

Don't Rush with Your Brush: An *In Vitro* Study on Toothbrush Hygiene

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ABSTRACT

Aim: To evaluate the effectiveness of audiovisual (AV) aids in creating awareness of toothbrush hygiene.

Materials and methods: This randomized trial study was conducted on 40 children aged 6–11 years. Children were given soft toothbrushes and then collected after brushing for 14 days. The toothbrushes were collected and sent to the laboratory for microbial culture evaluation. A questionnaire consisting of various questions regarding daily toothbrush hygiene practices was filled by the parents at the same time. After 14 days, brushes were collected, and the parents were shown an AV aid regarding toothbrush decontamination, parental awareness, and supervision. Those children were given new sets of toothbrushes and instructed to decontaminate them with chlorhexidine solution. After 14 days, the same questionnaire was filled out by the parents and toothbrushes were collected and immediately sent to the laboratory for microbial culture evaluation.

Results: All the sampled toothbrushes had significant ($p < 0.001$) bacterial growth after 14 days of use. The use of disinfectant led to a 99.98% reduction in microbial colony counts. Hence, showed a significant result. Questionnaire analysis showed a positive parental approach toward maintaining toothbrush hygiene.

Conclusion: Cleaning and disinfection of toothbrushes is crucial to stop the spread of disease since bacterial contamination cannot be 100% eliminated. AV aids can be effective tools for increasing awareness.

Clinical significance: Through this study, we want to emphasize toothbrush hygiene and create awareness for the same through AV aid because a healthy toothbrush leads to healthy oral health.

Keywords: Audiovisual aid, Disinfectant, Microbial count, Parental education, Toothbrush hygiene.

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INTRODUCTION

Toothbrushes are crucial for maintaining oral health as they remove plaque effectively. Additionally, they aid in the avoidance of periodontal disease and dental cavities.¹ Oral cavity, external environment, human hands, aerosol pollution, and storage containers can all contribute to the contamination of toothbrushes.² Contaminated toothbrushes have the potential to spread diseases that affect the whole body, including sepsis, gastrointestinal issues, cardiovascular problems, respiratory issues, and kidney problems.³ Oral health is essential for overall well-being and can reveal various disorders associated with the body.⁴

According to research, infected toothbrushes could endanger the health of those who are immunosuppressed, reinfect those who already have chronic periodontal disease, and possibly even reintroduce bacteria into healthy mouths of individuals.⁵ Centers for Disease Control and Prevention guidelines state that those with compromised immune systems would need to look for different ways to maintain their oral hygiene because even after giving toothbrushes a full rinse with tap water, some potentially harmful organisms may still be present.⁵

Researchers have proposed a relationship between the flu, sore throats, recurrent colds, and infected toothbrushes. It makes sense to consider methods to reduce or stop microorganisms from growing and spreading on toothbrushes, given the research that suggests oral bacteria may contribute to heart attacks, diabetes, and preterm delivery.⁵

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The bathroom is commonly regarded as the most contaminated room in a household. Toothbrushes that are communal, stored collectively, or positioned near the sink or toilet may serve as a breeding ground for dangerous germs.⁵ The release of even a few toilet drops causes the dissemination of millions of microorganisms into the air.² So, in order to maintain oral hygiene, it is important to clean your toothbrush.³

Overall, toothbrush disinfection should be easy, rapid, cost-effective, nontoxic, and efficient. Chemical reagents are still a practical and efficient way to disinfect toothbrushes. Numerous

studies have been conducted on toothbrush disinfection, including those utilizing microwave ovens, dishwashers, immersion disinfection, antibacterial solution sprays, and ultraviolet (UV) toothbrush sterilizers.³

Unfortunately, proper toothbrush care is frequently overlooked, and many of these toothbrushes are kept in restrooms, which are ideal habitats for a vast number of microorganisms. According to the theory, a lack of public awareness of toothbrush care is the cause of this.⁶ Information can be communicated to patients through means such as verbal communication, printed materials, and videotapes. The benefit of the video presentation is that patients can self-learn in comfort and privacy, making it more convenient for them.⁷ Therefore, the study aimed to assess the efficacy of audiovisual (AV) aids in raising awareness regarding toothbrush hygiene.

MATERIALS AND METHODS

The study received approval from the Ethical Committee. All individuals gave written informed consent before being enrolled. Nearly 90 children underwent screening at the outpatient section of pediatric and preventive dentistry. A total of 40 children aged 6–11 years were selected for the study following a preliminary assessment based on specific criteria.

Inclusion Criteria

- Children aged 6–11 of any gender.
- Children have good general health.
- Those who had at least 20 natural teeth per arch and who had complied with the tooth brushing instructions.
- Decayed, missing, filled teeth index <5.

Exclusion Criteria

- Children undergoing orthodontic treatment.

- Children who were on antibiotic therapy 3 months before the study.
- Those who had intraoral prostheses during the study period.
- Children have allergies to any materials utilized in the study.

Prepresentation

All the selected subjects were given a soft tufted toothbrush (Fig. 1A) and instructed to brush their teeth as they do in their daily routine. The subject's parents completed a questionnaire on their regular dental hygiene habits. After 14 days, the study participants were asked to return their used toothbrushes, which were collected in disposable sterile plastic pouches (Fig. 1B). The toothbrush heads were wrapped with polythene sheet to avoid any decontamination chances caused while sectioning the toothbrush head (Fig. 1C). The heads of the toothbrushes were uncovered and put into the precoded sterile container containing brain heart infusion broth agar for transportation (Fig. 1D). They were transported immediately for microbial analysis.

Audiovisual Aid

An AV aid was shown to the parents at the same time that explained the importance of toothbrush hygiene (Fig. 2A). The 10 minutes visual aid covered in detail various aspects of maintaining the toothbrush clean and decontaminated, the brushing frequency, brushing time, duration after which one should change the toothbrush were included in the AV aid. It also emphasized the various ways to keep the brush germ-free by suggesting avoiding multiple touches to avoid cross-infectivity and to keep the toothbrush dry and away from the toilets and sinks. Proper decontamination instructions were provided to the parents.



Figs 1A to D: (A) Toothbrush samples; (B) Toothbrush samples collected in sterilized plastic bags; (C) Sectioned toothbrush heads; (D) Toothbrush heads in the precoded sterile containers for transportation to the laboratory

Postpresentation

New sets of toothbrushes were provided to those same subjects. Participants were directed to brush their teeth twice daily (in the morning and at night) for a duration of 14 days. They were additionally advised to disinfect their toothbrushes using chlorhexidine solution by washing the toothbrush before and after brushing (Fig. 2). Patients were directed to clean their teeth for 2 minutes, with brushing periods for the entire mouth varying from 30 seconds to 6 minutes.⁸ Dental practitioners and the oral hygiene business often advise brushing for 2 minutes twice a day.⁹⁻¹¹



Figs 2A to C: (A) AV aid to parents; (B and C) Toothbrush sample disinfection in chlorhexidine solution

All participants were required to keep their toothbrushes in the unique container provided, which included vents to allow air circulation and prevent external contamination. Participants returned their toothbrushes in disposable sterile plastic packets after 14 days. The toothbrushes were promptly dispatched to the laboratory. The parents were asked to fill out the same questionnaire 14 days after showing AV aid to evaluate the efficacy of the same.

Comparing bacterial contamination of toothbrushes used for 14 days from persons before and after decontamination. The samples were equally distributed on a blood agar medium using the pouring cup technique. The Petri plates were incubated at 37°C for 24 hours to promote the growth of microbial colonies. The number of colonies was determined by calculating the total number of colony-forming units (CFU) per milliliter.

The samples were categorized into two groups:

- Group I: Presterilized toothbrush samples.
- Group II: Poststerilized toothbrush samples.

Statistical Analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 20 (IBM Corp., Armonk, New York, United States of America). Means, standard deviations, and medians for data of each microorganism after disinfection were calculated with descriptive statistics. A paired *t*-test was used to compare the quantity of bacteria before and after the surgery.

RESULTS

The CFU were quantified per milliliter for each group, and the data was organized into a table. The samples from each group were compared using paired *t*-tests in SPSS version 20 software.

Bacterial colonies were quantified from the incubated Petri dishes (Fig. 3). The Petri plates displayed growing colonies (Fig. 3A). Petri plates were analyzed before using disinfection of the toothbrushes, and significantly high growth of the microbial colony was observed, while after using disinfectants, there was a substantial decrease ($p < 0.001$) in bacterial growth in the group was seen (Fig. 3B).



Figs 3A and B: Microbial count analysis on blood agar plates. (A) Colony count before disinfection of toothbrushes; (B) Colony count after disinfection of toothbrushes

Data obtained from Table 1 showed a significant reduction of 99.98% of microbial count after using disinfectants.

In the questionnaire analysis, parents showed positive responses about maintaining toothbrush hygiene. The parents were asked the following questions regarding daily toothbrush hygiene.

The response to a few questions was statistically significant before and after AV aid (Fig. 4 and Table 2).

Table 1: Showing a statistically significant difference between the microbial colony count in both groups (pre- and postdisinfection groups)

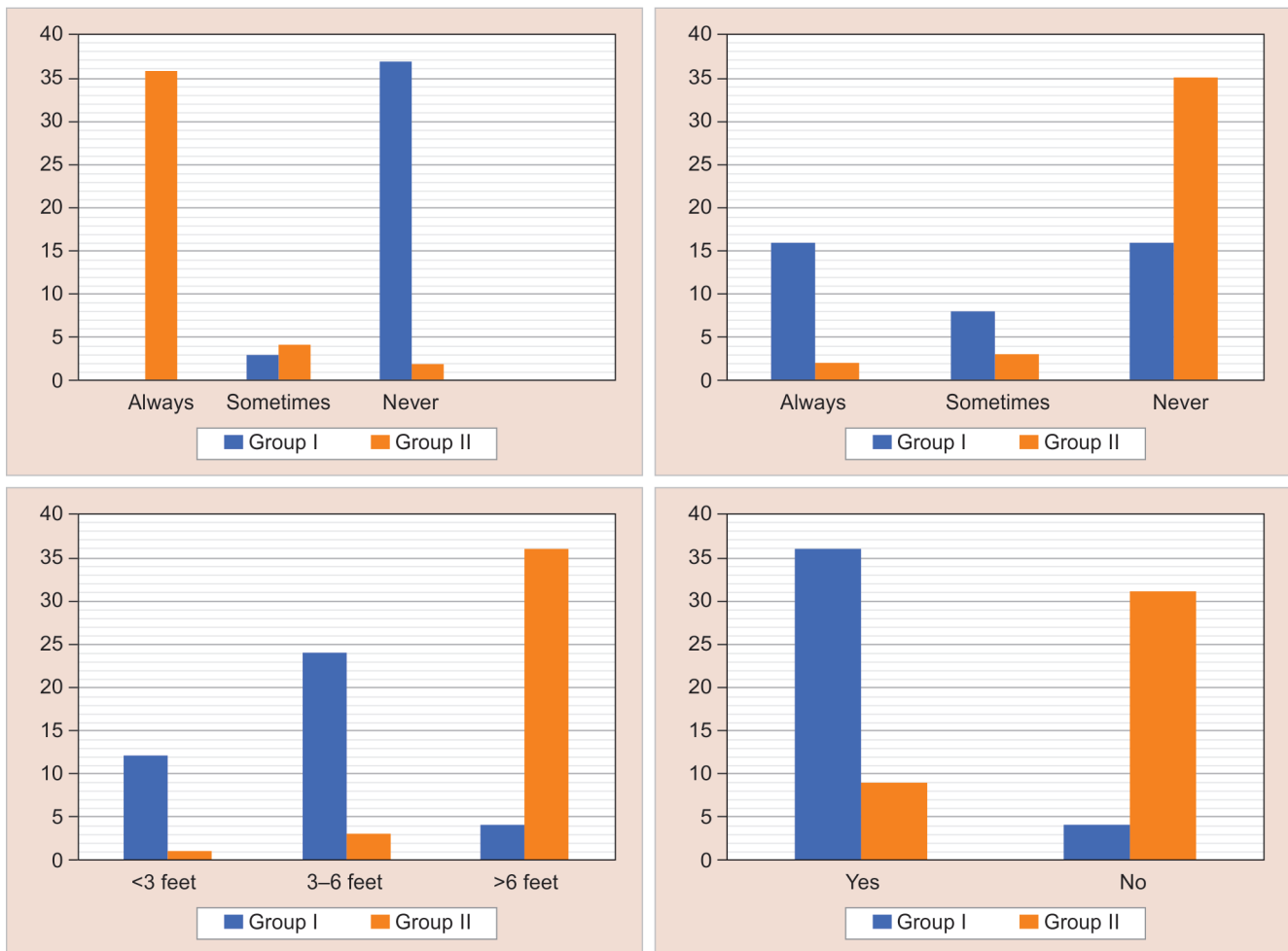
	Group I	Group II
N	40	40
Mean	536000.0000	100.9000
Standard deviation	229985.5068	56.39238
Standard error mean	72727.80303	17.83283
Correlation	0.908	
T-value	7.370	
p-value of t-test	0.000 (<0.001)	

DISCUSSION

Toothbrush contamination occurs as a result of using the toothbrush and storing it improperly, which might cause a variety of systemic and oral illnesses. The bristles frequently get worn out with prolonged use.¹² Hence, according to the American Dental Association, proper toothbrush maintenance and care are crucial factors for good oral health, and one should replace the toothbrush every 3–4 months. However, changing the toothbrush frequently drives up the maintenance costs, which becomes a burden. Thus, using disinfectant is more economical than changing toothbrushes frequently.¹

Commonly, after oral use, toothbrushes are rinsed with plain water and stored in the bathroom, and there is a high chance of cross-infection by sharing or keeping them in close proximity.¹ Several studies have also ruled out^{13–15} the role of contaminated toothbrushes and their causation in systemic infections.

Toothbrushes can contain bacteria, particularly fecal coliform bacteria, which may be transferred from the toilet or contaminated surfaces to the toothbrush when touched by the owner. There's a phenomenon in which a cloud of microscopic particles plumes into the air every time you flush your toilet, known as a "toilet plume." Most of us have our toilets in the bathroom; there is an explosion



Figs 4A to D: Graphical representation of questionnaire analysis; (A) Do you disinfect your toothbrush before and after every use? (B) Do you put a cover over your toothbrush head after use? (C) How far do you keep your toothbrush from the toilet bowl? (D) Do you keep multiple toothbrushes in a toothbrush holder?

Table 2: Showing the response of parents before and after showing AV aid

Questions	Group I (preinfection)	Group II (postinfection)
1 How many times do you brush your teeth?		
(a) Infrequent	(a) 4 (10%)	(a) 2 (5%)
(b) Once a day	(b) 25 (62.5%)	(b) 11 (27.5%)
(c) Twice a day	(c) 8 (20%)	(c) 25 (62.5%)
(d) More than twice a day	(d) 3 (7.5%)	(d) 2 (5%)
2 Do you disinfect your toothbrush before and after every use?		
(a) Always	(a) 0 (0%)	(a) 36 (90%)
(b) Sometimes	(b) 3 (7.5%)	(b) 4 (10%)
(c) Never	(c) 37 (92.5%)	(c) 0 (0%)
3 How long do you take to brush your teeth?		
(a) <1 minute	(a) 20 (50%)	(a) 6 (15%)
(b) About 1–2 minutes	(b) 23 (57.5%)	(b) 30 (75%)
(c) >2 minutes	(c) 7 (17.5%)	(c) 4 (10%)
4 Do you put a cover over your toothbrush head after use?		
(a) Always	(a) 16 (40%)	(a) 2 (5%)
(b) Sometimes	(b) 8 (20%)	(b) 3 (7.5%)
(c) Never	(c) 16 (40%)	(c) 35 (87.5%)
5 How far do you keep your toothbrush from the toilet bowl?		
(a) <3 feet	(a) 12 (30%)	(a) 1 (2.5%)
(b) 3–6 feet	(b) 24 (60%)	(b) 3 (7.5%)
(c) >6 feet	(c) 4 (10%)	(c) 36 (90%)
6 What is the bristle type of your toothbrush?		
(a) Soft	(a) 11 (27.5%)	(a) 33 (82.5%)
(b) Medium	(b) 14 (35%)	(b) 4 (10%)
(c) Hard	(c) 15 (37.5%)	(c) 3 (7.5%)
7 Do you keep multiple toothbrushes in a toothbrush holder?		
(a) Yes	(a) 36 (90%)	(a) 9 (22.5%)
(b) No	(b) 4 (10%)	(b) 31 (77.5%)

of airborne germs every time we flush! If our toothbrush is left out in the open, they can easily get into the bristles of the brush. About 100 million bacteria can lurk on an uncovered toothbrush.

To prevent contamination, keep at least 2 m/6 ft distance between the toilet bowl and the toothbrush. Always close the toilet lid before flushing. Keeping the toothbrush in a dry environment prevents the microbial growth. However, it can't be possible in areas with humid climates, so disinfectants play an important role here. Toothbrushes should be kept in separate holders to avoid multiple touches and cross-infection.

Disinfectants are typically considered to be liquid chemical formulations used to eradicate germs. Multiple research has highlighted the importance and effectiveness of toothbrush disinfection. Chlorhexidine was utilized as a disinfectant in this investigation. Chlorhexidine gluconate is a positively charged bis biguanide compound that is efficient in combating a variety of pathogens. At different concentrations, it can operate as both a bacteriostatic and bactericidal agent.¹² Chlorhexidine prevents bacteria from adhering to teeth and oral mucosa and harms microorganisms by making bacterial cell walls more permeable and altering the osmotic equilibrium. Both gram-negative and -positive bacteria, as well as yeasts, are inhibited by it.¹⁶ Several trademark UV sanitizers have become commercially available in recent years. The efficacy of these devices against bacteria and viruses has been investigated.¹⁷

In our study, we discovered that patients who received instructions using AV means resulted in a significant reduction of microbial count on their toothbrushes after using disinfectants. Parents showed a positive approach toward maintaining

toothbrush hygiene. Questionnaires have been proven to evaluate the effectiveness of AV aid.

CONCLUSION

Used toothbrushes act as microbial reservoirs and may be a major factor in the spread of disease to people. After being used for 14 days, it was discovered that every toothbrush used in the study had been infected with various germs. 0.2% chlorhexidine gluconate (83.3%) was proven to be efficient in disinfecting the toothbrushes by rinsing the toothbrush before and after brushing, whereas tap water was deemed ineffective.⁵ Toothbrushes must be renewed every 3 months. Sick individuals should change their toothbrushes often to prevent reinfection or spreading of infection. To maintain good oral hygiene and overall health, it is advisable for individuals to regularly disinfect their toothbrushes and store them in a clean, dry place.³

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