ORIGINAL RESEARCH

An in Vitro Investigation of the Effectiveness of Household Decontaminants for Toothbrushes

M Rajkumar[1], KC Vignesh[2], S Revathi[3], Akila Ganesh[4], P Kennedy Kumar[5], S Vijayanirmala[6]

Abstract

Background: Toothbrushes are essential for the removal of plaque and for promoting proper oral hygiene. Even though, many of them are employed in daily routines, there isn't general agreement on toothbrush disinfection methods. The purpose of this paper is to assess the efficacy of cleaning toothbrushes at home with tap water, rock salt, lime, and vinegar.

Materials and methods: An in vitro study was conducted involving 40 participants randomly allocated into four groups, each provided with toothbrushes and corresponding disinfection solutions: Group 1 (plain water control), Group 2 (rock salt solution), Group 3 (lime solution), and Group 4 (vinegar solution). Toothbrush samples were collected and subjected to microbiological analysis following a one-week usage period.

Toothbrush bristles were vortexed in 1ml peptone broth and serially diluted to 103 with peptone water. Subsequently, 0.01ml of diluted broth was inoculated onto 5% sheep blood agar using a calibrated loop for semi-quantitative analysis. Plates were incubated overnight and examined for bacterial growth, with colony morphology documented and Gram staining performed. Statistical analysis was conducted using one-way ANOVA (SPSS version 26.0) to determine significance between groups.

Results: Vinegar and lime showed statistically significant results for the decontamination of toothbrushes when compared to other agents.

Conclusion: This in vitro investigation demonstrated significant antimicrobial efficacy against S. mutans, S. aureus, and E. coli. Among the tested groups, lime and vinegar exhibited statistically significant bacterial reduction, representing accessible and economical household alternatives for disinfection purposes..

Keywords: Household decontaminants, Dentifrices, Oral hygiene, Disinfection and Streptococci species

Introduction

Toothbrush play a vital role in effective plaque removal and promotes effective oral hygiene[1]. Within a few seconds after the use of a toothbrush, it is invaded by a wide array of bacteria, viruses, yeast and fungi. The oral cavity and the place where toothbrushes are kept both contain the bacteria. Proper oral hygiene maintenance can greatly reduce these microbes and help in achieving superior dental health[2]. The studies have been conducted on the various household decontaminates and they are inexpensive. Rock salt has high antimicrobial properties and is often used as a preservative[3]. Lime has been proven to be used as a medicine against various infectious diseases[4] and prevents food contamination. Vinegar being a household material prevents contamination of bacteria in food[5]. Brushes can become contaminated when they come into contact with the environment, the oral cavity, and the containers in which they are housed[6].

Dayoub et al[7] observed increased bacterial contamination rates in toothbrushes stored in closed containers compared to those with air exposure. Similar findings were reported by Mehta et al[8], demonstrated that capped toothbrush storage resulted in enhanced microbial growth rates. Glass et al[9] documented that toothbrushes maintained in humid conditions demonstrated prolonged viability of Aggregatibacter actinomycetemcomitans and Herpes Simplex Virus 1 (HSV 1) for a minimum duration of three days[10]. Prolonged use of

Department and Institution Affiliation: [1]Department of Public Health Dentistry, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Porur, Chennai, [2]Department of Pediatric and Preventive Dentistry, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Porur, Chennai, [3]Department of Oral Medicine and Radiology, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Porur, Chennai, [4]Department of Public Health Dentistry, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Porur, Chennai, [5]Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Porur, Chennai [6]Department of Oral Pathology, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Porur, Chennai.

Corresponding Author: Dr. K. C. Vignesh, Senior Lecturer, Department of Pediatric and Preventive Dentistry, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), No 1, Ramachandra Nagar, Porur, Chennai, Email id - drkcvignesh@gmail.com

How to cite the article: Rajkumar M, Vignesh KC, Revathi S, Ganesh A, Kennedy Kumar P, Vijayanirmala S. An In vitro Investigation of the Effectiveness of Household Decontaminants for Toothbrushes. Oral Maxillofac Pathol J 2025; 16(2); 176-179.

Source of Support: Nil

Conflict of Interest: None

toothbrushes facilitates contamination by various microorganisms including Streptococcus mutans, Staphylococcus aureus, and Lactobacilli[11], with the retention and survival of these pathogens on toothbrush bristles post-brushing representing a potential source of oral re-contamination.

Rinsing the toothbrush using plain water after a single use may not eliminate all microorganisms present in the toothbrush[12]. A contaminated tooth brush can re-introduce microorganisms into the oral cavity[13]. The complex structure of a toothbrush can trap millions of microorganisms even after washing with plain water[14]. Additionally, as toothbrushes are kept in restrooms or close to washbasins, the storage location has a big impact on how contaminated they become. The release of even a small droplet from a wash basin can result in millions of microorganisms in the air[15]. In most households, toothbrushes are kept in a common container with some other household objects. Unfortunately, proper care and maintenance of the toothbrush is often neglected due to a lack of awareness among the people. Microbial contamination rates can be effectively controlled through regular replacement of toothbrushes[16], utilization of antiseptic solutions[17], and application of ultraviolet irradiation[18]. Despite the demonstrated effectiveness of these methods, their implementation may be limited by cost considerations and the technical complexity required for consistent application across diverse age groups and clinical settings. Rock salt, lime, and vinegar are three of the most common household materials, which have antimicrobial properties and are inexpensive. Hence, there is a need for a simple and inexpensive method for decontaminating toothbrushes that can be performed using household materials. The purpose of this study is to evaluate the effectiveness of household materials in the decontamination of toothbrushes.

Materials & Methods

An in vitro study was conducted following institutional ethics committee approval (CSP/22/MAR/108/193) within the Departments of Public Health Dentistry and Microbiology. The inclusion criteria comprised participants aged 20-30 years without systemic illness or dental pathology, while exclusion criteria included individuals outside this age range or those presenting with systemic or dental disorders. Forty new toothbrushes (Colgate soft toothbrush, Colgate Palmolive India Ltd, Chennai, Tamil Nadu) and 12×18 cm zip-lock covers (R K P Polybags Private Limited, Chennai, Tamil Nadu) were randomly allocated across four experimental groups: Group 1 (plain water control), Group 2 (rock salt), Group 3 (lemon), and Group 4 (vinegar), with ten participants per group.

Each group consists of ten participants and prior instructions were given on how to use the toothbrush along with the household decontaminates. Participants in Group I had to rinse their toothbrushes with water after using them. It has been considered as a control group. The toothbrush was recommended to be stored in the living room. The Group II participants were directed to soak their toothbrush for five minutes in a solution made by mixing two teaspoons (19 gram) of rock salt (Tata salt, India) in 100 ml of plain water. The participants were told to rinse the toothbrush under running water and then set it out in the living room. Throughout the week, the solution was modified every two days. Participants in Group III were instructed to use the extract of sliced, raw lemon for five minutes prior to rinsing the toothbrush under running water and preserving it in the living room. The extract was made by mixing 5 ml of lime juice with one 100 ml of plain water. Every two days in a week, the sample solution was replaced. Group IV participants were directed to use 5ml of commercially available vinegar (Suriya Food Products, Chennai, Tamil Nadu) for five minutes after which they instructed to rinse the toothbrush under a running tap water and store the toothbrush in the living room. Throughout the week, the solution was modified every two days. The participants have been instructed to brush their teeth twice a day and to store their toothbrushes in a vented container. In each group, the disinfectant solution should be changed on alternative days. After one week, participants returned their toothbrushes in a zip lock cover and the collected toothbrushes were sent to the laboratory on the same day for further microbial analysis.

The collected toothbrushes were separated as per the groups and labelled for identification. From the head of the toothbrush bristles, Group I (plain water) were cut aseptically using scissors (Martand Stainless Steel Foldable Scissor, India) and were added to the test tube which contained 10 ml of BHI (Brain Heart Infusion) broth vortexed for five minutes at 2400 rpm/ min. The vortexed broth was serially diluted to 103 using peptone water. A 0.01 ml aliquot of the diluted broth was inoculated onto 5% sheep blood agar plates using a calibrated loop. The plates were incubated overnight at 37°C under aerobic conditions. Following incubation, plates were examined for the presence or absence of bacterial growth. Colony morphology was documented, and Gram staining was performed on isolates from plates demonstrating growth. Bacterial identification to the genus level was conducted based on phenotypic characteristics and Matrix-Assisted Laser Desorption/Ionization (MALDI-TOF) technology. This methodology was consistently applied across all experimental groups.

Statistical analysis was performed using IBM statistical package for the social sciences (SPSS) (version 16.0) software (IBM Corp, Armonk, NY).

Results

The one-way analysis of variance (ANOVA) was used to determine the significant difference between the four groups. Means and standard deviations were calculated. A chi square test was used to compare the results between the groups ($p<0.0005$). p-value "Highly significant at $p<0.01$ The toothbrush kept in the control group contained higher colonies of microorganism. The distribution of colony-forming units (cfu) in treatment solutions is shown in Table 1. When compared to the other test and control agents, vinegar produced the best results for decontaminating the toothbrushes among the four groups. Among the test agents, which are shown in Table 2, vinegar demonstrated a statistically significant decrease in the microbial count compared to other test solutions. A chi-square test was employed to determine statistical significance, with a p-value of <0.0005 considered statistically significant.

Discussion

Toothbrushes, despite being integral to oral hygiene, are susceptible to microbial colonization, which may serve as a potential reservoir for re-infection and possibly even systemic diseases. The present study sought to assess the antimicrobial efficacy of readily available household decontaminants against microbial proliferation on used toothbrushes. Although all tested agents demonstrated statistically significant reduction in microbial loads following one-hour immersion, complete decontamination was not achieved by any intervention, indicating partial antimicrobial efficacy and highlighting the necessity for further optimization of disinfection protocols.

The present findings are consistent with those reported by Bezirtzoglou et al[18], who employed quantitative methodology to assess ozone efficacy against toothbrush-associated microbiota, demonstrating pronounced antimicrobial activity and significant reduction in total viable bacterial counts. However, the limited accessibility of ozone for routine household application renders domestic disinfection agents more practical, albeit less potent, alternatives. These results corroborate the concept that conventional household methods can achieve substantial microbial load reduction, though they lack the complete sterilization capacity demonstrated by clinical-grade interventions such as ozone therapy.

Suido et al[19] conducted a clinical investigation examining bacterial contamination of chlorhexidine-coated interdental brush filaments, with results emphasizing chlorhexidine's sustained antimicrobial effect attributed to its substantivity properties. Although the present study did not evaluate chlorhexidine-coated materials, the transient reduction in colony counts observed suggests that household agents lack the residual antimicrobial activity characteristic of professionally formulated antiseptics. These findings indicate that achieving long-term efficacy may require repeated application protocols or the utilization of more potent antimicrobial agents.

Additionally, Morris et al[20] investigated microbial contamination in power toothbrushes and demonstrated that brush head design significantly influenced microbial retention, with solid-head toothbrushes harboring fewer microorganisms compared to hollow-head designs, presumably due to reduced moisture retention capacity in the former. This observation corroborates our emphasis on post-use handling and storage protocols. Although the present study did not stratify results according to toothbrush design variations, these findings reinforce the critical role of desiccation and structural factors in contamination risk assessment, suggesting that household decontaminants may provide only partial mitigation when suboptimal design or storage conditions are present.

Furthermore, while the American Dental Association (ADA) recommends toothbrush replacement every three to four months, this recommendation primarily addresses bristle deterioration rather than microbial considerations. The present findings indicate that significant microbial accumulation occurs prior to visible physical degradation, suggesting that replacement intervals alone may be inadequate without concurrent disinfection protocols. Notably, none of the tested agents achieved complete microbial eradication within the one-hour immersion period, indicating limitations in either antimicrobial efficacy or protocol optimization. This partial effectiveness underscores the necessity for standardized, evidence-based guidelines for toothbrush disinfection that balance practical accessibility with antimicrobial performance.

Collectively, the existing literature establishes a growing consensus that although toothbrush contamination is unavoidable, diverse mitigation strategies encompassing mechanical design modifications and advanced disinfection protocols can substantially minimize associated risks. Nevertheless, additional research is warranted to establish optimal treatment parameters, including exposure duration, agent concentration, and potential synergistic combinations, with particular emphasis on protocols that are feasible for routine household implementation.

Limitation of the Present Study: Studies with larger sample sizes are required to establish standardization and achieve greater statistical significance. The investigation was limited to aerobic contaminants, specifically Staphylococcus species, Streptococcus species, and Escherichia coli, while anaerobic bacteria and fungal contaminants were not examined.

Table 1 - Kruskal -wallis test in Colony Forming Unit (CFU) of treatment solutions

Group	Mean CFU	Sample size	Mean Ranks	Standard Deviation (SD)
I	257.1	10	35.50	17.5
II	80.5	10	25.50	17.4
III	0.0	10	10.00	0.0
IV	0.2	10	11.00	0.6

Table 2: Grouping variable between groups

	CFU
Chi Square	36.911
DF	3
P-Value	0.0005

CFU- Colony Forming Unit
Df - Degrees of Freedom
p-value "Highly significance at p<0.01

Conclusions

This in vitro study demonstrated the antimicrobial efficacy of the tested disinfectant against S. mutans, S. aureus, and E. coli. Among the four experimental groups evaluated, lime and vinegar exhibited statistically significant reductions in bacterial load compared to controls. Given their accessibility and cost-effectiveness as household products, these findings suggest that lime and vinegar may serve as viable alternatives for antimicrobial applications.

Contributors

All authors jointly formulated the major concepts of this study and approved the final version. KCV and RS initially drafted and edited sections of this manuscript. MR, AG, KKP, VNS made critical revisions for important scientific content. MR & KCV provided overall supervision of the study. All authors provided information and reference for this paper.

References

1. Turner LA, McCombs GB, Hynes WL, Tolle SL. A novel approach to controlling bacterial contamination on toothbrushes: chlorhexidine coating. International journal of dental hygiene. 2009 Nov;7(4):241-5.
2. Löe H. Oral hygiene in the prevention of caries and periodontal disease. International dental journal. 2000 Jun;50(3):129-39.
3. Wijnker JJ, Koop G, Lipman LJ. Antimicrobial properties of salt (NaCl) used for the preservation of natural casings. Food Microbiology. 2006 Oct 1;23(7):657-62.
4. Hindi NK, Chabuck ZA. Antimicrobial activity of different aqueous lemon extracts. Journal of Applied Pharmaceutical Science. 2013 Jun 27;3(6):074-8.
5. Rahmani AH, Albutti AS, Aly SM. Therapeutics role of olive fruits/oil in the prevention of diseases via modulation of anti-oxidant, anti-tumour and genetic activity. International journal of clinical and experimental medicine. 2014 Apr 15;7(4):799.
6. American Dental Association. ADA statement on toothbrush care: Cleaning, storage and replacement. Council on Scientific Affairs. Positions and Statements. 2005 Nov.
7. Caudry SD, Klitorinos A, Chan EC. Contaminated toothbrushes and their disinfection. Journal (Canadian Dental Association). 1995 Jun 1;61(6):511-6.
8. Dayoub MB, Rusilko D, Gross A. Microbial contamination of toothbrushes. Journal of dental research. 1977 Jun;56(6):706
9. Mehta A, Sequeira PS, Bhat G. Bacterial contamination and decontamination of toothbrushes after use. New York State Dental Journal. 2007 Apr 1;73(3):20.
10. Glass RT, Carson SR, Barker RL, Peiper SC, Shapiro S. Detection of HIV proviral DNA on toothbrushes: a preliminary study. Journal-Oklahoma Dental Association. 1994 Jan 1;84(3):17-20.
11. Blaustein RA, Michelitsch LM, Glawe AJ, Lee H, Huttelmaier S, Hellgeth N, Ben Maamar S, Hartmann EM. Toothbrush microbiomes feature a meeting ground for human oral and environmental microbiota. Microbiome. 2021 Dec; 9:1-4.
12. Sconyers JR, Crawford JJ, Moriarty JD. Relationship of bacteremia to toothbrushing in patients with periodontitis. The Journal of the American Dental Association. 1973 Sep 1;87(3):616-22.
13. Basman A, Peker İL, Akca G, Alkurt MT, Sarikir C, Celik I. Evaluation of toothbrush disinfection via different methods. Brazilian oral research. 2015 Dec 15;30.
14. Matreja P, Anand M, Shetty S, Samuel SR, Tho BS. Is your Tooth Cleaner, Clean? Global Journal of Medical researchDentistry and tolaryngology. 2013 Jan 1;13(2):18-23.
15. Peker I, Akca G, Sarikir C, Toraman Alkurt M, Celik I. Effectiveness of alternative methods for toothbrush disinfection: an in vitro study. The Scientific World Journal. 2014;2014(1):726190.
16. Pai V. Effect of a single-use toothbrush on plaque microflora. Indian Journal of Dental Research. 2009 Oct 1;20(4):404-6.
17. Sato S, Pedrazzi V, Lara EH, Panzeri H, Ito IY. Antimicrobial spray for toothbrush disinfection: an in vivo evaluation. Quintessence international. 2005 Nov 1;36(10).
18. Bezirtzoglou E, Cretoiu SM, Moldoveanu M, Alexopoulos A, Lazar V, Nakou M. A quantitative approach to the effectiveness of ozone against microbiota organisms colonizing toothbrushes. Journal of dentistry. 2008 Aug 1;36(8):600-5.
19. Suido H, Offenbacher S, Arnold RR. A clinical study of bacterial contamination of chlorhexidine-coated filaments of an interdental brush. The Journal of Clinical Dentistry. 1998 Jan 1;9(4):105-9.
20. Morris DW, Goldschmidt M, Keene H, Cron SG. Microbial contamination of power toothbrushes: A comparison of solid-head versus hollow-head designs. American Dental Hygienists' Association. 2014 Aug 1;88(4):237-42.