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RESEARCH ARTICLE

Qualitative and quantitative assay of microbial contamination of tooth brushes stored in different sanitary settings

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Abstract

Background: Microbial contamination was not given any attention while recommending the frequency for change of toothbrush. **Aim:** To qualitatively and quantitatively assess the tooth brushes preserved in different sanitary settings for microbial contamination. **Materials and method:** The study was conducted on thirty participants preserving their tooth brushes in three different sanitary settings (outside the bathroom, within the bathroom without attached toilets, and within the bathroom with attached toilets). In each group, five participants were healthy and other five were having mild to moderate periodontitis. The tooth brush samples were collected after one month from their households and subjected to qualitative and quantitative assay of microorganisms. The data from different groups were compared using chi-square test and t-test. **Results:** The tooth brushes stored outside the bathrooms demonstrated the presence of Candida, Streptococci, Klebsiella, Staphylococcus aureus and Lactobacillus. Pseudomonas, Candida, Streptococci, Staphylococcus aureus and Lactobacillus were demonstrable in the tooth brush samples collected from participants who stored their brush in bathrooms without attached toilets. Pseudomonas, Candida, Streptococci, Klebsiella, Staphylococcus aureus, Lactobacillus, Proteus and E.coli were demonstrable in the tooth brush samples collected from both healthy and diseased participants who preserved their brush in bathrooms with attached toilets. **Conclusion:** The tooth brushes preserved in unsanitary conditions can be a source of contamination and call for proper preservation of tooth brush. Homecare procedures such as air drying, dipping the tooth brush in salt water and use of portable sanitizers may be advised by dentists as part of routine oral hygiene instructions.

INTRODUCTION

The compartmentalization involved in viewing the mouth separately from the rest of the body must cease because oral health affects general health by causing considerable pain and suffering and by changing what people eat, their

speech and their quality of life and well-being. Oral health also has an effect on other chronic diseases (Petersen PE, 2003). Oral diseases can be greatly controlled by reducing the microbial load in the oral cavity and this can be achieved by maintaining proper oral hygiene (Bhat S, 2003). Tooth brushes are an important component of routine oral hygiene aids used in promotion of oral health and prevention of oral diseases (Carranza FA Jr, Newman MG, 1996).

Regrettably, tooth brushes are often preserved in bathrooms which are a good place to harbor millions of different pathogenic micro-organisms. This neglect in the proper maintenance of tooth brushes is attributed to lack of public awareness on the possibilities of tooth brush contamination while stored in sanitary settings (Karbasappa GN et al 2011).

The colonization of these pathogenic micro-organisms on toothbrush while being stored in unsanitary conditions represents a potential cause of re-contamination of the oral cavity (Wetzel WE, 2005). The tooth brush may get contaminated by Streptococcus, Staphylococcus, (Taji SS, 1998) and lactobacilli (Fernandez V and Cesar D, 2006). These bacteria are implicated in the causation of many life threatening diseases such as infective endocarditis besides influencing the occurrence of oral diseases such as dental caries and gingivitis (Wetzel WE, 2005; Boylan R, 2008).

A manual tooth brush lasts approximately 3 months after which the bristles may get flared. This will reduce the effectiveness of tooth brush bristle in removing the dental plaque (Yankell SL and Saxer UP 2004). Hence, American Dental Association recommends change of tooth brush every 3 - 4 months. The average life span of a manual toothbrush is approximately 3 months (Glaze PM and Wade AB, 1986). However, microbial contamination was not given any attention while recommending the frequency for change of toothbrush. The studies demonstrating the contamination of toothbrushes preserved in sanitary settings are sparse. In this background, the present study was undertaken to qualitatively and quantitatively assess the tooth brushes preserved in different sanitary settings for microbial contamination.

Materials and method:

This study was carried out among thirty participants visiting the department of Public Health Dentistry, Government Dental College, Hyderabad. The ethical clearance was obtained from the Institutional Ethics Committee. A clinical oral examination was carried out by a dentist to assess their oral health status besides collecting desired information on other systemic diseases. The list of participants free from systemic diseases visiting the department over a period of ten days from 1st to 10th September 2014 was prepared. These participants were recalled for a health education program that was organized by the department in the third week of September 2014. The participants were appraised on benefits of good oral hygiene in the maintenance of oral and general health by a Public Health Dentist. All the participants were given an oral hygiene kit containing 100 gram tooth paste and a soft bristled brush. The participants were given a demonstration of Modified Bass Technique and they were requested to brush twice a day. The participant's practice of preserving the tooth brush following brushing was recorded at this time. Based on this information, the participants were grouped into three categories.

Group 1: Participants with the practice of preserving their brush outside the bathroom,

Group 2: Participants with the practice of preserving their brush within the bathroom without attached toilets

Group 3: Participants with the practice of preserving their brush within the bathroom with attached toilets.

Ten participants were recruited for each of these groups. Within each group, five of the ten participants were healthy and the remaining five were with mild to moderate periodontitis. Then, these participants were informed that their brush will be collected at the end of one month for a microbiological assay and their consent obtained. The participants were requested not use any antimicrobial mouth rinses during the study period. Each of these participants was given a sterile transparent zip lock plastic pouch. They were requested to place their brush in the zip lock pouch after using their brush in the morning on day thirty.

Sample collection: At the end of one month, samples were collected in the morning from each participant. On the intended day for sample collection, the participants were instructed to rinse the brush in tap water after brushing and place it in the zip lock pouch. Five investigators collected these samples from the participant's households and sent them for laboratory investigations on the same day. At the time of sample collection, the information on how and where the brush was preserved during the last thirty days was obtained. This information helped in ascertaining any deviation from their routine practice of storing the brush.

Isolation of organisms: Handles of toothbrushes were cut off using heat sterile scissors. Heads of the brushes (containing the bristles) were then soaked in 10 ml of sterile tryptone soya broth (TSB) for 60 minutes. This was followed by vortex mixing for 1 min to dislodge suspected adherent bacteria. The bacterial suspension was serially diluted to obtain dilution factors of up to 10^3. The spread plate technique was employed. One milliliter (1 ml) each

of the dilution factors was obtained using a sterile pipette and plated on agar plate. MacConkey agar and Mannitol salt agar media were used for the isolation of non-fastidious bacteria, coliforms and staphylococci, respectively. Plates were incubated aerobically at 37^0C for 24- 48 h.

Identification of isolates: Total viable counts of bacterial population were enumerated. Morphological characteristics of isolates were observed and Gram's staining was performed for each isolate.

A. Gram positive cocci of Manitol salt agar were further identified as Staphylococcus aureus and Staphylococcus epidermidis by several biochemical tests such as Catalase test, Oxidase test, Coagulase test, Carbohydrates fermentation test and others.

B. Gram negative bacilli on MacConkey plates were identified as follows: **a**. Gram negative, non lactose fermenting, oxidase positive colonies were considered as Pseudomonas species **b**. Gram negative, lactose fermenting, oxidase negative colonies were considered as Coliform species.

Statistical analysis: The data was entered on to a personal computer and statistical analysis was carried out using SPSS version 20. The mean CFU of bacteria between the healthy and periodontitis subjects in each of these groups was compared using independent sample t-test and statistical significance was fixed at 0.05.

Results:

A total of thirty participants with a mean age of 28.9 ± 7.9 years participated in the study. The age distribution of healthy and diseased individuals in each of the three groups is denoted in table 1.

The tooth brushes stored outside the bathrooms demonstrated the presence of *Candida, Streptococci, Klebsiella, Staphylococcus aureus and Lactobacillus*. These microorganisms were demonstrable in the tooth brushes of healthy as well as diseased individuals. The mean bacterial load of *Candida* (table 2, p = 0.017) and *Staphylococcus aureus* (table 2, p = 0.001) was significantly higher in the tooth brush samples collected from diseased individuals compared their healthy counterparts. The samples collected from group 1 participants failed to demonstrate the presence of *Pseudomonas, Proteus* and *E.coli*.

Pseudomonas, Candida, Streptococci, Staphylococcus aureus and Lactobacillus were demonstrable in the tooth brush samples collected from group two participants who stored their brush in bathrooms without attached toilets. These bacteria were demonstrable in samples of both healthy and diseased participants in the group. However, the mean colony forming units (CFU) of *Pseudomonas* (table 3, p = 0.001), *Candida* (table 3, p = 0.017) and *Staphylococcus aureus* (table 3, p = 0.001) was significantly higher in the samples of diseased individuals compared to healthy individuals.

Pseudomonas, Candida, Streptococci, Klebsiella, Staphylococcus aureus, Lactobacillus, Proteus and E.coli were demonstrable in the tooth brush samples collected from both healthy and diseased participants in group three who preserved their brush in bathrooms with attached toilets. The mean CFU of Pseudomonas (table 4, p = 0.001) and Candida (table 4, p = 0.017) were significantly higher in tooth brush samples of diseased individuals compared to their healthy participants in the group.

Table 1: Age distribution of study participants in different groups

Group	Health status	Number of participants	Mean Age
Group 1	Healthy	5	23.4 ± 0.6
	Diseased	5	32.0 ± 4.4
Group 2	Healthy	5	21.4 ± 1.5
	Diseased	5	39.6 ± 3.6
Group 3	Healthy	5	21.8 ± 1.9
	Diseased	5	35.4 ± 5.9
Total		30	28.9 ± 7.9

Table 2: Microbial contamination of tooth brushes stored outside the bathroom (group 1)

Health status	*Pseudo*	*Candida*	*Strepto*	*Klebsl*	*Staphy*	*Lactobac*	*Proteus*
	Mean Colony Forming Units / ml ± Standard deviation						
Healthy	NS	0.4 ± 0.1 X 10	1.8 ± 0.4 $X10^6$	4.0 ± 0.2 X 10^2	5.4 ± 1.4 $X10^2$	1.8 ± 0.4 X 10^2	NS
Diseased	NS	1.0 ± 0.4X 10	1.8 ± 0.4 X 10^6	27.6 ± 0.8 X 10^2	36.8 ± 2.2 X 10^2	1.8 ± 0.4 X 10^2	NS
Statistical inference		t=-3.0 df= 8 p=0.017	t=0 df= 8 p=1.000	t=-62.8 df= 8 p=1.000	t=-26.6 df= 8 p=0.001	t=0 df= 8 p=1.000	

Table 3: Microbial contamination of tooth brushes stored inside the bathroom without attached toilets (group 2)

Health status	*Pseudo*	*Candida*	*Strepto*	*Klebsl*	*Staphy*	*Lactobac*	*Proteus*
Healthy	4.8 ± 1.4 $X10^2$	0.4 ± 0.2 X 10	1.8 ± 0.3 X 10^6	NS	5.4 ± 1.4 $X10^2$	1.8 ± 0.4 $X10^2$	NS
Diseased	15.5 ± 1.3 X 10^2	1.0 ± 0.4 X 10	1.8 ± 0.4 X 10^6	NS	36.8 ± 2.2 $X10^2$	1.8 ± 0.4 $X10^2$	NS
Statistical inference	t = -12.7 df = 8 p = 0.001	t = -3.0 df = 8 p = 0.017	t = 0.0 df = 8 p = 1.000		t = -26.6 df = 8 p = 0.001	t = 0.0 df = 8 p = 1.000	

Table 4: Microbial contamination of tooth brushes stored inside the bathroom with attached toilets (group 3)

Health status	*Pseudo*	*Candida*	*Strepto*	*Klebsl*	*Staphy*	*Lactobac*	*Proteus*
Healthy	4.8 ± 1.4 X 10^2	0.4 ± 0.2 X 10	1.8 ± 0.4 X 10^6	1.6 ± 0.5 $X10^2$	5.4 ± 1.4 $X10^2$	1.8 ± 0.4 $X10^2$	1.4 ± 0.6 $X10^2$
Diseased	15.5 ± 1.3 X 10^2	1.0 ± 0.4 X 10	1.8 ± 0.4 X 10^6	1.6 ± 0.5 $X10^2$	36.8 ± 2.2 $X10^2$	1.8 ± 0.4 $X10^2$	1.4 ± 0.6 $X10^2$
Statistical inference	t = -12.7 df = 8 p = 0.001	t = -3.0 df = 8 p = 0.017	t = 0.0 df = 8 p = 1.000	t = 0.0 df = 8 p = 1.000	t = - 26.6 df = 8 p = 0.001	t = 0.0 df = 8 p = 1.000	t = 0.0 df = 8 p = 1.000

Discussion:

The results of the present study demonstrated that the tooth brushes are a source of bacterial contamination and adequate care needs to be exercised in the preservation of tooth brushes following oral hygiene practice. The contamination of tooth brush could create a significant risk of propagation of infection for certain patients such as immunosuppressed, cardiopathic, organ transplant recipients (Sumasogi HP et al, 2002). *Candida, Streptococcus pyogens, Klebsiella, Staphylococcus aureus and Lactobacillus* was found in the tooth brush samples preserved outside bathrooms. *Pseudomonas, Candida, Streptococci, Staphylococcus aureus and Lactobacillus* was demonstrable in the tooth brush samples preserved in bathrooms without attached toilets. These results were in agreement with studies by Karibasappa GN et al (2011) and Wetzel WE et al (2005). *Pseudomonas, Candida, Streptococcus pyogens, Klebsiella, Staphylococcus aureus, Lactobacillus, Proteus and E.coli* was demonstrable in the tooth brush samples preserved in bathrooms with attached toilets. These results were similar to the results of study by Sumasogi HP et al (2002). The micro-organisms isolated from the tooth brush samples in the present study can cause many general and oral diseases. Streptococcus and Lactobacillus are a part of normal oral Microflora and found in environment as well. Hence, these bacteria are found in samples of tooth brushes collected from all the three settings. *Streptococcus mutans* is the principle microorganism involved in the causation of dental caries in human beings (Chandrashekar et al, 2015). *Lactobacilli* are involved in the progression of dental caries (Shivakumar KM et al 2009). Candida causes candidiasis (Brandt ME 2002). *Pseudomonas* is commonly seen in environment although not part of normal oral or enteric flora. It can cause suppurative otitis, eye infections, urinary tract

infections, burn infections, etc (Karibasappa GN et al 2011). Klebsiella causes pyogenic infections, septicemia, pneumonia, diarrhea, urinary tract infections. It is a part of normal enteric flora and may get acquired on tooth brushes due to aerosol contamination. Streptococcus pyogenes causes urinary tract infections, rheumatic fever, glomerulonephritis (Karibasappa GN et al 2011). *Staphylococcus* is a part of oral flora, seen in environment and non enteric flora and it causes boils, carbuncle, pustules, abscess, osteomyelitis, endocarditis and septicemia (Abd-ulnabi RM 2012). *E.coli* is seen only in gut (enteric flora), but can get transmitted through aerosol contamination. Hence present only in brushes stored in bathrooms with attached toilets. *E.coli* is not influenced by the oral condition of the person but related only to storage place. Proteus is seen only in enteric flora, and aerosol contamination is a possible explanation in the samples of tooth brushes preserved in bathrooms with attached toilets (Karibasappa GN et al 2011).

The bacteria isolated in the present study not only are oral pathogens but also general pathogens. Improper storage of toothbrushes that were kept in the bathrooms with or without attached toilet, exposing them to the unfavorable surrounding external environment may be a source of contamination by general pathogens (Long SR et al, 2000). Oral commensals could also have contributed for contamination of toothbrushes. Toothbrushes contaminated with the micro-organisms such as Pseudomonas, Sta. aureus and Klebseilla pose a serious threat to oral and general health. Anti-microbial solutions, air drying and toothbrush sanitizer are some of the recent methods employed for preventing tooth brush contamination. Some of the commercially available antimicrobial solutions are 0.2% Chlorhexidine, 2% Triclosan, 1% Sodium hypochlorite, 3% Hydrogen peroxide, Dettolin, etc. Home-made microbial solutions like 3% neem, salt water may be recommended as anti-microbial agents for preservation of toothbrushes.

Conclusion:

The tooth brushes preserved in unsanitary conditions can be a source of contamination that can predispose to oral and general diseases especially among immunocompromized individuals. Homecare procedures such as air drying, dipping the tooth brush in salt water and use of portable sanitizers may be advised by dentists as part of routine oral hygiene instructions.

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