Review Article

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Evaluating Sanitization of Toothbrushes Using Various Decontamination Methods: A Meta-Analysis

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ABSTRACT

Toothbrushes play an essential role in personal oral hygiene by effective plaque removal.However, they get heavily contaminated by bacteria, viruses, yeasts, and fungi which may originate from the oral cavity after every use as well as from the environment where they are stored.This systematic review was conducted to identify various decontamination interventions attempted scientifically and it summarizes the efficacy of each. Meta-analysis illustrated that the use of Ultra-violet rays and Microwave had a significant effect on reduction of the microbial count of a used toothbrush with a mean difference of -2.61 and CI (-4.66,-0.76) with I^2=98%. When compared with non-active treatment group, the natural agents (garlic, green tree and tea-tree oil) proved to sterilize the toothbrushes effectively with mean difference of -483.34, CI (-914.79, -51.88) and I^2=100%.In contrast, chlorhexidine showed the insignificant result with a mean difference of -347.55 and CI (-951.90, 256.80) with I^2=100%. The evidence from this review suggests that decontaminating toothbrush reduces bacterial load. Toothbrushes exposed to radiation and natural agents proved to sanitize them effectively but chlorhexidine rendered insignificant results.

Keywords: Chemical agent; disinfection; radiation; microbial load; natural agent; toothbrush.

INTRODUCTION

Toothbrushes play an essential role in personal oral hygiene by effective plaque removal.[1-5] They also help in the prevention of dental caries and periodontal disease.[6] However, toothbrushes may be the cause of disease transmission and increase the risk of infection since they can serve as a reservoir for microorganisms in healthy,diseased and medically ill adults.[3,7-10] Sharing or storing the toothbrushes together mostly increases the contamination.[11-14] Studies have reported 70% of the used toothbrushes being heavily contaminated with different pathogenic microorganisms.[15,16]

The risk of infection or re-infection can be prevented by various decontamination methods such as, radiation (Microwave, ultraviolet rays) and antimicrobial solutions (Chemicals:chlorhexidine, triclosan, cetylpyridinium chloride, Listerine and several dentifrices; natural agents: garlic and tea tree oil extracts).[2,17] However, there is no systematic review attempted to look at the effectiveness of decontamination of tooth brush and also the effective intervention for decontamination. Thus, the present systematic review was directed to provide an evidence on the effectiveness of decontaminating toothbrush on the bacterial overload.

METHODS

An electronic search was conducted in March 2016 which included six databases (PubMed, Cochrane CENTRAL, Ovid-MEDLINE, Scopus, CINAHL, and Web of Science). The databases were searched for the period of 1996 to 4th of April 2016. (The oldest article that could be accessed on this subject was carried out in 1996). Keywords are given in appendix 1 and search strategy for the PubMed is given in appendix 2. For this review randomized controlled trials, non-randomized controlled trials and in-vitro studies were included. Studies with individuals irrespective of their oral health having at least 20 teeth in the oral cavity and using manual toothbrushes were included. The review included the studies only with interventions provided for the following disinfection; Chemical agents: Chlorhexidine digluconate, white vinegar, sodium hypochlorite, hydrogen peroxide, Listerine or any dentifrice solution; Natural agents: Garlic, tea tree oil, green tea etc. and Radiation therapy: Microwave and UV rays.Whereas, studies done on participants on antibiotic therapy for any reasons were excluded. Antimicrobial effectiveness of all the

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chemicals was compared with non-active treatment like distilled water and saline or no treatment. This review analysed the effectiveness of all the different interventions in terms of Colony Forming Unit count (CFU count), Minimally Inhibitory Concentration (MIC), adverse effects of active treatment and their cost effectiveness.

For the study selection, two review authors (S.K.A and S.D) were involved in title screening. Abstract and full-text screening was carried out independently. Full-text copies of all eligible and potentially eligible studies were obtained, which was further evaluated in detail by two review authors to identify studies that met the inclusion criteria. Possible disagreements were moderated by third review author (T.V.B) and expert opinion was taken by the fourth review author (N.S.N). The details of the included studies from the titles to full texts are described as Preferred Reporting Items for Systematic review and Meta-analysis (Figure 1). Likewise, data extraction and management were carried out by the same authors independently using a standard data extraction form adapted from Cochrane Handbook for systematic reviews of Interventions 5.0.2.[18] The extracted data includes: general information of the studies, risk of bias and outcome measures (Form format in Appendix 2).

Methodological quality assessment of all the included RCTs, Crossover studies and Non-RCT's were independently assessed by two authors based on three tools namely "The Cochrane Collaboration's tool for assessing Risk of bias"[19] for RCT's, "Effective Practice and Organization of Care (EPOC)-specific resources for review authors" [20] for Non-RCTs and the tool developed by Ding H et al., 2005[21] for Crossover trials. Each assessor assessed the study as 'high risk' 'low risk' or 'unclear risk' for each of the domains included in the tool.Meta-analysis was carried out for studies having similar outcome measures. For continuous outcome (CFU/ml and log CFU), mean and standard deviation were used to summarize the data for each study. Mean difference was calculated using random effects model in Review Manager 5.3. Heterogeneity was assessed using Q statistics and I^2 Results were expressed in terms of mean difference, 95% confidence interval, and forest plot.

RESULTS

Total of 1115 citations were retrieved from six databases and after removing duplication, 940 articles remained for title screening. After title and abstract screening, 30 articles were qualified for full-text screening. Out of them, 10 studies were excluded because control group was not present in those studies. Final selection of 20 studies were done for data extraction. Among included studies, there were eight RCTs, six N-RCTs, four crossover and two *in-vitro* studies. Out of eight RCTs, only four had sufficient data for meta-analysis. The selection of studies has been shown through PRISMA flow diagram in Figure 1.

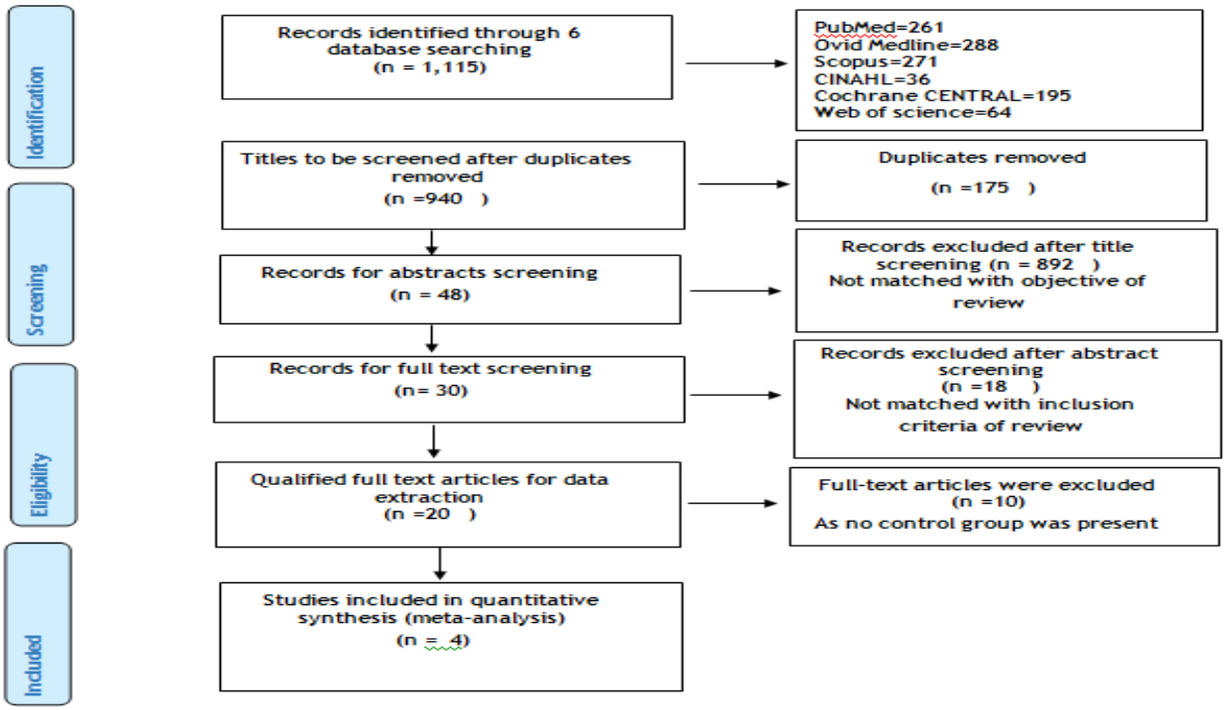

Figure 1. PRISMA flow diagram.

Table 1. Shows characteristics of all the included studies. The number of RCTs included for this review[2,17,22-27] were carried out between the year 2007 and 2016. Amongst the included studies, five were conducted in India, two in Turkey and one from New York. There were total of 629 participants from the included studies which involved children and adults of age ranged from 24 months to 65 years. The decontamination methods were chlorhexidine mouthwash, spray, cetylpyridinium chloride, Dettol, Listerine, UV rays, microwave and natural agents like tea tree oil, garlic extracts with water or saline. The number of Non-RCTs[28-33] included in this review were conducted in the year 2000 to 2007 which was done in Turkey, Brazil, India, and the US. The total number of participants was 140 which involved children and adults. Amongst six Non-RCTs, one study has not clearly mentioned about the number of participants.[31] The interventions in NRCTs included dentifrices with fluoride and triclosan, chlorhexidine, sodium hydroxide, tetrasodium EDTA, cetylpyridinium spray, hydrogen peroxide and dettolin. Crossover studies[34,35-37] published in the year 2006-2015 carried out in Brazil and Turkey recruited 136 participants analyzing the sanitizing agents like periguard, periobio, cetylpyridinium chloride, brushtox and experimental solution (1% polyaminopropyl biguanide), *M. glomerata* and *M. Laevigata*. In vitro studies[38,39] published in the year 2011 and 2012 were carried out in Brazil and India respectively using 120 toothbrushes which were contaminated with *S mutans* and divided into three treatment groups, chlorhexidine solution, microwave, and sterile tap water.

Each assessor assessed the study as 'Unclear or Low or High risk' for each of the domains included in the tool (Figure 2 and 3). Although most of the studies reported random allocation done in their study interventions, only one out of eight RCT studies had specified the allocation method. Allocation concealment was done in none of the studies. Only two out of eight studies reported blinding of participants, personnel and outcome assessment. All four crossover studies had appropriate crossover design, randomized treatment order and blinding done. Only one study[34] analyzed the carry-over effect and had allocation concealment done.

The first two domains, "sequence generation and allocation concealment" are not specific for Non-randomized studies as they have no random allocation. All the non-randomized trials had baseline characteristics and baseline outcome measurement done except one study[31] where baseline characteristics of the study population were not mentioned and also had incomplete outcome data reported. A study[30] had no baseline outcome data mentioned. Only two NRCT studies[28,33] had carried out blinding. All the studies indicated low risk of bias in selective outcome reporting.

Table 1. Characteristics of included studies in the systematic review on evaluation of toothbrush using various decontamination methods on toothbrush sanitization.

Design	Participants	Age in years	Intervention
RCT	24	7	Chlorhexidine mouth wash and Chlorhexidine spray[27]
RCT	25	21-65	Violight tooth brush holder[22]
RCT	30	22-28	Microwave and UV[2]
RCT	15	21-50	0.2% Chlorhexidine and UV rays[26]
RCT	75	18-21	3% Neem,garlic, green tea , 0.2%CHX[23]
RCT	210	18-25	0.2% Tea tree oil, 3% Garlic extract,0.2% CHX, 0.05% CPC, UV[17]
RCT	50	08-11	Hexidine, 3.0% Hydrogen peroxide, Dettol, Listerine[24]
RCT	200	24-72 months	0.12% Chlorhexidine gluconate[25]
N-RCT	40	05-07	Dentifrice with fluoridated and triclosan[33]
N-RCT	19	05-12	0.12% Chlorhexidine gluconate, 1% sodium hydroxide[29]
N-RCT	21	05-12	0.12% Chlorhexidine gluconate and 1% sodium hydroxide[30]
N-RCT	Not clear	Not clear	Tetrasodium EDTA[31]
N-RCT	30	23 to 56	Cetylpyridinium spray and basic formulation only with spray[28]
N-RCT	30	12-14	Hexidine mouth wash, Hydrogen peroxide and Dettolin[32]

Cross-over	52	28-42 months	Periogard (0.12% chlorhexidine solution, Brustox antiseptic toothbrush cleaner spray (activated ethanol), Experimental solution containing 1% polyaminopropyl biguanide, 0.1% bronopol, EDTA, propyleneglycol, polyvinylpirrolidone K30, ethanol 96%, 0.5M sodium hydroxide solution, distilled water and blue no. 1 dye[35]
Cross-over	30	Not mentioned	0.12% CHX solution[34]
Cross-over	38	02-05	0.12%CHX (periogard), 2.5% solution of M glomerata ethanol,2.5% M. Laevigata ethanol[36]
Cross-over	16	20.33 (Mean)	Chlorhexidine gluconate 0.12% (Periobio and periogard) and0.05% cetylyridinium chloride[37]
In Vitro	60	NA	CHX solution, microwave oven[39]
In Vitro	60	NA	0.12% Chlorhexidine and Microwave oven[38]

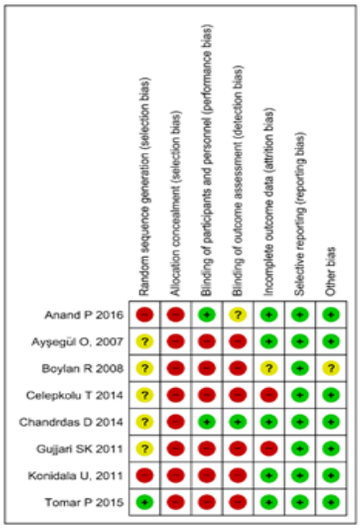

Figure 2. Risk of Bias of RCTs.

Figure 3 shows the meta-analysis of two RCT studies[17,23] comparing mean and standard deviation of 0.2% chlorhexidine with distilled water following random effect model. The studies independently showed immersion of toothbrush in 0.2% chlorhexidine for 12 to effectively reduce microbial load. In contrast, the result of meta-analysis after combining two studies had shown the insignificant result with a mean difference of -347.55 and CI (-951.90, 256.80) with I^2=100%.

A study was done by Gujjari SK et al.,[2] showed the use of UV light toothbrush sanitizer and microwave oven as means for decontamination of toothbrush. Sanitization was carried out by placing the brush in the receptacle and the head of the toothbrush was exposed for 12 minutes to UV radiation. Toothbrushes in the microwave group were sanitized by placing the brush in a microwave oven (2450MHz) for 5 minutes.[2]Other study carried out by Boylan et al.,[22] showed sanitization via the use of UV light holder. Meta-analysis illustrated that the use of both ultra-violet rays and microwave had significant effect on reduction of the microbial count of the used toothbrush with a mean difference of -2.61 and CI (-4.66,-0.76) with I^2=98%(Figure 4).

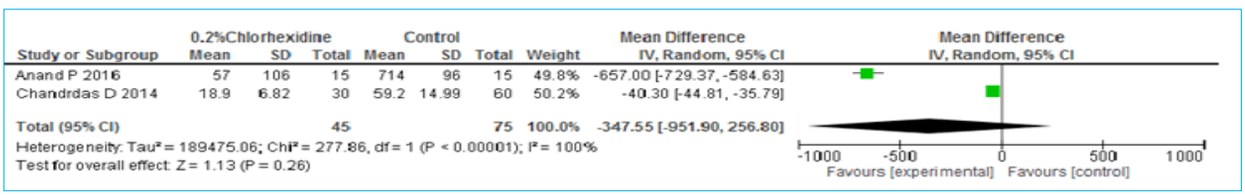

Figure 3. Meta-analysis of mean difference for Chlorhexidine with Distilled water.

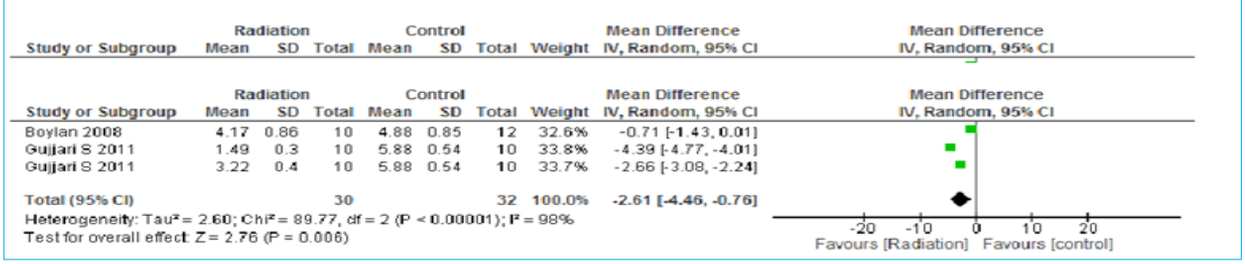

Figure 4. Meta-analysis of mean difference for Radiation with Tap water.

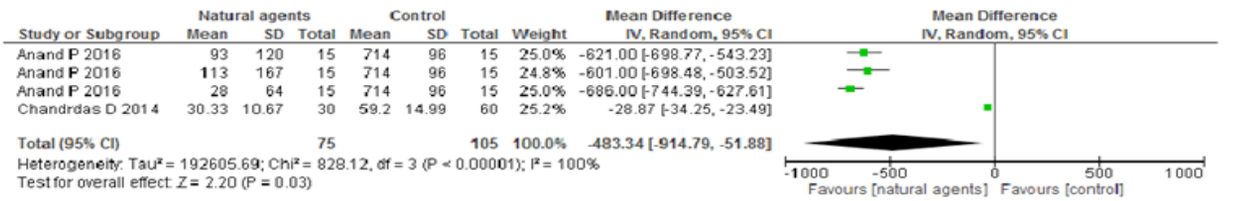

Figure 5. Meta-analysis of mean difference for Natural agents with Distilled water.

Two studies[17,23] showed decontamination of the toothbrush by immersion in agents such as Garlic, Green tea and Tea tree oil for 12 hours. Meta-analysis elucidated that all the agents effectively sterilize the toothbrushes. When comparing mean CFU/ml between the control group and each natural agents, the difference noted were all statistically significant. The mean difference was -483.34, CI (-914.79, -51.88) and I^2=100% (Figure 5).

The included studies using chemical, radiation and natural agents displayed a significant reduction in microbial load on used toothbrushes. In vitro studies[38,39] indicated that 0.12% Chlorhexidine and Microwave were equally effective. Crossover studies[34,35-37] showed the antimicrobial activity of the sanitizing agents like periguard, periobio, cetylpyridinium chloride, brushtox and experimental solution (1% polyaminopropyl biguanide), *M. glomerata* and *M. Laevigata*. All the studies proved chlorhexidine solution as an effective disinfectant. An experimental solution and *M. glomerata* were equally competent as chlorhexidine but had better results as compared to Brushtox. All the disinfectants used in Non-RCTs (0.2% hexidine, 0.12% chlorhexidine, Dettolin, Hydrogen peroxide and 1% sodium hypochlorite) were successful in producing desired result. Chlorhexine solution (0.2%) has shown 100% reduction of *mutans streptococci*. When toothbrushes were emerged in EDTA for 16 hours, the total viable count was reduced by more than 99%.[31] An intervention done with hydrogen peroxide resulted in no microbial growth at any interval i.e., 0-28 days. It can be considered to be safe as a disinfectant.[32]

The MIC is defined as the lowest concentration of test samples which inhibited visible growth of the microorganism tested. MIC values for the ethanol extract and solution of *M. glomerata* were 400 and 125 µg/mL, respectively, whereas those for the ethanol extract and solution of *M. laevig*ata were 400 and 14 µg/mL, respectively. A study carried out by Lessa FCR et al., 2012 showed that both solutions were able to reduce the formation of *m. streptococci* by 37.3 ± 23.7% and 28.7 ± 25.1% respectively.[36] Neither of the included studies mentioned about any adverse effects of the used disinfectants except for study done by Anand P et al.,[23] 2016that briefly discussed the unpleasant taste of garlic which caused halitosis and nausea.

UV light toothbrush holder is commercially available, expensive and may not be much cost effective. More studies are required to find out the cost-effectiveness of UV chamber (toothbrush holder). Chlorhexidine is easily available in the market and is cost effective.[26] A study conducted by Aysegul O et al.,[27]2007 highlighted the fact that although chlorhexidine solution is inexpensive, some bacteria get resistant to this chemical and the solution needs to be changed frequently because of which it is not so cost effective. Although CHX spray costs more than the mouthwash, the former is easier to use and provides longer preventive benefits.[27]The total cost of chemicals such as hexidine, 3% hydrogen peroxide and listerine per month INR. 68, 18 and 94 respectively. Among these disinfectants, 3% hydrogen peroxide is the most economical and it can be recommended for daily use.[24]

DISCUSSION

There is no systematic review attempted to look at the effectiveness of decontamination of tooth brush and also the effective intervention for decontamination. This systematic review was carried out to assess the effectiveness of various decontamination methods in decreasing the microbial load on the toothbrush. A toothbrush is the most common device used for oral hygiene maintenance.[17] Commonly, after oral use, toothbrushes are rinsed with plain water and stored in bathroom[26] and there is a high chance of cross-infection by sharing or keeping them in close proximity.[17,40] According to American Dental Association, for sound oral hygiene, appropriate toothbrush care and maintenance are important considerations and a person should change his/her toothbrush every three to four months.[41] However, frequent change of toothbrush increases the maintenance cost which becomes a burden. So, instead of changing toothbrushes in short intervals, use of disinfectant is more economical.[24] This is the first systematic review and meta-analysis demonstrating the clinical significance of various disinfectants used for sanitization of a toothbrush. All the eight RCTs[2,17,22-27] which compared different

disinfectants with the control group were at high risk of bias. The studies included in meta-analysis showed 98-100% statistical heterogeneity. However, meta-analysis has been performed because of the homogeneity in the methodology of the included studies.

On analysis, radiation and natural agents were proved to be effective in reducing the microbial colonization of toothbrush bristles. Antimicrobial activity of microwave irradiation at 2450 MHz for five minutes, destruction of microorganisms occurs due to its thermal effect on the cellular contents which finally leads to cell lysis.[2,42,43] UV radiation reveal that it is more effective in reduction of the total viable count on toothbrushes. As per manufacturer's instructions, exposure of toothbrush to UV rays for seven minutes inactivates the microorganisms by disrupting the chemical bonds that hold the DNA atom[26,44] and exposure upto 12 minutes can further lead to complete destruction of microorganisms.[2,45]

Two studies[17,23] were analyzed for natural disinfectants (herbal extracts) which showed that natural agents can effectively reduce the toothbrush contamination. Garlic extract showed the maximum reduction (96%) in the *Streptococcus mutans* count when immersed in 4.15 mg/ml for 12 hours. The antibacterial activity of garlic is due to the presence of allicin that possesses strong anti-*Streptococcus mutans* activity.[23,46] Also, use of 3% garlic extract has shown 100% reduction of the microbial count.[17] Green tea polyphenols have significant antioxidant, anti-carcinogenic, anti-inflammatory, thermogenic, probiotic, and antimicrobial properties. A study demonstrated its effectiveness as it reduced 84% of the *Streptococcus mutans* count from the contaminated toothbrush.[23] The presence of polyphenolic compounds is responsible for its anti-*Streptococcus mutans*property which inhibits growth, acid production, metabolism, and glucosyltransferase enzyme activity of *Streptococcus mutans*.[23, 47] Toothbrushes soaked in 0.2% of Tea tree oil solution (TTO) for 12 hours showed significant reduction in *S. mutans* count (71.9%).This oil is also effective against gram positive, gram negative bacteria's, viruses as well as fungi.[17,47] Treatment of *S. aureus* with tea tree oil (TTO) results in the leakage of potassium ions, inhibition of respiration. *S. aureus*cells get sensitized to sodium chloride, and apparently produces morphological changes under electron microscopy.[48-50] The evidence from this review suggests that decontaminating toothbrush reduces bacterial load. Disinfecting agents used in the included studies proved to be effective in reducing microbial count on a used toothbrush. However, definitive findings were difficult to draw due to less number of studies for analysis and difference in their outcomes with regard to colony forming unit. Also, the comparison of different agents could not be revealed through meta-analysis. Further, well-designed and high-quality intervention studies are required which follows respective reporting guidelines to better understand the comparative effectiveness of these interventions.

CONCLUSIONS

Present review reveals that disinfectants like radiation (UV rays and microwave) and natural agents (Garlic, green tea and tea tree oil) effectively reduce the microbial load on used toothbrush. On the other hand, 0.2% Chlorhexidine as a chemical disinfectant provides an insignificant result. However, the comparison between the effectiveness of chlorhexidine, natural agents and radiation could not be performed because of the heterogeneity in outcome measures. Remaining 16 studies showed that the disinfecting agents used were effective in reducing the microbial count.

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REFERENCES

1. Terézhalmy G, Biesbrock A, Walters P, Grender J, Bartizek R. Clinical evaluation of brushing time and plaque removal potential of two manual toothbrushes. Int J Dent Hyg. 2008;6(4):321-7. [DOI]
2. Gujjari SK, Gujjari AK, Patel PV, Shubhashini PV. Comparative evaluation of ultraviolet and microwave sanitization techniques for toothbrush decontamination. J Int Soc Prev Community Dent. 2011;1(1):20-6.[PubMed]
3. Frazelle MR, Munro CL. Toothbrush contamination: a review of the literature. Nurs Res Pract. 2012;2012:6. [DOI]
4. Stiller S, Bosma M, Shi X, Spirgel C, Yankell S. Interproximal access efficacy of three manual toothbrushes with extended, x-angled or flat multitufted bristles. Int J Dent Hyg. 2010;8(3):244-8.[DOI]
5. Sharma N, Qaqish J, Walters P, Grender J, Biesbrock A. A clinical evaluation of the plaque removal efficacy of five manual toothbrushes. J Clin Dent. 2010;21(1):8. [Google Scholar]

6. Komiyama EY, Back-Brito GN, Balducci I, Koga-Ito CY. Evaluation of alternative methods for the disinfection of toothbrushes. Braz Oral Res. 2010;24(1):28-33.[DOI]
7. Karibasappa G, Nagesh L, Sujatha B. Assessment of microbial contamination of toothbrush head: an in vitro study. Indian J Dent Res. 2011;22(1):2.[Link]
8. Ankola A, Hebbal M, Eshwar S. How clean is the toothbrush that cleans your tooth? Int J Dent Hyg. 2009;7(4):237-40. [DOI]
9. Efstratiou M, Papaioannou W, Nakou M, Ktenas E, Vrotsos I, Panis V. Contamination of a toothbrush with antibacterial properties by oral microorganisms. J Dent. 2007;35(4):331-7.[DOI]
10. Cobb CM. The tooth brush as a cause of repeated infections of the mouth. The Boston Medical and Surgical Journal. 1920;183(9):263-4.[DOI]
11. Peker I, Akca G, Sarikir C, Alkurt MT, Celik I. Effectiveness of Alternative Methods for Toothbrush Disinfection: An In Vitro Study. Scientific World Journal. 2014.[DOI]
12. Warren DP, GOLDSCHMIDT MC, THOMPSON MB, ADLER-STORTHZ K, KEENE HJ. The effects of toothpastes on the residual microbial contamination of toothbrushes. J Am Dent Assoc. 2001;132(9):1241-5. [DOI]
13. Glass RT SS. Oral inflammatory disease and the tooth brush. . J Ala Dent Assoc. 1993;77(4):12-6.
14. Verran J, Leahy-Gilmartin AA. Investigations into the microbial contamination of toothbrushes. Microbios. 1996;85(345):231-8.[Link]
15. Mehta A, Sequeira P, Bhat G. Bacterial contamination and decontamination of toothbrushes after use. NY State Dent J. 2007;73(3):20-2.[Link]
16. Taji S, Rogers A. The microbial contamination of toothbrushes. A pilot study. Aust Dent J. 1998;43(2):128-30.[DOI]
17. Chandrdas D, Jayakumar H, Chandra M, Katodia L, Sreedevi A. Evaluation of antimicrobial efficacy of garlic, tea tree oil, cetylpyridinium chloride, chlorhexidine, and ultraviolet sanitizing device in the decontamination of toothbrush. Indian J Dent. 2014;5(4):183. [PubMed]
18. Higgins JPT DJ. Selecting studies and collecting data. In: Higgins JPT DJ, editor. Cochran handbook for systematic review of interventions. New York: Wiley Online Liberary; 2008. p. 151-83.[DOI]
19. GREEN S. Assessing risk of bias in included studies. In: Higgins JPT. Cochrane Handbook for Systematic Reviews of Interventions Version 50 1, The Cochrane Collaboration. 2008:187-235.[Link]
20. Collaboration C. Suggested risk of bias criteria for EPOC reviews. 2013.
21. Ding H, Hu GL, Zheng XY, Chen Q, Threapleton DE, Zhou ZH. The method quality of cross-over studies involved in Cochrane Systematic Reviews. PloS ONE. 2015;10(4):e0120519. [DOI]
22. Boylan R, Li Y, Simeonova L, Sherwin G, Kreismann J, Craig RG, et al. Reduction in bacterial contamination of toothbrushes using the Violight ultraviolet light activated toothbrush sanitizer. Am J Dent. 2008;21(5):313.[Link]
23. Anand PS, Athira S, Chandramohan S, Ranjith K, Raj VV, Manjula V. Comparison of efficacy of herbal disinfectants with chlorhexidine mouthwash on decontamination of toothbrushes: An experimental trial. J Int Soc Prev Community Dent. 2016;6(1):22.[PubMed]
24. Konidala U, Nuvvula S, Mohapatra A, Nirmala S. Efficacy of various disinfectants on microbially contaminated toothbrushes due to brushing. Contemp Clin Dent. 2011;2(4):302.[PubMed]
25. Celepkolu T, Toptancı İR, Bucaktepe PGE, Sen V, Dogan MS, Kars V, et al. A microbiological assessment of the oral hygiene of 24-72-month-old kindergarten children and disinfection of their toothbrushes. BMC Oral Health. 2014;14(1):94.[DOI]
26. Tomar P, Hongal S, Saxena V, Jain M, Rana K, Ganavadiya R. Evaluating sanitization of toothbrushes using ultra violet rays and 0.2% chlorhexidine solution: A comparative clinical study. J Basic Clin Pharm. 2014;6(1):12.[PubMed]
27. Ayşegül Ö, Elgin IE, Gulcin A, Nedim S. The efficacy of chlorhexidine spray vs mouthwash in the microbial contamination of child toothbrushes. J Dent Child. 2007;74(3):177-81. [Link]
28. Sato S, Ito IY, Lara EHG, Panzeri H, Albuquerque Junior RFd, Pedrazzi V. Bacterial survival rate on toothbrushes and their decontamination with antimicrobial solutions. J Appl Oral Sci. 2004;12(2):99-103. [DOI]
29. Nelson Filho P, Macari S, Faria G, Assed S, Ito I. Microbial contamination of toothbrushes and their decontamination. Pediatr Dent. 2000;22(5):381-4.[FullText]
30. BHAT S HKS, GEORGE R M. Microbial contamination of tooth brushes and their decontamination. J Indian Soc Pedo Prev Dent. 2003;21(3):108-12[Link]
31. Devine D, Percival R, Wood D, Tuthill T, Kite P, Killington R, et al. Inhibition of biofilms associated with dentures and toothbrushes by tetrasodium EDTA. J Appl Microbiol. 2007;103(6):2516-24.[DOI]

32. Sogi SH SV, Kiran SN. Contamination of toothbrush at different time intervals and effectiveness of various disinfecting solutions in reducing the contamination of toothbrush. J Indian Soc Pedo Prev Dent. 2002;20(3):81-5.[Link]

33. Nelson-Filho P, Isper AR, Assed S, Faria G, Ito IY. Effect of triclosan dentifrice on toothbrush contamination. Pediatr Dent. 2004;26(1):11-6.[Link]

34. Rodrigues LK, Motter CW, Pegoraro DA, Menoli APV, Menolli RA. Microbiological contamination of toothbrushes and identification of a decontamination protocol using chlorhexidine spray. Revista Odonto Ciência. 2012;27(3):213-7.[DOI]

35. Nelson-Filho P, Faria G, da Silva RAB, Rossi MA, Ito IY. Evaluation of the contamination and disinfection methods of toothbrushes used by 24-to 48-month-old children. J Dent Child. 2006;73(3):152-8.[Link]

36. Lessa FC, Grillo CH, Pinto FE, Lorençon B, Martins JD, Bertolucci SK, et al. Efficacy of guaco mouthwashes (Mikania glomerata and Mikania laevigata) on the disinfection of toothbrushes. Revista Brasileira de Farmacognosia. 2012;22(6):1330-7. [Link]

37. do Nascimento C, Trinca NN, Pita MS, Pedrazzi V. Genomic identification and quantification of microbial species adhering to toothbrush bristles after disinfection: a cross-over study. Archives of oral biology. 2015;60(7):1039-47.

38. Nelson-Filho P, da Silva LAB, da Silva LL, Ferreira PDF, Ito IY. Efficacy of microwaves and chlorhexidine on the disinfection of pacifiers and toothbrushes: an in vitro study. Pediatr Dent. 2011;33(1):10-3. [Link]

39. Chamele J, Bhat C, Saraf T, Jadhav A, Beg A, Jagtap C, et al. Efficacy of microwaves and chlorhexidine for disinfection of pacifiers and toothbrushes: an in vitro study group. J Contemp Dent Pract. 2012;3:0.05.[Link]

40. Balappanavar AY, Nagesh L, Ankola AV, Tangade PS, Kakodkar P, Varun S. Antimicrobial Efficacy of Various Disinfecting Solutions in Reducing the Contamination of the Toothbrush-A Comparative Study. Oral Health Prev Dent. 2009;7(2). [Link]

41. Smiles M GR. Toothbrush Care: Cleaning, Storing and Replacement. American Dental Association2016[Link]

42. Vela G, Wu J. Mechanism of lethal action of 2,450-MHz radiation on microorganisms. Appl Env Microbiol. 1979;37(3):550-3. [Link]

43. Janković SM, Milošev MZ, Novaković ML. The effects of microwave radiation on microbial cultures. Hospital Pharmacology-International Multidisciplinary Journal. 2014;1(2):102-8. [Link]

44. Blatchley E, Dumoutier N, Halaby T, Levi Y, Laine J. Bacterial responses to ultraviolet irradiation. Water Sci Technol. 2001;43(10):179.

45. Bélanger-Giguère K, Giguère S, Bélanger M. Disinfection of toothbrushes contaminated with Streptococcus mutans. Am J Dent. 2011;24(3):155-8.[Link]

46. Lu X, Rasco BA, Jabal JM, Aston DE, Lin M, Konkel ME. Investigating antibacterial mechanisms of garlic (Allium sativum) concentrate and garlic-derived organosulfur compounds on Campylobacter jejuni using FT-IR spectroscopy, Raman spectroscopy and electron microscope. Appl Env Microbiol. 2011:AEM. 02845-10. [Link]

47. Mistry KS, Sanghvi Z, Parmar G, Shah S. The antimicrobial activity of Azadirachta indica, Mimusops elengi, Tinospora cardifolia, Ocimum sanctum and 2% chlorhexidine gluconate on common endodontic pathogens: An in vitro study. Eur J Dent. 2014;8(2):172.[PubMed]

48. Carson C, Hammer K, Riley T. Melaleuca alternifolia (tea tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev. 2006;19(1):50-62. [Link]

49. Carson CF, Mee BJ, Riley TV. Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrobiol Agents Chemother. 2002;46(6):1914-20.[Link]

50. Carson CF, Cookson BD, Farrelly H, Riley TV. Susceptibility of methicillin-resistant Staphylococcus aureus to the essential oil of Melaleuca alternifolia. J Antimicrobiol Chemother. 1995;35(3):421-4. [DOI]