



Effectiveness of Alternate Methods of Toothbrush Disinfection: A Systematic Review

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ABSTRACT

Toothbrushes are used every day in the home and become part and parcel of our lives. Yet we do not know the proper way to maintain, store and disinfect the toothbrushes. Because of this many oral infections can easily spread. To eradicate the unawareness, it is necessary to know a cheap method of toothbrush disinfection which can be used every day in home. The aim of the review is to assess the effectiveness of alternate methods of toothbrush disinfection. The Databases of PubMed, Cochrane, LILACS, Science direct, Metapress and were searched up to December 2018 for the related topic. In vitro studies in which the effectiveness of alternate methods of toothbrush disinfection have been evaluated. The systematic search revealed a total of 832 publications from PubMed, Cochrane, LILACS, Science direct, Metapress and which were scrutinized based on preset inclusion and exclusion criteria. Three publications fulfilled all the inclusion criteria, and 829 publications were excluded from the review. All the three studies used Microbiological analysis for determination of effectiveness of toothbrush disinfection via colony forming units. All the three studies had high risk of bias with level 6 evidence. Three studies reported statistically significant differences in favour of microwave disinfection compared to control groups or sterile water or tap water. There was not a significant difference between microwave and chemical methods of disinfection. With the available evidence, based on quality assessment and evidence level of selected articles, it can be concluded that microwave disinfection is an effective method of disinfection than tap water and its cheap also. But there has to more studies done on disinfection against microorganisms.

Key words: Toothbrush, Disinfection, Contamination, Microwave disinfection, Colony forming units

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INTRODUCTION

Dental plaque is a community of microorganisms found on a tooth surface as a biofilm, embedded in a matrix of polymers of host and bacterial origin [1,2]. The structure of the plaque biofilm might restrict the penetration of antimicrobial agents, but bacteria growing on a surface grow slowly and display a novel phenotype, one consequence of which is a reduced sensitivity to inhibitors [3]. Plaque is natural and contributes (like the resident microflora of all other sites in the body) to the normal development of the physiology and defences of the host [4]. Dental plaque forms via an ordered sequence of events,

resulting in a structurally- and functionally-organized, species-rich microbial community [5].

Microscopic studies have shown that during the first two days of plaque formation, the resident Gram-positive flora proliferates, and an increasing number of Gram-negative cocci and rods appear. The second phase occurs after 2-4 days and is characterized by a proliferation of fusobacteria and filamentous bacteria in addition to the organisms already present. During the third phase (4-9 days), vibrio-like organisms and spirochaetes are added so that a complex flora is formed, consisting ultimately of approximately 50% Gram-negative organisms [6].

The oral cavity is free of microorganisms at birth because the fetus develops in sterile conditions. There is a great variety of microbes in the oral cavity during the first day of life, such

as Streptococcus, Staphylococcus, Neisseria, Candida, Lactobacillus, Veillonella and coliforms [1]. However, mutans streptococci (MS), which is the primary etiological agent of dental caries in humans, is present only after dental eruption, because it establishes on hard surfaces [7]. Toothbrushes are manufactured free of microorganisms. After a single use for periods varying from 30 seconds to 4 minutes, however, toothbrushes may become contaminated by a wide array of bacteria, 2-10 viruses, yeasts, and fungi, which are present both in the oral cavity and in the external environment [8]. Contamination of toothbrush can occur from a variety of sources such as the oral cavity, environment, aerosols, and storage containers. Since toothbrushes are usually kept in the bathroom, they become more prone to contamination as even small droplets from the toilet lead to the release of millions of bacteria in the atmosphere [9]. Since modern dentistry emphasizes prevention and infection control, toothbrushes should be correctly stored, disinfected, and changed at regular intervals. However, the literature presents few articles on the disinfection of toothbrushes [10].

As early as 1920, Cobb has reported the toothbrush to be a cause of repeated infections of the mouth. Svanberg et al. found that toothbrushes can be heavily infected by MS after 24 h. According to Glass, microorganisms not only adhere to and reproduce on used toothbrushes but also can transmit organisms responsible for both local and systemic diseases. He also reported that herpes simplex type I survived for 48 h on toothbrushes that had been artificially air-dried and for 7 days or more on moist toothbrushes. Caudry et al. reported that despite the millions of toothbrushes sold each year in North America, there is little public awareness that their bristles may become contaminated by microorganisms with use. The author also believes that contaminated bristles may play an important role in the transmission and inoculation of the contaminating microorganisms through abrasions of the gingiva, as well as through existing lesions. Glass and Lare said that toothbrushes could be an important means of transmission of pathogenic microorganisms to patients submitted to organ transplantation or with immunological depression, via gingival lesions [11-15].

Under normal conditions of storage, toothbrushes can be a source, or a vector for transmission or re-infection of diseases such as herpes or periodontopathogen microorganisms, and coliforms from the bathroom environment. Toothbrushes can become contaminated from the oral cavity, environmental life, hands, aerosol contamination, and storage containers. Bacteria which attach to, accumulate, and survive on toothbrushes may be transmitted to the individual causing disease [14]. There is a need for standardized nursing guidelines to prevent toothbrush contamination, which may increase the risk of infections from potentially pathogenic microorganisms and is clinically relevant for assessing the risks and benefits of oral care [16]. The American Dental Association ADA recommends changing toothbrushes once every 3 months [17].

The possible methods of toothbrush disinfection are Soaking the toothbrush in alcohol was one of the first recommended procedures for toothbrush disinfection in 1920. Later, in 1929, Kauffmann listed some methods for sanitation and drying of toothbrushes such as sunlight and table salt to absorb their moisture and to keep the brush inside a closed container with a preparation containing formaldehyde gas for its disinfection.

Other methods included the use of ultraviolet light, immersion in a disinfecting solution, spraying of antimicrobial solutions on the bristles, use of a microwave oven 3 and washing of the toothbrush in a dishwasher [11,14,18-24].

In the department of public health dentistry, we have successfully completed numerous epidemiological studies for the betterment of our community [25-42]. So, the aim of the present study is about the various methods of toothbrush disinfection to know which one is more suitable.

MATERIALS AND METHOD

Studies including randomized control trials, *in vitro* trials and clinical trials evaluating effectiveness of alternate methods of toothbrush disinfection. Cross sectional studies, animal studies, literature reviews and systematic reviews were excluded. The Databases of Pubmed, Cochrane, LILACS, Google scholar and

ongoing trials registries were searched up to December 2018. Only articles in English, done in human species and in vitro trials were applied during the electronic search to include all the possible trials that are relevant for the search phase of the systematic review. Reference lists of the identified randomized trials were also checked for possible additional studies.

Electronic search was carried out using the keywords in the Search engines- PubMed, Science Direct, Cochrane, LILACS, google scholar and clinical trials.gov which yielded a total of 835 articles. Based on preset inclusion and exclusion criteria, the titles of the studies identified from the search were assessed independently by two review authors. Seven articles were excluded for duplications. Conflicts concerning inclusion of the studies were resolved by discussion. Ten articles titles were identified from the search after reading the titles and selected for reading abstracts. Abstracts of selected articles were reviewed independently. Six were excluded after reading the abstract. Full text articles were

retrieved for four relevant studies. One study was excluded after reading the complete article. After reviewing the articles independently, finally 3 articles were selected based on eligibility criteria [43–45].

The reference list of the full text articles was reviewed for identifying additional studies. Titles of articles relevant to the review were selected by discussion. Abstracts of the two selected articles were reviewed. Difference of opinion concerning inclusion of a study was resolved by discussion and all two articles eliminated after reviewing abstracts. The flowchart of the procedure of article selection was given in figure 1. Quality Assessment criteria to evaluate the studies were decided by two review authors in accordance with CRIS guidelines [46] which is modified CONSORT guidelines for in vitro studies. The risk of bias for each study was independently assessed by the review authors and conflicts concerning risk of bias were sorted by discussion.

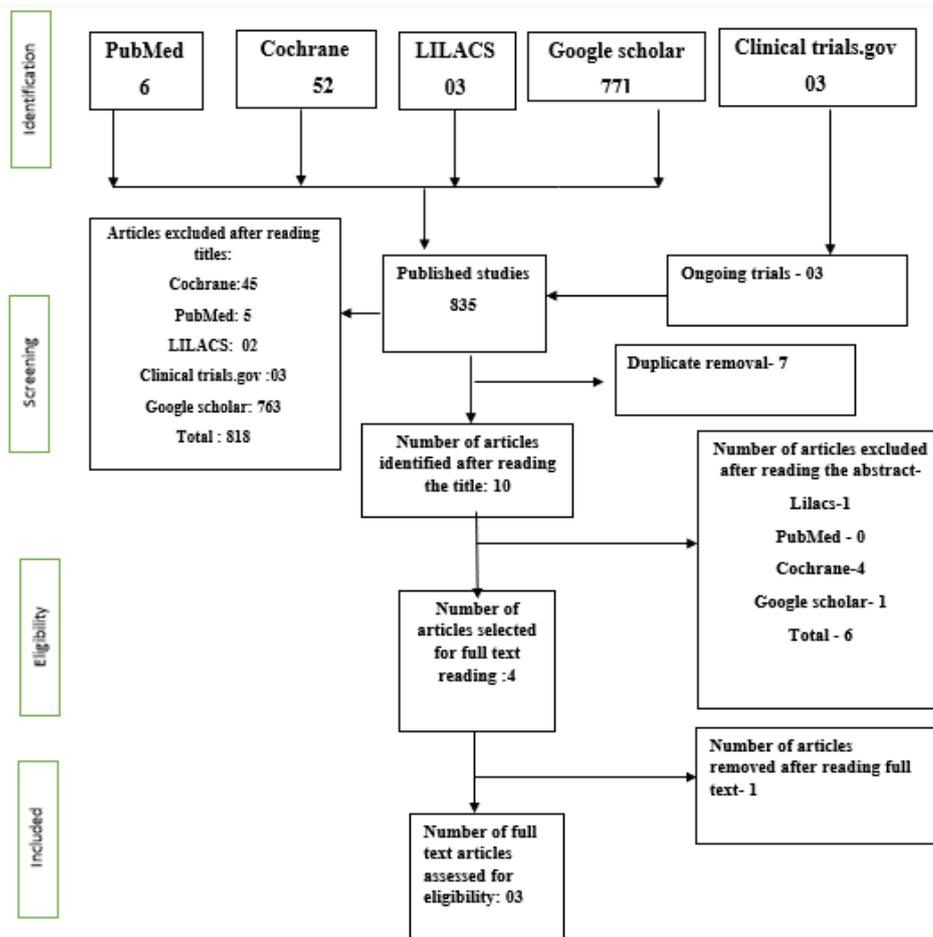


Figure 1: Flowchart of search.

Two authors independently extracted the following data from each of the included studies: Author and Journal citation details, Study Design, Sample Size, Participants and Group, Methodology, Parameters, Statistical Analysis, Results.

RESULTS AND DISCUSSION

A systematic literature search yielded 835 publications from various databases. Seven articles were excluded for duplications. Conflicts concerning inclusion of the studies were resolved by discussion. Ten articles titles were identified from the search after reading the titles and selected for reading abstracts. Abstracts of selected articles were reviewed independently. Six were excluded after reading the abstract. Full text articles were retrieved for four relevant studies. One study was excluded after reading the complete article. After reviewing the articles independently, finally 3 articles were selected based on eligibility criteria. The studies were characterised based on factors such as the study location, type of study design, study setting,

year of publication, age of the study population, measurement tool and the duration of the study. Table 1 describes the general characteristics and data of all the included studies.

In the selected 3 studies, microwave disinfection method was used. In one study, microwave, NaOCl, white vinegar, UV and MCP were used. In the second study, Microwave, chlorhexidine, and sterile water were used. In the third study, Microwave, control without treatment, air dry hours, Crest Pro-Health mouthwash, Listerine mouthwash, dishwasher and UV methods were used. Table 1 explains the general characteristics and the summation of the selected articles. Quality Assessment was done according to CRIS guidelines which is modified CONSORT guidelines for in vitro studies (Table 2). Out of 5 categories in assessing the quality, if all 5 criteria were fulfilled, then the study was given low risk; if 3-4 criteria were fulfilled then it was given medium risk and if less than 3 criteria were fulfilled, then the study was given low risk. In our present review, one study was categorized under low risk and 2 were categorized under medium risk.

Table 1: Data extraction table and summation of the included studies.

S.No	Article	Author and journal	Study design	Sample size	Methodology	Parameter	Outcome	Inference
1	Effectiveness of alternative methods for toothbrush Disinfection: An In Vitro Study	Ilkay Peker et al Scientific World Journal 2014 Article ID 72619043	In vitro	280	The toothbrushes were divided into 7 groups and were by standardised suspensions of Lactobacillus rhamnosus, Streptococcus mutans, Staphylococcus aureus and E.Coli	1% sodium hypochlorite (NaOCl), 100% and 50% white vinegar, microwave (MW) oven, ultraviolet (UV) sanitizer, and mouth rinse-containing propolis (MCP)	There were statistically significant differences between all groups	Each agent was most effective for separate microorganisms
2	Efficacy of Microwaves and Chlorhexidine for Disinfection of Pacifiers and Toothbrushes: An in vitro Study	Jay Chamele et al. The Journal of Contemporary Dental Practice, September-October 2012;13(5):690-69444	In vitro	60 toothbrushes and 60 pacifiers	60 pacifiers and 60 toothbrushes were contaminated with S. mutans	Chlorhexidine, Microwave and Sterile tap water	Chlorhexidine and Microwave were statistically similar to each other and differed from group sterile water	Chlorhexidine and Microwave have similar efficiency
3	Disinfection of toothbrushes contaminated with Streptococcus mutans	Kim Bélanger-Giguère, American Journal of Dentistry, 2011; 24(3)45	In vitro	7	7 tooth brushes (1 for control and 1 for test group) were contaminated S.Mutans.	Control without treatment; air dry for 4 hours; Crest Pro-Health mouthwash for 20 minutes; Listerine mouthwash for 20 minutes; normal cleaning cycle in a dishwasher; microwave on high power for 5 minutes; and ultraviolet light using the DenTek Toothbrush Sanitizer for 10 minutes	The Crest Pro-Health mouthwash and the dishwasher almost completely eliminated S. mutans.	Crest Pro Health Mouthwash and the dishwasher were more effective than microwave, and others
							Microwave	
							The Listerine mouthwash and the air dry	
							UV	

Table 2: Risk of bias of the selected studies.

S.No	Study	Sample size calculation	Meaningful difference between groups	Sample preparation and handling	Allocation sequence, randomization and blinding	Statistical analysis	Risk of bias
1	Ilkay Peker et al. [43]	Yes	Yes	Yes	No	Yes	Medium
2	Jay Chamele et al. [44]	Yes	Yes	Yes	Yes	Yes	Low
3	Kim Bélanger-giguère et al. [45]	No	Yes	Yes	No	Yes	Medium

Pathogenic microorganisms can be present on toothbrushes after brushing and could potentially cause disease. Changing toothbrushes every day is not economical. Common oral hygiene practices such as leaving the toothbrush to dry after brushing or covering it with a cap might not be sufficient or have a detrimental effect. In a study, the use of a cap covering the toothbrush head promoted growth of pathogens which thrive in a humid environment [47]. Moreover, biofilm developed on heads and bristles of conventional and antimicrobial toothbrushes containing triclosan after repeated usage [48]. Several UV toothbrush sanitizing devices have been marketed, however, the efficacy of only a few have been independently tested.

The degree of toothbrush contamination varies depending on how the toothbrush was stored after daily use, and the toothbrush can be highly contaminated by microorganisms according to oral conditions, environment, hand hygiene, aerosol contamination, and storage container. The toothbrush which is generally stored in the bathroom, and bacteria grow well under such a humid and warm condition. Therefore, the importance of storing and disinfecting the toothbrush after use is further emphasized. In this study, the antimicrobial effect on the toothbrush was investigated, and as a result, the antimicrobial effect was found to be greater in the order of CHX, PVI, UV toothbrush sterilizer, and sodium bicarbonate-normal saline. It was confirmed that various methods of toothbrush disinfection are extremely helpful in preventing toothbrush infection or cross-infection, and CHX and PVI disinfectants are the most effective methods for sterilizing harmful microorganisms that remain in the toothbrush [49].

Cobb reported that toothbrushes were the cause of repeated infections in the mouth. It is necessary to recognize the importance of proper toothbrush disinfection to ensure a healthy oral environment, urge the public in recognizing the necessity of toothbrush disinfection in order to

prevent oral infections due to bacteria in the toothbrush, and implement a simple and efficient disinfection method after tooth brushing. Based on this, it is important for dental hygienists to educate patients about toothbrush disinfection and motivate them on proper toothbrush storage, so they can develop a habit of toothbrush disinfection [11].

Interpretation of results

The review included three studies, which assessed the effectiveness of alternate methods of toothbrush disinfection with microwave disinfection compared to others.

In the present review out of three studies, Ilkay Peker et al. study, there were statistically significant differences between all test groups for all microorganisms. MW was the most effective for *L. rhamnosus* and 100% white vinegar was the most effective method for *S. mutans* and *S. aureus*. NaOCl was the most effective for *E. coli*. In Jay Chamele et al study, the results of both types of evaluation showed many *S. mutans* colonies after spraying with sterile tap water, and chlorhexidine spraying and microwaving were effective in eliminating colonies. Groups 1 and 2 were statistically like each other ($p > 0.05$) and differed significantly from group 3 ($p < 0.05$). In Kim Bélanger-Giguère et al. study, The Crest Pro-Health mouthwash and the dishwasher almost eliminated *S. mutans*. The second most effective treatment was the microwave. The Listerine mouthwash group and the air-dry group were not significantly different from each other and ranked third. Although UV light significantly decreased the number of bacteria compared to the control, reduction in the number of *S. mutans* CFU was significantly lower than that of all the other treatments evaluated. The limitation of the study was very less number studies and there were no separate study for specific microorganisms.

CONCLUSION

The studies show microwave disinfection

is significantly better than sterile water disinfection or using tap water. However, there is no significant data to prove it is better than other methods or it eliminates a specific type of bacteria, Additional studies will be needed to determine the effectiveness of these products in eradication of other significant oral pathogens including their effect on mixed cultures and biofilms. As observed with disinfection with antibiotics, biofilms formed on toothbrushes might be more resistant than individual cells to eradication.

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CONFLICT OF INTEREST

Nil.

REFERENCES

- Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. *Periodontol* 2002; 28:12–55.
- Marsh PD. Dental plaque as a microbial biofilm. *Caries Res* 2004; 38:204–211.
- Gilbert P, Maira-Litran T, McBain AJ, et al. The physiology and collective recalcitrance of microbial biofilm communities. *Adv Microb Physiol* 2002; 46:202–256.
- Marsh PD. Role of the oral microflora in health. *Microb Ecol Health Dis* 2000; 12:130–137.
- Marsh PD. Dental plaque as a biofilm and a microbial community – implications for health and disease. *BMC Oral Health* 2006; 6:1-7.
- Löe H. The role of bacteria in periodontal diseases. *Bull World Health Organ* 1981; 59:821–825.
- Talib R, Alnaimi R, Mustafa E. The microbial contamination of toothbrushes and their disinfection by antimicrobial solutions. *Al-Rafidain Dent J* 2008; 8:144–150.
- Nelson-Filho P, Faria G, da Silva RAB, et al. Evaluation of the contamination and disinfection methods of toothbrushes used by 24- to 48-month-old children. *J Dent Child* 2006; 73:152–158.
- Ankola AV, Hebbal M, Eshwar S. How clean is the toothbrush that cleans your tooth? *Int J Dent Hyg* 2009; 7:237–240.
- Balappanavar AY, Nagesh L, Ankola AV, et al. Antimicrobial efficacy of various disinfecting solutions in reducing the contamination of the toothbrush-A comparative study. *Oral Health Prev Dent* 2009; 7:137–145.
- Cobb CM. The Toothbrush as a cause of repeated infections of the mouth. *Boston Med Surg J* 1920; 183:263–264.
- Svanberg M. Contamination of toothpaste and toothbrush by *Streptococcus mutans*. *Eur J Oral Sci* 1978; 86:412–414.
- Glass RT. The infected toothbrush, the infected denture, and transmission of disease: A review. *Compendium* 1992; 13:592-598.
- Caudry SD, Klitorinos A, Chan EC. Contaminated toothbrushes, and their disinfection. *J Can Dent Assoc* 1995; 61:511–516.
- Glass RT, Lare MM. Toothbrush contamination: A potential health risk? *Quintessence Int* 1986; 17:39–42.
- Frazelle MR, Munro CL. Toothbrush contamination: A review of the literature. *Nurs Res Pract* 2012; 2012:420630.
- Sanitizers T. Toothbrush care, cleaning and replacement. *J Am Dent Assoc* 2006; 137:415.
- Sabarish R, Chaparala SR, Yelisetty PP, et al. An In-vitro assessment of the physical and chemical properties of toothbrush bristle following decontamination by three different methods: A pilot study. *Cureus* 2019; 11.
- Neal PR, Rippin JW. The efficacy of a toothbrush disinfectant spray—an in vitro study. *J Dent* 2003; 31:153–157.
- Nelson Filho P, Macari S, Faria G, et al. Microbial contamination of toothbrushes and their decontamination. *Pediatr Dent* 2000; 22:381–384.
- Quirynen M, De Soete M, Pauwels M, et al. Can toothpaste or a toothbrush with antibacterial tufts prevent toothbrush contamination? *J Periodontol* 2003; 74:312–322.
- Quirynen M, de Soete M, Pauwels M, et al. Bacterial survival rate on tooth and interdental brushes in relation to the use of toothpaste. *J Clin Periodontol* 2001; 28:1106–1114.
- Suido H, Offenbacher S, Arnold RR. A clinical study of bacterial contamination of chlorhexidine-coated filaments of an interdental brush. *J Clin Dent* 1998; 9:105–109.
- Zolnowski-Casey M. An infection control procedure that is the patient's responsibility. *J Am Dent Assoc* 1998; 129:616–617.
- Prabakar J, John J, Arumugham IM, et al. Comparing the effectiveness of probiotic, green tea, and chlorhexidine- and fluoride-containing dentifrices on oral microbial flora: A double-blind, randomized clinical trial. *Contemp Clin Dent* 2018; 9:560–569.
- Prabakar J, John J, Arumugham IM, et al. Comparative evaluation of the viscosity and length of resin tags of conventional and hydrophilic pit and fissure sealants on permanent molars: An study. *Contemp Clin Dent* 2018; 9:388–394.
- Prabakar J, John J, Arumugham IM, et al. Comparative evaluation of retention, cariostatic effect and

- discoloration of conventional and hydrophilic sealants-A single blinded randomized split mouth clinical trial. *Contemp Clin Dent* 2018; 9:S233-S239.
28. Shenoy RP, Salam TAA, Varghese S. Prevalence, and clinical parameters of cervical abrasion as a function of population, age, gender, and toothbrushing habits: A systematic review. *World J Dent* 2019; 10:470-480.
29. Manchery N, John J, Nagappan N, et al. Remineralization potential of dentifrice containing nanohydroxyapatite on artificial carious lesions of enamel: A comparative in vitro study. *Dent Res J* 2019; 16:310.
30. Vishnu Prasad S, Kumar M, Ramakrishnan M, et al. Report on oral health status and treatment needs of 5-15 years old children with sensory deficits in Chennai, India. *Spec Care Dentist* 2018; 38:58-59.
31. Khatri SG, Madan KA, Srinivasan SR, et al. Retention of moisture-tolerant fluoride-releasing sealant and amorphous calcium phosphate-containing sealant in 6-9-year-old children: A randomized controlled trial. *J Indian Soc Pedod Prev Dent* 2019; 37:92-98.
32. Prabakar J, John J, Srisakthi D. Prevalence of dental caries and treatment needs among school going children of Chandigarh. *Indian J Dent Res* 2016; 27:547-552.
33. Kumar RP, Pradeep Kumar R, Vijayalakshmi B. Assessment of fluoride concentration in ground water in Madurai District, Tamil Nadu, India. *Res J Pharmacy Technol* 2017; 10:309.
34. Kannan SSD, Kumar VS, Rathinavelu PK, et al. Awareness and attitude towards mass disaster and its management among house surgeons in a dental college and hospital in CHENNAI, INDIA. In: *Disaster Management and Human Health Risk V*. Southampton UK: WIT Press, 121-129.
35. Kumar RP, Preethi R. Assessment of water quality and pollution of porur, chembarambakkam and puzhal lake. *J Adv Pharm Technol Res* 2017; 10:2157.
36. Samuel SR, Acharya S, Rao JC. School interventions-based prevention of early-childhood caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial. *J Public Health Dent* 2020; 80:51-60.
37. Mathew MG, Samuel SR, Soni AJ, et al. Evaluation of adhesion of *Streptococcus mutans*, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: Randomized controlled trial. *Clin Oral Investig* 2020; 18:1-6.
38. Pavithra RP, Preethi Pavithra R, et al. Influence of naturally occurring phytochemicals on oral health. *Res J Pharm Technol* 2019; 12:3979.
39. Neralla M, Jayabalan J, George R, et al. Role of nutrition in rehabilitation of patients following surgery for oral squamous cell carcinoma. *Int J Res Pharma Sci* 2019; 10:3197-3203.
40. Mohapatra S, Pradeep Kumar R, Meignana Arumugham I, et al. Assessment of microhardness of enamel carious like lesions after treatment with nova min, bio min and remin pro containing toothpastes: An in Vitro study. *Indian J Public Health Res Development* 2019; 10:375.
41. Harini G, Leelavathi L. Nicotine replacement therapy for smoking cessation-An overview. *Ind Jour of Publ Health Rese Develop* 2019; 10:3588.
42. Pratha AA, Prabakar J. Comparing the effect of carbonated and energy drinks on salivary pH-In Vivo randomized controlled trial. *Res J Pharm Technol* 2019; 12: 4699-4702.
43. Peker I, Akca G, Sarikir C, et al. Effectiveness of alternative methods for toothbrush disinfection: An In Vitro study. *Scientific World J* 2014; 2014:1-9.
44. Bhat C, Chamele J, Saraf T, et al. Efficacy of microwaves and chlorhexidine for disinfection of pacifiers and toothbrushes: An in vitro Study. *J Contemporary Dent Practice* 2012; 13:690-694.
45. Bélanger-Giguère K, Giguère S, Bélanger M. Disinfection of toothbrushes contaminated with ***Streptococcus mutans***. *Am J Dent* 2011; 24:155-158.
46. Krithikadatta J, Gopikrishna V, Datta M. CRIS Guidelines (Checklist for Reporting In-vitro Studies): A concept note on the need for standardized guidelines for improving quality and transparency in reporting in-vitro studies in experimental dental research. *J Conserv Dent* 2014; 17:301-304.
47. Mehta A, Sequeira PS, Bhat G. Bacterial contamination and decontamination of toothbrushes after use. *State Dent J* 2007; 73:20-22.
48. Sammons RL, Kaur D, Neal P. Bacterial survival, and biofilm formation on conventional and antibacterial toothbrushes. *Biofilms* 2004; 1:123-130.
49. Shin AR, Nam SH. Antimicrobial effects of various methods for the disinfection of contaminated toothbrushes. *Biomed Res* 2018; 29.