brushing is the most popular and effective way of cleaning tooth surfaces off dental plaque. Besides being easy to use, toothbrush is cheap and affordable by almost everybody. To obtain the maximum result out of a toothbrush, one is advice to change a toothbrush every three months or so. However, except from washing the toothbrush with tap water, there has never been any suggestion as to how the toothbrush should be kept after use to ensure that it is clean before it is used again. It was reported that depending on storage conditions, a toothbrush in regular use can serve as a reservoir for the reintroduction of potential pathogens from the mouth,\(^1\)\(^2\) and also those from the storage environments.\(^3\)

In this study we would like see whether washing toothbrush with normal tap water is effective enough in avoiding bacterial contamination. We would compare these results with those obtained from toothbrush washed with deionized water. Comparisons of results were also made with toothbrush post-rinsed with mouthwash containing chlorhexidine gluconate. In addition to that, we would also determine if toothbrushes carry pathogenic microorganisms from the mouth itself, or from the environment where it is being stored.

Materials And Methods

Chlorhexidine gluconate (0.12%)
A mouthwash purchased from a local pharmacy.

Toothbrush
Standard designed toothbrushes bought from a local shop.

Growth media
Brain heart infusion agar (BHI) was used as the agar base for chocolate agar (CA) and blood agar (BA).

Experimental design. A volunteer was given three toothbrushes to be used in a period of three weeks. The volunteer was advised to use Toothbrush A (TA) in the morning, Toothbrush B (TB) after lunch and Toothbrush C (TC) after dinner. TA was always washed with deionized water after used. TB with tap water and TC with chlorhexidine gluconate. The order of use of the three toothbrushes was rotated the following day with TB, TC and TA in the morning, after lunch and after dinner, respectively. This regime was repeated and followed closely for three weeks to ensure all toothbrushes have been fairly used.

Isolation Assay. The head of the toothbrush was carefully immersed in a culture bottle containing sterile distilled water and strongly shaken to detached any bacteria adhering to the bristles. The toothbrush was then discarded and 20µl of the bacterial suspension was pipetted out and inoculated into a culture tube containing 5ml of BHI broth. The tubes were incubated at 37°C for 18hours. Following the incubation period, 20µl of the growth suspension was taken for inoculation on to agar plates; BA, CA and BHI consecutively. The plates were incubated for 18hours at 37°C. The population of bacteria colonies observed following incubation was counted. For identification purposes, pure colonies of each of the bacteria present in each plate were made. Gram staining was the carried out on each of the different types of colonies. Those that stained as blue coccus were subjected to the catalase and coagulase tests. Bacteria were further identified down to the species level using the API identification System (BioMereux, France).
Results And Discussions

Isolation and quantitation of individual genera was attempted using blood and chocolate agar plates. Blood agar was used because it would allow the maximum growth of oral microorganisms carried over from the mouth. Since most oral microorganisms are fastidious, therefore a rich media such as the blood agar has to be used. Chocolate agar on the other hand, was used as it is the best media allowing the growth of contaminants especially those of the staphylococci. In addition to that, chocolate agar was used to grow contaminants originating from the water system as well as from dust in the environment.

Total population of bacterial contaminants on toothbrush

Results obtained from the total bacterial population showed an 8.6% (62.6x10^6 CFU) reduction in CFU counts from TB compared to that from TA (74.4x10^6 CFU). Post-rinsing the toothbrush with CHX (TC) showed the least CFU counts (2.4x10^6 CFU) with a reduction of 93.8% compared to TA (Figure 1).

Bacterial contaminants of toothbrush

Deionized water is free of ions and microorganisms which may interfere with bacterial growth. It was expected that TA would harbor bacteria of the mouth and thus, represented the maximum number of bacteria carried over from the mouth. Different from what have been reported in earlier studies, Peptostreptococcus spp. and not streptococci were the majority originating from the oral cavity. At the same time, the toothbrush would have also acquired contaminant(s) from the environment where the toothbrush has been kept throughout the experiment (for example within which there is a toilet). And in accordance with results obtained by other researchers, Staphylococcus spp. are the most commonly isolated contaminant. In this study S. aureus made up to about 40% of the total CFU isolated from TA.

<table>
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<td>CHX post rinsing</td>
<td>Actinomyces naeslundii, Clostridium spp.</td>
<td>Contaminant from dust, water</td>
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Table 1: Bacterial contaminants on treated used toothbrushes

Tap water has gone through treatment processes with chlorine and fluoride before they are supplied for human consumption. The presence of additional ions such as chlorines and fluorides may have suppressive effect on the oral bacteria such as Peptostreptococcus spp. but it somehow promoted the growth another oral bacteria, Actinomyces naeslundii. S. aureus in this case formed a larger component of contaminant of about 90% compared to the above TA. Additional to those acquired from the dust in the environment, S. aureus could have easily come from the tap water used for rinsing the toothbrush. Clostridium spp. was another contaminant isolated from TB, however it formed very minor existence of about 2%.

Post-rinsing used toothbrush with CHX gluconate (TC) proved an effective way to reduced contamination by A. naeslundii. The growth of A. naeslundii was showed to dropped about 52% in TC compared to TB. However, with respect to Clostridium spp., post-rinsing the used toothbrush with CHX gluconate showed no effect at all. On the other hand, it is the best way of controlling contamination by S. aureus as none were isolated from TC.

Conclusions
All toothbrushes were found to be contaminated with bacteria either from the oral cavity, or/and from dust and water within the environment. *Staphylococcus* spp., comparable to other studies²,³ is the most common contaminant on used toothbrushes. Although a proper mechanism for sterilization of toothbrush need to be develop, rinsing a toothbrush with mouthwash such as chlorhexidine gluconate proved an effective procedure to follow.

**References**