Original

Assessment of Effectiveness of 30% Rock Salt Solution in Toothbrush Decontamination - An In Vivo Study

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Abstract

Introduction: Toothbrushes are the most commonly used intra oral cleaning aids for personal hygiene maintenance. Salt is a time tested natural disinfectant and itfulfills the requirements of a domestic disinfectant. Objective: To assess the effectiveness of domestic ingredient, rock salt in 30% concentration as an antimicrobial agent for toothbrush decontamination. Materials and Methods: 50 samples of toothbrushes with standardized dimensions, brand and bristle hardness and 50 identical dentifrices were distributed to systemically healthy, 18 to 25year old volunteers. Volunteers were instructed to maintain identical oral hygiene practices and storage conditions. The toothbrushes were retrieved after one week in sterilized pouches, randomly divided into two groups of 25 each. Group A was set as Control. Brushes in Group A were immersed in tap water for a duration of 5 mins Group B samples were immersed in 30% rock salt solution for 5 mins. The number of colony forming units of microbes present in Group B was assessed microscopically and was compared with that of the Group A. Results: 78% of samples belonging to Group-A that were immersed in 30% Rock salt solution were completely devoid of bacteria and microbial contamination was evident in all the brushes from control group. To conclude, Addressing issue of toothbrush decontamination with Rock salt is an effective, economical and realistic solution as demonstrated by this study.

Introduction

Health of the mouth reflects that of one's body. Oral Cavity is the entrance to the alimentary canal. It houses multitude of complex microbial communities. Oral microbiota is primarily composed of species of Streptococci, Staphylococci, Lactobacilli, fungi like Candida etc. Balance between host defense and microbial virulence determines the overall health of an individual.

Tooth brushing is a simple procedure performed on a daily basis. It causes gingival crevicular tissue disruption to a great extent thereby increasing the possibility of bacteremia [1]. This simple procedure performed on a daily basis is capable of causing bacteremia to the same extent as dental extraction [2].

Toothbrushes are the most commonly used intra oral cleaning aids for personal hygiene maintenance. Ironically, this device used for oral hygiene maintenance acts a fomite [3]. The bristles and tuft ends of the toothbrushes exposed to environment following usage provide an ideal niche for microbial contamination. When two toothbrushes are placed in direct contact, cross contamination is likely.

Contaminated toothbrushes act as a source for perpetuation of oral and other systemic diseases. Three main pathways linking oral infection to secondary disease were suggested: (1) metastatic infection, secondary to the oral infection, due totransient bacteremia (presumably resulting primarily in endocarditis); (2) systemic inflammation from immunologic injury caused by oral bacteria; and (3) systemic vascular injury due to oral microbial endotoxins [4].

Toothbrushes with antibacterial tufts have been experimented but have proven to be futile. Toothpaste formulations with increased amounts of Triclosan to reduce residual bacterial load on toothbrushes following usage was experimented and did not produce desired results [5,6].

Regular decontamination of toothbrushes in hence indispensable. Various chemical disinfectants like Chlorhexidine, EDTA, Sodium Hypochlorite have proven to be effective [7] but are not available for domestic usage.

There is paucity of data regarding effectiveness of Rock Salt as an antimicrobial agent. The aim of this study is to assess the effectiveness of domestic, economical ingredient, rock salt in 30% concentration as an antimicrobial agent for toothbrush decontamination.

Materials and Methods

44 hostel student volunteers belonging to age group of 18-25 years, who were free of systemic disease were included as participants after obtaining informed consent. Study subjects were restricted to hostel students to standardize the source of water supply. Soft bristled toothbrushes and dentifrices of the same brand were distributed and volunteers were asked to brush twice daily, rinse them under running water and store them outside the toilet open to air.

Ethical consent was obtained from the Institutional Ethical Committee, Sri Ramachandra University prior to beginning the study. The obtained tooth brush samples were analyzed at the Department of Microbiology, Sri Ramachandra University.

Toothbrushes were retrieved following one week of usage in sterilized pouches and were transferred to laboratory within 24 hours. They were then divided randomly into 2 groups of 22 each. Group A as study samples and Group B as control.

Group-A study samples were immersed in 100ml of 30% Rock Salt solution and Group-B in 100 ml tap water using sterilized glass bottles of dimension of 20cm height and 6cm base diameter for 5 minutes.

Tooth brush samples of both groups were then transferred into sterile Mac-Cartney bottles containing 10ml Phosphate Buffer Solution and left for one hour with intermittent mechanical agitation at intervals of 5 minutes.

With the help of standardized 4mm diameter loop, a volume of 0.1ml of Phosphate Buffer Solution was taken from each sample and plated into Trypticase soy agar plates using Zig Zag streaking method for semi-quantification of bacterial count. Plates were then incubated at 37◦C for 48 hours. At the end of 48 hours, readings of plates were recorded and quantified. The colony morphology and count was noted and gram staining was performed from the colony to ascertain grams reaction and further biochemical tests were performed to identify bacteria at the genus level.

Results

Upon microbiological examination of toothbrushes that were collected after one week of usage, 78% of samples belonging to Group-A that were immersed in 30% Rock salt solution were completely devoid of bacteria and microbial contamination was evident in all the brushes from control group. Further, upon Gram staining it was found that contaminant bacterial flora comprised predominantly of Gram Positive Cocci and Bacilli in the control group. In, 22% of study samples that exhibited bacterial growth, Micrococci was isolated.

Staphylococcus (Coagulase positive and negative) was the major contaminant isolated from 11 out of 22 control samples. Bacillus Subtilis was found in 5 control samples and Micrococci in the remaining 6 control samples.

Table 1: Number of Colony Forming Units of Bacteria

S. No	GROUP A	GROUP B
1.	NO GROWTH	100 CFU
2.	NO GROWTH	10000 CFU
3.	1000 CFU	100000 CFU
4.	NO GROWTH	100 CFU
5.	NO GROWTH	10000 CFU
6.	NO GROWTH	100 CFU
7.	100 CFU	100 CFU
8.	NO GROWTH	10000 CFU
9.	NO GROWTH	100000 CFU
10.	NO GROWTH	10000 CFU
11.	NO GROWTH	1000 CFU
12.	NO GROWTH	100 CFU
13.	NO GROWTH	100000 CFU
14.	100 CFU	100000 CFU
15.	NO GROWTH	1000 CFU
16.	NO GROWTH	10000 CFU
17.	NO GROWTH	10000 CFU
18.	100 CFU	10000 CFU
19.	NO GROWTH	1000 CFU
20.	NO GROWTH	1000 CFU
21.	NO GROWTH	100 CFU
22.	100 CFU	1000 CFU

*CFU- Colony forming units

Discussion

Toothbrushes are the most commonly employed devices for oral hygiene maintenance. Since inception, various changes and modification have been incorporated into toothbrushes. Despite various advances, toothbrush maintenance remains a grey area. Cobb C.M et.al reported that toothbrush is a major cause of repeated infections in the mouth [8].

Oral cavity houses multitude of complex microbial communities. Overall health of an individual is consequent with the orchestration of balance between microbial virulence and host defense. Poor oral health can predispose to various systemic conditions. Biofilms constitute an aggregation of organisms that exhibit autopoiesis, homeostasis, synergy and communality [9]. It is this biofilm forming characteristic of Staphylococcus that acts a mechanism of adherence to toothbrushes, (Aguilar et al., 2001) [3].

Salt (Sodium Chloride) is a time tested natural disinfectant. It is found in abundance in nature. Salt in its non-iodized form exhibits antimicrobial effect. The action of NaCl as an antimicrobial agent is based primarily on its plasmolytic effect on microbial cell membrane. High levels of NaCl in culture broths osmotically stress microbial cells resulting to decreased turgor pressure of the cell membrane, release of microbial cytoplasmic water, and cell shrinkage. Dehydration occurs as a consequence and the medium is rendered unsuitable for microbial growth.

In the present study, there was a complete elimination of microbial contamination following one week of usage in 78% of the samples and evidence of bacterial contamination in all the control samples. However, in a study conducted by Farah Rami Saleh [10] it is shown that the microbial contamination ratio was 37.5% when salt water was employed as a disinfectant. Other studies by Bhat S [8], Rajiv Saini [11] demonstrate similar

microbial contamination of toothbrushes as evident in the control samples of this study.

In this study, species of staphylococci were the predominant flora that was isolated from the control group, this concord with findings of study conducted by S. S. Taji [12]. Inhibition of growth of species of Staphylococci by Rock Salt solution observed in the study group is concurrent with findings of study by Monalisa M Bayani [13]. In the present study, the limitations are: Bacterial flora resident in oral cavity is vary greatly between individuals hence, extent of contamination and nature of contaminant is dissimilar across study and control groups and oral hygiene practices are likely to vary between individuals.

Conclusion

Addressing issue of toothbrush decontamination with Rock salt is an effective, economical and realistic solution as demonstrated by this study.

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