RETAINED MICROBIAL FLORA OF TOOTH BRUSHES USED BY A GROUP OF HEALTHY DENTAL STUDENTS

J.A.M.S.JAYATILAKE* AND H.N.FERNANDO

Division of Microbiology, Faculty of Dental Sciences, University of Peradeniya

Introduction

Many microorganisms including oral commensals survive on tooth brushes posing a threat of cross infection and self inoculation (Taji and Rogers, 1998; Warren et al., 2001). However, there have been no previous studies on the retained microbial flora (RMF) of tooth brushes used by Sri Lankans. Therefore, this project was designed to obtain preliminary data on the RMF of tooth brushes used by a group of healthy dental students.

Methodology

Study group comprised of randomly selected fifteen dental students (eight males and seven females) in the age of 22-23 years. All of them had healthy periodontium and minimum of twenty eight teeth in the mouth. Those who had undergone any antibiotic treatment or dental scaling three months prior to the experiment were excluded. Individuals having prosthodontic or orthodontic appliance therapy were also excluded. After obtaining the informed consent, their tooth brushes were collected within one hour of last tooth brushing and they were replaced with new brushes. Information regarding the type of tooth brush, duration of use, tooth paste, toothbrush cleaning and storage practices and also brushing habits were recorded. Microbial cultures of collected tooth brushes were done within two hours of collection following a modified method of Brunetel et al., (2000). In summary, microbes from the brush-head and bristles were dislodged into 10ml of sterile phosphate buffered saline (PBS) by vortexing, for 3 min. Resultant suspension was centrifuged at 1500g and the pellate was resuspended in 1ml of sterile PBS. Afterwards, ten fold dilutions were made using PBS. 75µl of 10^{-3} dilution was inoculated in triplicate into blood agar (BA), MacConkey agar (MA) and Sabouraud dextrose agar (SDA) plates. Plates were incubated aerobically and anaerobically at 37°C and saturated humidity for 48 h, and colony forming units per ml (CFU/ml) were counted. Colony morphology, hemolysis on blood agar, Gram stain, catalase test, coagulase test, oxidase test and lactose fermentation test were used to identify different bacteria cultures. Correlations among CFU counts of aerobic BA anaerobic BA and MA were determined using Pearson's correlation (SPSS 11.5; SPSS Inc. Chicago, IL, USA).

Results

All tooth brushes were of similar type and they had been used at least for one month duration prior to the day of collection. All participants used fluoridated tooth paste and brushing frequency was twice a day. Four participants (27%) did not wash tooth brush prior to use. Six participants (40%) stated that they store their brushes after tooth brushing everyday in the bath room. Others kept their brushes outside. All used tap water to wash their tooth brushes. Except one, all tooth brushes showed microbial growth on culture plates. RMF levels accounted up to $10^4 - 10^6$ CFU/ml on aerobic BA anaerobic BA and MA. Many tooth brushes produced 10^5 CFU/ml in any of the culture media used. These colonies gave about five to ten morphotypes per one tooth brush. Only one tooth brush showed colony growth on SDA. Gram staining showed that RMF is full of Gram positive and negative organisms of different morphologies arranged in numerous patterns. Gram positive cocci in clusters that gave positive catalase and coagulase tests were isolated from four tooth brushes. Several lactose fermenting Gram negative bacilli were detected on MA cultures. Gram negative, nonlactose fermenting bacilli with positive oxidase reaction were isolated from two tooth brushes. Filamentous Gram positive rods that

give characteristic yellow granular colonies on BA and Gram negative spindle shaped rods were detected separately from two brushes. One tooth brush carried gram positive budding yeast. CFU counts of the RMF on aerobic BA, anaerobic BA and MA were positively correlated ($r1 = 0.79$, $r_2 = 0.85$, $r_3 = 0.77$; $p < 0.05$).

Discussion

Present data is comparable with that of Taji and Rogers (1998) who have investigated on RMF of tooth brushes in a group of ten healthy individuals. These investigators postulated that RMF of tooth brushes used by healthy people lies within 10^4 - 10^5 CFU/ml. Similarly, current data showed that $10^4 - 10^6$ CFU/ml microorganisms with different characteristics (aerobic, anaerobic, Gram positive and negative) thrive on tooth brushes used by healthy subjects. On the other hand, CFU counts of RMF on aerobic BA, anaerobic BA and MA were positively correlated showing that bacteria with different characteristics proliferate in a similar pattern on tooth brushes. Gram positive cocci with catalase and coagulase positive reaction were determined as *Staphylococcus aureus* while Gram negative non lactose fermenting bacilli with positive oxidase reaction were identified as *Pseudomonas* species (SLCM Laboratory Manual, 2001). Filamentous Gram positive rod that gives characteristic yellow granular colonies on BA and Gram negative spindle shaped rods are morphologically similar to *Actinomyces* species and *Fusobacterium* species respectively. Gram positive budding yeast resembles *Candida* species. However, further confirmatory tests are needed to identify these organisms up to species level.

More than 25% of this study group did not clean their brush prior to use. This can increase the risk of microbial self inoculation. Moreover, some stored their tooth brushes in the bath room. This may permit free growth of microbes unaffected by drying and sunlight. Finally, these data demonstrates that tooth brushes used by this group of Sri Lankans do harbor RMF belonging to various types and growth characteristics. This may facilitate evolution of virulent strains of microbes that are resistant to antibiotics, dentifrices and environmental stimuli. Self inoculation of such organisms may be hazardous with regard to oral and systemic infections particularly in patients whose immunity is compromised.

References

Bunetel L, Tricot-Doleux S, Agnani G, Bonnaure-Mallet M (2000). In vitro evaluation of the retention of three species of pathogenic microorganisms by three different types of toothbrush. *Oral Microbiol Immunol* 15:313-316.

Taji SS, Rogers AH (1998) ADRF Trebitsch Scholarship. The microbial contamination of toothbrushes. A pilot study. *Aust Dent J* 43:128-30.

Warren DP, Goldschmidt MC, Thompson MB, Adler-storths K, Keene HJ (2001) The effects of toothpastes on the residual microbial contamination of toothbrushes. *J Am Dent Assoc* 132:1241-45.