



Microbial Contamination of Tooth Brushes Stored in Different Settings before and After Disinfection with Chlorhexidine—A Comparative Study

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ABSTRACT

Objective: To qualitatively and quantitatively assess the microbial contamination of tooth brushes preserved in different sanitary settings before and after disinfection with 0.2% chlorhexidine. **Materials and Method:** The study was carried out in two phases among thirty participants visiting a dental hospital. These participants were assigned to one of the three groups based on the practice of preserving the tooth brush. Group 1: Participant's who preserved their brush outside the bathroom. Group 2: Participant's who preserved their brush within the bathroom without attached toilets. Group 3: Participants who preserved their brush within the bathroom with attached toilets. Participants were given oral hygiene kits containing a brush and paste in the first phase. The brush samples were collected on day thirty for qualitative and quantitative estimation of microbial contamination. In the second phase, participants were requested to rinse their brushes in 0.2% chlorhexidine after brushing and before placing it back. The mean CFU of different bacteria was compared using independent sample t-test and paired sample 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* was demonstrable in the tooth brush samples of group 2. *Pseudomonas*, *Candida*, *Streptococci*, *Klebsiella*, *Staphylococcus aureus*, *Lactobacillus*, *Proteus* and *E.coli* were all demonstrable in group 3. However, the disinfection with 0.2% chlorhexidine reduced the microbial load substantially. **Conclusion:** Rinsing the tooth brush with 0.2% chlorhexidine substantially reduced the microbial contamination in all the three settings.

Key words: Chlorhexidine, *Lactobacillus*, Microbial contamination, *Streptococcus mutans*, Tooth brush.

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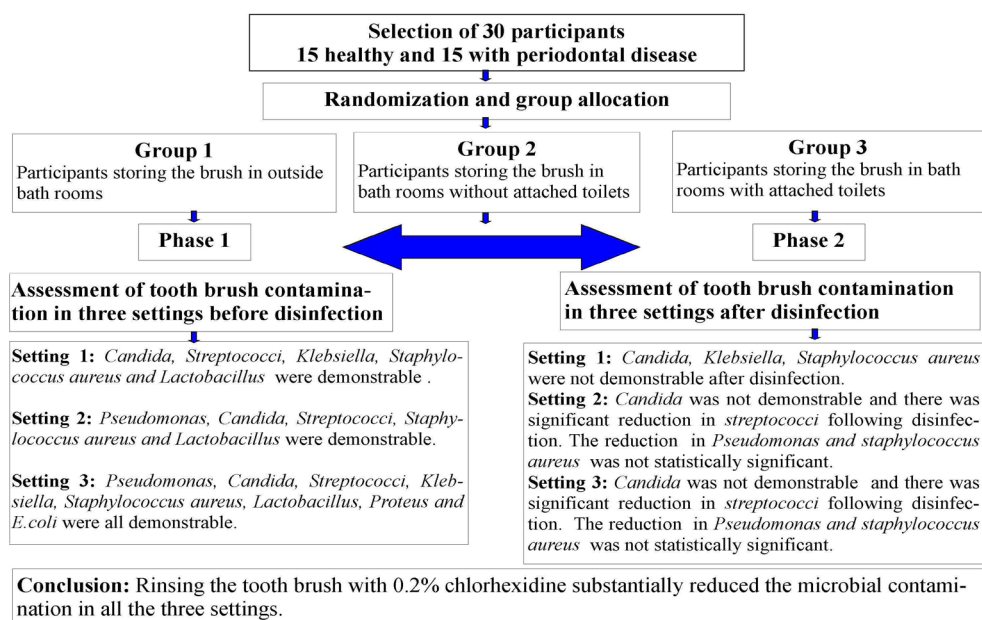
DOI:
10.5530/jyp.2015.4s.11

INTRODUCTION

Tooth brushes are a vital component of routine oral hygiene aids used in promotion of oral health and prevention of oral diseases.¹ Unfortunately, they are often stored in unsanitary conditions such as bathrooms.

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Graphical Abstract

These unsanitary conditions are the settings that harbor millions of different pathogenic microbes. The neglect in the appropriate maintenance of tooth brushes is attributed to lack of public awareness on the possibilities of tooth brush contamination while they are stored after brushing.² The colonization of pathogenic micro-organisms on toothbrush while being stored in unsanitary conditions represents a potential cause of re-contamination of the oral cavity.³ The tooth brush may get contaminated by *Streptococcus*, *Staphylococcus* and *Lactobacilli*.^{4,5} 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.^{3,6}

The effectiveness in removing dental plaque of a manual tooth brush lasting approximately three months or more may reduce owing to the flaring of tooth brush bristles.⁷ Hence, American Dental Association (ADA) recommends change of tooth brush every 3-4 months. The average life span of a manual toothbrush is approximately 3 months.⁸ 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 before and after simple disinfection process are sparse. In this background, the present study was undertaken to qualitatively and quantitatively assess the microbial contamination of tooth brushes preserved in different sanitary settings before and after disinfection with 0.2% chlorhexidine.

MATERIALS AND METHODS

This comparative study was carried out among thirty participants visiting the department of Public Health Dentistry, Government Dental College, Hyderabad after the ethical clearance was obtained from the Institutional Ethics Committee vide letter number GDCH/ETHC/4/2015. At the preliminary visit, 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 who visited the department between 1st and 10th September 2014 was prepared. These participants were recalled for a health education program organized by the department in the third week of September 2014. The participants were educated on benefits of good oral hygiene for optimal 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 following the completion of health education session. Modified Bass Technique was demonstrated to the participants and they were directed to brush twice daily. The information on where the participants preserved their brush after brushing was recorded at this time. Based on this information, the participants were assigned to one of the three categories.

Group 1: Participant's who preserved their brush outside the bathroom

Group 2: Participant's who preserved their brush within the bathroom without attached toilets

Group 3: Participants who preserved their brush within the bathroom with attached toilets.

Ten participants were recruited in each group and within each group; five were healthy while the others 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 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, use of any antibiotics during the study period was obtained. This information helped in making exclusions. The individuals using any antibiotics during the study period and deviating from the assigned practice of preserving the brush were excluded.

Sample collection following disinfection

In the second phase of the study, each participant was given a oral hygiene kit containing tooth brush, tooth paste and a disinfecting solution (100 ml of 0.2% chlorhexidine). The participants were directed to use the tooth brush and preserve it in the routine setting. However, they were directed to immerse their brush in 5 ml of the disinfecting solution for 20 seconds before placing the brush back in the location intended for its preservation. Antimicrobial solution was distributed at ten days interval. This ensured that the participants were reinforced about the disinfection of their tooth brush and facilitated compliance on the recommendation given to them. The brushes were collected on day thirty and sent for microbial assay.

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°C 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.

- 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.
- 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 while the mean CFU of bacteria before and after disinfection in each group was compared using paired sample t-test. The statistical significance was fixed at 0.05. The CONSORT flow diagram is depicted as Figure 1.

RESULTS

Thirty participants aged between 19 to 45 years, with a mean of 28.9 ± 7.9 years participated in the study with no exclusions made from the baseline. 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

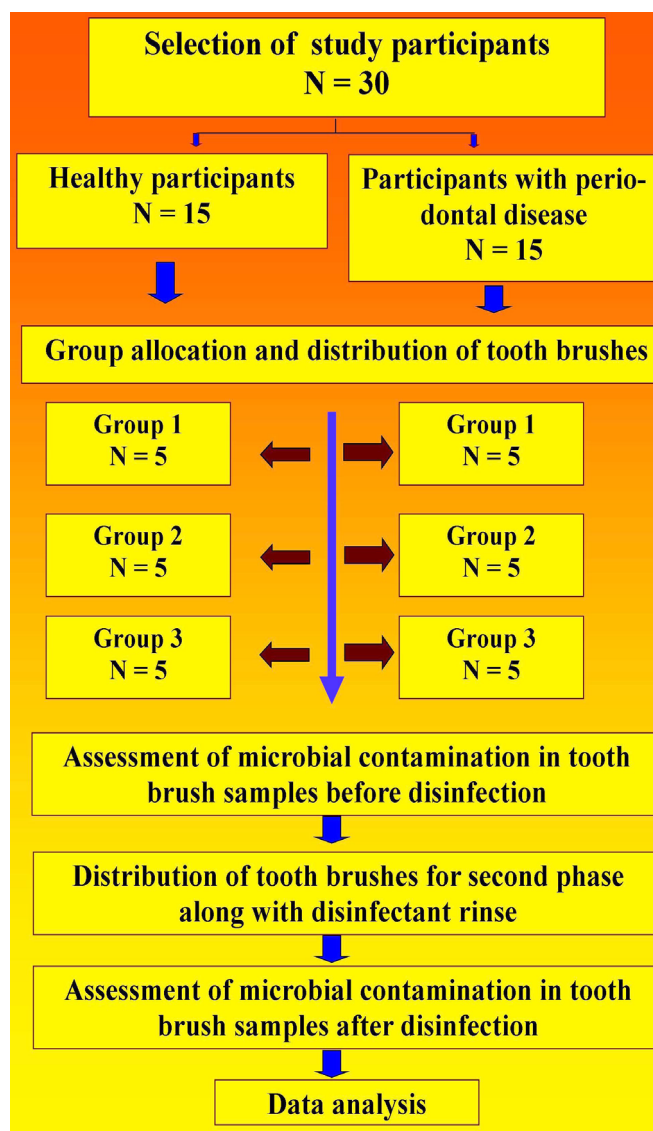


Figure 1: Consort Flow Diagram Depicting Methodology in the Present Study

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

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*.

However, following disinfection with 0.2% chlorhexidine in the second phase of the study, *Candida*, *Klebsiella*, *Staphylococcus aureus* which were demonstrable in the first phase were not demonstrable. The mean CFU of *streptococcus* were significantly lower among tooth brush samples collected both healthy (Table 2, $p=0.01$,) and diseased individuals (Table 2, $p=0.01$) compared to the results of the first phase where no disinfection was followed before preserving the brush in the intended location. Although, the mean CFU of *lactobacillus* was reduced compared to baseline levels, the reduction observed was not statistically significant in both the subgroups.

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

Table 2: Microbial contamination of tooth brushes stored outside the bathroom (group 1) before and after using disinfectant

Intervention	Health status	<i>Pseudo</i>	<i>Candida</i>	<i>Strepto</i>	<i>Klebsl</i>	<i>Staphy</i>	<i>Lactobac</i>	<i>Proteus</i>	<i>E.coli</i>
Mean Colony Forming Units/ml ± Standard deviation									
Before	Healthy	NS	0.4±0.1 X10	1.8±0.4 X10 ⁶	4.0±0.2X 10 ²	5.4±1.4 X10 ²	1.8±0.4X 10 ²	NS	NS
	Diseased	NS	1.0±0.4 X10	1.8±0.4X 10 ⁶	27.6±0.8X 10 ²	36.8±2.2X 10 ²	1.8±0.4X 10 ²	NS	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			
After	Healthy	NS	NS	1.2±0.2X 10 ⁶	NS	NS	1.1±0.6X 10 ²	NS	NS
	Diseased	NS	NS	1.2±0.2X 10 ⁶	NS	NS	1.1±0.6X 10 ²	NS	NS
Statistical inference				t=0 df=8 p=1.000			t=0 df=8 p=1.000		

Table 3: Microbial contamination of tooth brushes stored inside the bathroom without attached toilets (group 2) before and after using disinfectant

Intervention	Health status	<i>Pseudo</i>	<i>Candida</i>	<i>Strepto</i>	<i>Klebsl</i>	<i>Staphy</i>	<i>Lactobac</i>	<i>Proteus</i>	<i>E.coli</i>
Before	Healthy	4.8±1.4 X10 ²	0.4±0.2 X10	1.8±0.3X 10 ⁶	NS	5.4±1.4 X10 ²	1.8±0.4 X10 ²	NS	NS
	Diseased	15.5±1.3X 10 ²	1.0±0.4 X10	1.8±0.4X 10 ⁶	NS	36.8±2.2 X10 ²	1.8±0.4 X10 ²	NS	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		
After	Healthy	3.9±0.7X 10 ²	NS	1.2±0.2X 10 ⁶	NS	4.0±0.2 X10 ²	1.1±0.6 X10 ²	NS	NS
	Diseased	11.6±1.2X 10 ²	NS	1.2±0.2X 10 ⁶	NS	27.6±0.8 X10 ²	1.1±0.6 X10 ²	NS	NS
Statistical inference		t=-12.5 df=8 p=0.001		t=-0.0 df=8 p=1.000		t=-62.8 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) before and after using disinfectant

Intervention	Health status	<i>Pseudo</i>	<i>Candida</i>	<i>Strepto</i>	<i>Klebsl</i>	<i>Staphy</i>	<i>Lactobac</i>	<i>Proteus</i>	<i>E.coli</i>
Before	Healthy	4.8±1.4 X 10 ²	0.4±0.2 X10	1.8±0.4X 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	1.2±0.2 X10 ²
	Diseased	15.5±1.3X 10 ²	1.0±0.4 X10	1.8±0.4X 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	1.2±0.2 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	t=0.0 df=8 p=1.000
After	Healthy	3.9±0.7X 10 ²	NS	1.2±0.2X 10 ⁶	NS	4.0±0.2 X10 ²	1.1±0.6 X10 ²	NS	NS
	Diseased	11.6±1.2X 10 ²	NS	1.2±0.2X 10 ⁶	NS	27.6±0.8 X10 ²	1.1±0.6 X10 ²	NS	NS
Statistical inference		t=-12.5 df=8 p=0.001		t=-0.0 df=8 p=1.000		t=-62.8 df=8 p=0.001	t=-0.0 df=8 p=1.000		

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.

Following disinfection, *Candida* was not demonstrable in the samples collected from both healthy and diseased participants in this group. There was a significant reduction in the mean CFU of *Streptococcus* in the samples collected from both healthy (Table 3, p=0.004) and diseased individuals (Table 3, p=0.012) in this group compared to their baseline levels. Although, there was a reduction in the mean CFU of *Pseudomonas* and *staphylococcus aureus* following disinfection, the reduction was significant among the samples collected from diseased individuals (Table 3,

p=0.001). The reduction in the mean CFU of *Lactobacillus* was not statistically significant in both the subgroups.

Pseudomonas, *Candida*, *Streptococci*, *Klebsiella*, *Staphylococcus aureus*, *Lactobacillus*, *Proteus* and *E.coli* were all 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 healthy participants in the group.

Candida was not demonstrable in the tooth brush samples collected following disinfection in both the subgroups. There was a significant reduction in the mean CFU of *Streptococcus* in both subgroups following disinfection

(Table 4, $p=0.01$). Although, there was a reduction in the mean CFU of *Pseudomonas* and *staphylococcus aureus* following disinfection, the reduction was significant among the samples collected from diseased individuals (Table 4, $p=0.001$). The reduction in the mean CFU of *Klebsiella*, *Lactobacillus*, *Proteus* and *E.coli* following disinfection was not statistically significant in both the subgroups.

DISCUSSION

The results of the present study established that the location at which tooth brushes are stored after brushing can act as a potential source of bacterial contamination highlighting the importance of adequate care. The appropriate methods of preservation of tooth brushes following oral hygiene practice needs to be stressed upon. The contaminated tooth brush could transmit infection among immunosuppressed, cardiopathic, organ transplant recipients.⁹ *Candida*, *Streptococcus pyogenes*, *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 similar to the findings of other studies.^{2,3} *Pseudomonas*, *Candida*, *Streptococcus pyogenes*, *Klebsiella*, *Staphylococcus aureus*, *Lactobacillus*, *Proteus* and *E.coli* was demonstrable in the tooth brush samples preserved in bathrooms with attached toilets similar to the findings of a study by Sumasogi HP *et al* (2002).⁹ The micro-organisms isolated from the tooth brush samples in our study are potential risk factors in the etiology of many general and oral diseases. *Streptococcus* and *Lactobacillus*, found in samples of tooth brushes collected from all the three settings are principle microorganisms involved in the causation of dental caries in human beings.¹⁰⁻¹² *Candida* causes candidiasis.¹³ *Pseudomonas* can cause suppurative otitis, eye infections, urinary tract infections, burn infections, etc.² *Klebsiella* causes pyogenic infections, septicemia, pneumonia, diarrhea, urinary tract infections. *Streptococcus pyogenes* causes urinary tract infections, rheumatic fever, glomerulonephritis.² *Staphylococcus* causes boils, carbuncle, pustules, abscess, osteomyelitis, endocarditis and septicemia.¹⁴ *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.²

The bacteria isolated in the present study not only are oral pathogens but also general pathogens. Inappropriate storage of toothbrushes 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.¹⁵ Oral commensals could also have contributed for contamination of toothbrushes. Toothbrushes contaminated with the micro-organisms such as *Pseudomonas*, *Sta. aureus* and *Klebsiella* pose a serious threat to oral and general health.

The simple practice of immersing the tooth brushes in a disinfectant solution for 20 seconds can bring about a substantial reduction in the mean CFU of *Streptococci* besides completely eliminating *Candida*. Although, the reduction in the mean CFU of all other bacteria was not statistically significant, the small reduction noted was encouraging. These results were in agreement with the results of other studies.^{9,16}

Limitations

The smaller sample size in the present study could be an explanation for not observing the reduction in the mean CFU as significant. In this context, our study could be considered as a pilot study and further studies with larger sample are recommended to validate the present results.

The use of anti-microbial solutions, air drying and toothbrush sanitizer are some of the recent methods available for preserving tooth brushes. Besides commercially available antimicrobial solutions such as 0.2% Chlorhexidine, 2% Triclosan, 1% Sodium hypochlorite, 3% Hydrogen peroxide, Dettol, etc, some home-made microbial solutions like 3% neem, salt water may be recommended for preservation of toothbrushes. Their efficacy in tooth brush sanitation may be compared with physical methods in future research.

CONCLUSION

The tooth brushes preserved in unsanitary conditions are a potential source of contamination that can predispose to oral and general diseases especially among immunocompromized individuals. Rinsing the tooth brush with 0.2% chlorhexidine substantially reduced the microbial contamination in all the three settings. Dipping the tooth brushes in any antimicrobial solutions may be considered as an option to reduce microbial contamination. Besides, 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.

ACKNOWLEDGEMENTS

The investigators sincerely thank all the participants for their kind cooperation and participation.

ABBREVIATION

CFU:	Colony Forming Units
ADA:	American Dental Association
TSB:	Tryptone soya broth
CONSORT:	Consolidated Standards of Reporting Trials

Highlights of Paper

- The article assessed the microbial contamination of tooth brushes preserved in three different sanitary settings before and after disinfection with 0.2% chlorhexidine.
- The microbiological assay of tooth brush samples collected from three different settings before intervention demonstrated the difference in the microbial contamination occurring in different settings.
- The post disinfection microbial assay demonstrated the disinfecting efficacy of 0.2% chlorhexidine in reducing such contamination.

Author Profile



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