Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.11.03.Art030

Microbial Contamination of Toothbrush and Methods to Overcome - A Review

Murukesan S[1*], Saranya varadarajan[2], Raghunathan Jagannathan[3], Swaminathan Rajendran[4], Deepika Rajendiran[5], Thodur Madapusi Balaji[6]

[1]*Research Scholar, Texila University, Chennai, India*

[2]*Reader, Department of Oral Pathology, Sree Venkateshwara Dental College, Thalambur , Chennai, India*

[3]*Raghunathan Jagannathan, Senior Lecturer, Tagore Dental College and Hospital, Chennai, India*

[4] *Swaminathan Rajendran, Senior Lecturer, Sree venkateswara Dental College and Hospital, Chennai.*

[5]*Deepika R, Consultant Endodontist, Craniofacial Clinic (P) Ltd, Chennai, India*

[6]*Thodur Madapusi Balaji, Adjunct Professor, Tagore Dental College and Hospital, Chennai, India*

Abstract

Numerous microbes found in household toothbrushes can lead to contamination of the oral cavity. These microorganisms can survive on the toothbrush for several days or weeks. Several studies have found a link between tooth decay and toothbrush bacterial residues. As a result, toothbrush disinfection is critical for toothbrush and oral hygiene maintenance. Furthermore, brush disinfection is required to prevent disease transmission, especially in children, the elderly, and high-risk patients, such as those with immunodeficiency or undergoing organ transplants or chemotherapy. This paper aims to analyse various methods of decontaminating toothbrushes. Numerous techniques have been discussed in the literature regarding decontamination of toothbrushes between uses. However, no single completely efficacious technique has been found to the said effect. Therefore, the search is still on for a rapidly effective, cost-effective, nontoxic, and easily implementable suitable alternative.

Keywords: *Chemical, Contamination, Decontamination, Physical, Toothbrush.*

Introduction

Numerous microbes found in household toothbrushes can lead to contamination of the oral cavity [1]. These microorganisms can survive on the toothbrush for several days or weeks [2]. Several studies have found a link between tooth decay and toothbrush bacterial residues [3]. The toothbrush is typically rinsed with running water and stored after brushing. As a result, whether or not a toothbrush is rinsed after brushing determines the amount of bacterial contamination [4]. Contaminated toothbrushes have been linked to sepsis, GI disorders, cardiac disease, respiratory problems, and kidney disease [5]. Brushing teeth for 30 seconds to four minutes can cause the toothbrush to become contaminated with bacteria, viruses, and fungi found in the oral cavity [6].

The oral cavity is one of most bacteria-laden regions in the human body.[7] Furthermore, toothbrushes contain 4x108 CFU/mL of bacteria, the vast majority of which are Staphylococci (64%), Escherichia (57%), and Pseudomonas (28%). Brushes could become heavily polluted with pathogens in the air as a

Received: 13.08.2023 ***Accepted: 22.09.2023*** ***Published on: 29.09.2023***

****Corresponding Author: murukesan.subramanian.1970@gmail.com***

result of improper storage or poor hand hygiene. Moreover, toothbrushes are generally placed in the toilet, where they become heavily contaminated by airborne gut flora [8]. As a result, toothbrush disinfection is critical for toothbrush and oral hygiene maintenance [9].

Furthermore, brush disinfection is required to prevent disease transmission, especially in children, the elderly and high-risk patients, such as those with immunodeficiency or undergoing organ transplants or chemotherapy [5].

Reduced toothbrush contamination may also aid in the treatment of a variety of oral diseases [10]. Several toothbrush disinfection studies are currently underway, including those that employ an ultraviolet (UV) toothbrush steriliser, immersion disinfection, antibacterial solution sprays, microwave ovens, and dishwashers. Overall, toothbrush disinfection should be quick, effective, cheap, non-toxic, and straightforward. Chemical reagents are still an effective and simple technique for disinfecting toothbrushes [11]. According to a previous study, brushes showed very little bacteria after brushing teeth after using an antimicrobial mouthwash. After 20 minutes of submerging a toothbrush in Listerine mouthwash, microbes in the mouth were drastically decreased.8 Several paediatric research studies have discovered that disinfecting toothbrushes with other 0.12% chlorhexidine-containing solutions, such as mouthwashes and sprays, is effective [12]. Another experiment reported no statistical difference between chlorhexidine-coated filament toothbrushes and the control group devoid of coating [13].

[7] were able to detect *Pseudomonas*, *S. aureus*, *S. epidermidis*, and yeast colonies on the toothbrushes of healthy individuals. According to Bhat et al. *S. mutans* were found to be the most common species on contaminated toothbrush samples, as cultured on Mitis Salivarius agar plates [14]. Yet another study reported the following species.

1. *Bordetella* spp.,
2. *Salmonella*,
3. *Candida*,
4. *Klebsiella*,
5. *Proteus*,
6. *Pseudomonas* spp.,
7. *Citrobacter*,
8. *S. aureus*,
9. *Providencia*,
10. *Lactobacillus*,
11. *Chromobacterium*,
12. *B. cereus*,
13. Enterococci,
14. Non-hemolytic streptococci [15].

Methods of Decontamination

Chemical

Antibacterial Chemicals

According to a study, 1% sodium hypochlorite greatly reduced the number of microorganisms detected and destroyed almost all streptococci; the same concentration also killed E. coli. Furthermore, the effects on microbial flora of 2% chlorhexidine digluconate and 50% vinegar were comparable. The methodical use of these substances in dentistry for prosthesis decontamination is likely to enhance infection control and minimize the likelihood of cross-contamination [16].

Due to its toxicity, white vinegar is not commonly used for tooth disinfection; however, it is a good option for cleaning agents against a variety of bacterial strains [17]. There have, however, been few studies on the use of white vinegar in dentistry.

At 50% and 100% concentrations, white vinegar is commonly used to disinfect toothbrushes and acrylic paints. On acrylic resins, 100% acetic acid (white vinegar) demonstrated excellent antibacterial activity against Candida albicans and Staphylococcus aureus. It was also as effective against C. albicans, *E. coli*, and *S. mutans* as 1% sodium hypochlorite and 2% chlorhexidine digluconate solutions. Komiyama et al. discovered that 50% white vinegar killed *S. aureus, S. mutans,* and *S. pyogenes* but not *C. albicans*. Immersion of a

toothbrush in 50% and 100% white vinegar for 10 minutes killed all microbes and was the most effective therapy against *S. mutans* and *S. aureus* [18-20].

Mouthwashes

The toothpaste, mouthwash, and oral antiseptics all decrease bacteria on toothbrushes [21]. The toothbrushes and potential disinfection methods in healthy adults [22]. The toothbrushes became heavily contaminated after use, according to their findings. By submerging the toothbrush in Listerine for twenty minutes before and after brushing, the microbial load was reduced. The use of antimicrobial-coated toothbrushes to avoid toothbrush contamination in adults with oral disease [23].

Triclosan showed excellent pathogen reduction on toothbrushes [6]. Three toothbrushes were placed in Streptococcus mutans and Candida albicans suspensions: silver nanoparticle, chlorhexidine-coated, and a control. So far, no antimicrobial toothbrush has delivered the claimed 99.9% reduction in CFU with microorganisms. The incorporation of chlorhexidine in toothbrush bristles has seemed to be the most promising solution for toothbrush self-disinfection thus far. The effectiveness of coated tufts and dentrifice in adult patients with oral diseases was investigated while coated foods could not prevent contamination, using toothpaste [24].

The toothbrushes soaking in chlorhexidine gluconate overnight were highly successful at decreasing toothbrush contamination, and chlorhexidine was far more effective than Listerine in lowering microbial load [25]. The toothbrushes washed with tap water left high levels of contaminants and biofilm [26]. The regular and triclosan-containing dentifrices resulted in lesser toothbrush contamination [27].

Bacterial counts were measured before and after sterilisation with 0.1% Listerine, 0.2% chlorhexidine gluconate, 70% white alcohol, 1% sodium hypochlorite, 10% povidone-iodine, 2% glutaraldehyde, ultraviolet radiation, microwave irradiation, 100% white vinegar, and 3% hydrogen peroxide, with a tap water rinse used as a control. Both before and after sterilisation, the microbial count was drastically decreased. All sterilisation techniques were effective for toothbrush disinfection. The mean bacterial count reduction and percentage reduction in total bacterial count were greatest after sterilisation with 2% glutaraldehyde and 3% hydrogen peroxide solutions, respectively [11].

The toothbrushes immersed in 0.2% chlorhexidine for 20 minutes a day were effective for disinfection [18]. Microbes could be completely removed by immersing in 0.12% chlorhexidine solution for two hours, followed by 0.2% chlorhexidine solution for 20 hours [16, 28]. After sterilisation, the reduction in bacterial count was greater in the 2% glutaraldehyde (90.16% reduction) and 3% hydrogen peroxide (87.03% reduction) groups. Bacterial colonies were reduced by 76.91% and 73.43%, respectively, when a UV steriliser and a 10% PVI solution were used. The toothbrushes disinfected with 10% PVI for 10 minutes had an antibacterial effect like chlorhexidine disinfectants [29].

Phytochemicals

With the rising prevalence of drug resistance in common pathogens and the risks associated with therapeutic drugs, finding alternatives to existing drugs is critical [30]. These alternative medications may be best obtained from herbs with known pharmaceutical properties [31]. In recent years, antimicrobial properties of medicinal plants have been reported from all over the world [32].

Neem has a wide range of medicinal qualities and is widely available in both rural and urban areas of most developing countries. In this study, 3% neem was found to be an effective antimicrobial answer against *Streptococcus mutans* on toothbrush bristles (87% reduction). This matched the findings of [2], [33]. This could be because polyphenolic tannins in the extract bind to surface-associated bacterial

proteins, resulting in bacterial agglomeration and loss of glucosyltransferase activity. This bacterial aggregate effectively reduces *Streptococcus mutans*. Aarati et al. discovered that neem extracts, both aqueous and alcoholic, have significant antibacterial properties against *Streptococcus mutans* [34].

Garlic had the greatest reduction in *Streptococcus mutans* count (96%) in the current study. The presence of allicin in garlic is responsible for its antibacterial activity. There haven't been many studies that have used garlic to decontaminate toothbrushes, according to the literature search. The findings of this study agree with those of [35-37] Allicin has anti-*Streptococcus mutans* activity, according to [38] According to Fani et al., garlic-infused mouthwash might serve to prevent dental caries [39]. Garlic extract, despite its antimicrobial properties, has been associated with unpleasant taste, halitosis, and nausea [40].

Green tea polyphenols have been shown to have significant antitumor, antioxidant, thermogenic, anti-inflammatory, probiotic, and antibacterial properties in numerous human, animal, and in vitro studies [39]. Green tea reduced the *Streptococcus mutans* count by 84% and has been shown to be effective in disinfecting toothbrushes.

The presence of polyphenols in green tea makes it a good antibacterial agent against *Streptococcus mutans*. There are not much of studies using green tea to decontaminate toothbrushes. Several studies have found that green tea inhibits the growth, acid production, metabolism, and glucosyltransferase enzyme activity of *Streptococcus mutans* [33].

Physical Methods

UV Irradiation

The use of chlorhexidine, UV, and saline sterilisation, found that varying solution dilutions and UV light intensities lowered the average bacterial count [41]. UV light can effectively inhibit microorganisms by destroying the chemical bonds containing DNA atoms. Exposing toothbrush samples to UV light for 7 minutes lowered bacterial count by 76.91%. Sustained exposure to UV radiation can destroy microorganisms [42]. The effectiveness of UV sanitization devices against bacteria and viruses has also been studied. VIO light and HIGHDENT reduce Gram-negative and Gram-positive bacteria by 83% and 100%, respectively [43].

Using DenTek UV for 10 minutes, on the other hand, did not work against *S. mutans*. Even though prolonged UV light exposure kills more microbes, the equipment closes after 10 minutes [44]. The UV toothbrush holder was the most expensive of all the toothbrush disinfection kits, used in one study. As a result, the affordability of UV toothbrush holders should be investigated [45]. Glass and Jensen studied ultraviolet light as a method of decontamination and discovered that it was effective in reducing the number of microbes on toothbrushes [46].

Microwave Irradiation

Microwave radiation is an effective way to sterilise acrylic resins. Microwave irradiation for 6-10 minutes had an antibacterial effect on a removable prosthesis contaminated with *S. epidermidis, S. aureus, K. pneumoniae, B. subtilis*, and *Candida albicans* [28].

Another study discovered that sterilising S. mutans-infected toothbrushes in a microwave at high power for 5 minutes was adequate; however, this method failed to eliminate all microorganisms. Furthermore, the toothbrush was destroyed after five minutes of irradiation [47].

Design and Storage Factors

Toothbrushes can become contaminated when they encounter the environment, and toothbrush storage containers have an impact on bacterial survival. Brushes put in closed containers and exposed to contaminated surfaces had higher bacterial counts than those left open to air [48]. Storing toothbrushes with a cap increased bacteria survival [26]. Increasing the

humidity in the environment enhanced bacterial viability on toothbrushes. Furthermore, the bacteria could survive in the presence of moisture for more than 24 hours [49].

Toothbrushes come in many different shapes and sizes. Toothbrush bristles range in hardness from soft to hard and are offered in an array of cluster patterns and plastic shapes, while toothbrush handles are available in a variety of plastic shapes and decorative mouldings. Some of the studies focused on various toothbrush design elements. Bacteria become entangled inside the toothbrush bristles, and bacterial viability is dependent on the bacteria (aerobic versus anaerobic) and toothbrush design [21]. Moreover, the researchers discovered that solid handles held fewer bacteria and that the microbial load increased with surface area. Efstratiou et al. found that bacterial retention was influenced by the type of filament [23].

Frayed and tightly packed toothbrush bristles trap and retain more bacteria [49]. Glass's study, which looked at the level of bacterial retention based on toothbrush brand, colour, and bristle pattern, supported this finding [50]. The toothbrushes with the least contamination were soft and round, clear, and had two bristle rows. After only a short period of exposure, Glass discovered that pathogenic bacteria adhere to plastic. According to [22] bacteria adhere strongly to the bristles. Keeping moisture and oral debris in the bristles improved bacterial survival, according to [25]. Table 1 shows the summary of available evidence regarding the susceptibility of various pathogens to the methods of decontamination discussed so far.

Table 1. Susceptibility of Various Microbes/Pathogens to Methods of Decontamination

Contaminant	**Techniques of decontamination**				
	Chemicals	**Mouthwash**	**Phyto-chemicals**	**UV irradiation**	**Microwave Irradiation**
Bordetella spp.,	No report available	No report available	No report available	No report available	No report available
Salmonella,	No report available	No report available	No report available	No report available	No report available
Candida,	**Yes [18]**	**Yes [6]**	No report available	No report available	**Yes [28]**
Klebsiella	No report available	No report available	No report available	No report available	**Yes [28]**
Proteus	No report available	No report available	No report available	No report available	No report available
Pseudomonas spp	No report available	No report available	No report available	No report available	No report available
Citrobacter	**Yes [18]**	No report available	No report available	No report available	No report available
S. aureus	**Yes [18]**	Yes [6]	No report available	No report available	Yes [28]
S. Mutans	**Yes [18]**	**Yes [6]**	**Yes [34]**	**No [46]**	No report available
Providencia	No report available	No report available	No report available	No report available	No report available
Lactobacillus	No report available	No report available	No report available	No report available	No report available

Chromobacterium	No report available	No report available	No report available	No report available	No report available
B. cereus,	No report available	No report available	No report available	No report available	No report available
Enterococci,	No report available	No report available	No report available	No report available	No report available
Non-hemolytic streptococci.	No report available	No report available	No report available	No report available	No report available

Conclusion

Toothbrushes are an integral part of any oral hygiene maintenance kit and tooth-brushing plays a pivotal role in plaque control. On the contrary, toothbrushes can also serve as a reservoir for microorganisms and, thus, may play a significant role in the causation and transmission of infection. Numerous techniques have been discussed in the literature regarding decontamination of toothbrushes between uses. However, no single completely efficient technique has been found to the said effect. Therefore, the search is still on for a rapidly effective, cost-effective, nontoxic, and easily implementable suitable alternative.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The authors thank Dr. S. Rangachari, retired professor of Orthodontics, at Madras Dental College for his kind encouragement and guidance.

References

[1] Aarati, N., Ranganath, N. N., Soumya, B. G., Kishore, B., & Mithun, K.,2011, Evaluation of antibacterial and anticandidial efficacy of aqueous and alcoholic extract of neem (Azadirachta indica, An in vitro study. *International Journal of Research in Ayurveda and Pharmacy*, 2, 230–235.

[2] Abd-Ulnabi, R. M.,2012, Bacterial contamination of toothbrushes with comparison of healthy and dental patients. *Basrah J. Sci*., 30, 120–130.

[3] Ahmad, I., & Beg, A. Z.,2001, Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology*, 74(2), 113–123.

[4] Aldigs, E. K.,2013, Toothbrushes: A potential health risk. *Egyptian Journal of Experimental Biology (Botany)*, 9, 259–263.

[5] Amin, M., Kazemi, M., & Rasaie, N.,2012, In vitro comparison of the effects of garlic juice and chlorhexidine mouthwash on oral pathogens. *Jundishapur Journal of Microbiology*, 5(2), 398–400.

[6] Amirabadi, F., & Sasannejad, S.,2016, Evaluation of the antimicrobial effects of various methods to disinfect toothbrushes contaminated with Streptococcus mutans. *International Journal of Medical Research and Health Sciences*, 5, 536–540.

[7] Anand, P. J., Athira, S., Chandramohan, S., Ranjith, K., Raj, V. V., & Manjula, V. D.,2016, January–February, Comparison of efficacy of herbal disinfectants with chlorhexidine mouthwash on decontamination of toothbrushes: An experimental trial. *Journal of International Society of Preventive and Community Dentistry*, 6(1), 22–27.

[8] Assari, A. S., Mohammed Mahrous, M., Ahmad, Y. A., Alotaibi, F., Alshammari, M., AlTurki, F., & AlShammari, T.,2022, January 11, Efficacy of different sterilization techniques for toothbrush decontamination: *An ex vivo study*. *Cureus*, 14(1), e21117.

[9] Balappanavar, A. Y., Nagesh, L., Ankola, A. V., Tangade, P. S., Kakodkar, P., & Varun, S.,2009, June 1, Antimicrobial efficacy of various disinfecting solutions in reducing the contamination of the

toothbrush-A comparative study. *Oral Health and Preventive Dentistry*, 7(2), 137–145.

[10] Bélanger-Giguère, K., Giguère, S., & Bélanger, M.,2011, Disinfection of toothbrushes contaminated with Streptococcus mutans. *American Journal of Dentistry*, 24(3), 155–158.

[11] Bhat, P. K., Badiyani, B. K., Sarkar, S., Chengappa, S., & Bhaskar, N. N.,2012, Effectiveness of antimicrobial solutions on streptococcus mutans in used toothbrushes. *World Journal Dent*, 3, 6–10.

[12] Bhat, S. S., Hegde, K. S., & George, R. M.,2003, Microbial contamination of toothbrushes and their decontamination. *Journal of the Indian Society of Pedodontics and Preventive Dentistry*, 21(3), 108–112.

[13] Bunetel, L., Tricot-Doleux, S., Agnani, G., & Bonnaure-Mallet, M.,2000, In vitro evaluation of the retention of three species of pathogenic microorganisms by three different types of toothbrush. *Oral Microbiology and Immunology*, 15(5), 313–316. https://doi.org/10.1034/j.1399-302x.2000.150508.x

[14] Caudry, S. D., Klitorinos, A., & Chan, E. C.,1995, Contaminated toothbrushes and their disinfection. *Journal. Canadian Dental Association*, 61(6), 511–516.

[15] Chen, Y.-Y., Chiu, H.-C., & Wang, Y.-B.,2009, Effect of garlic extract on acid production and growth of streptococcus mutans. *Journal of Food and Drug Analysis*, 17(1), 59–63. https://doi.org/10.38212/2224-6614.2309

[16] Chibebe, J., Jr., & Pallos, D.,2001, Evaluation of sterilization of toothbrushes in a microwave oven (in vitro study, *Rev. Biociênc*, 7, 39–42.

[17] Cugini, M. A., Warren, P. R., & Cugini, M.,2006, May 1, The Oral-B Cross Action manual toothbrush: A 5-year literature review. *Journal*, 72(4), 323.

[18] da Silva, F. C., Kimpara, E. T., Mancini, M. N., Balducci, I., Jorge, A. O., & Koga-Ito, C. Y.,2008, Effectiveness of six different disinfectants on removing five microbial species and effects on the topographic characteristics of acrylic resin. *Journal of Prosthodontics*, 17(8), 627–633.

[19] Dalirsani, Z., Aghazadeh, M., Adibpour, M., Amirchaghm, M., Pakfetrat, A., Mozaffari, P. M., Mehdipour, M., & Zenooz, A. T.,2011, In vitro comparison of the antimicrobial activity of ten herbal extracts against Streptococcus mutans with chlorhexidine. *Journal of Applied Sciences*, 11(5), 878–882.

[20] Dayoub, M. B., Rusilko, D., & Gross, A.,1977, Microbial contamination of toothbrushes. *Journal of Dental Research*, 56(6) (6, article 706), 706.

[21] Efstratiou, M., Papaioannou, W., Nakou, M., Ktenas, E., Vrotsos, I. A., & Panis, V.,2007, Contamination of a toothbrush with antibacterial properties by oral microorganisms. *Journal of Dentistry*, 35(4), 331–337.

[22] Fani, M. M., Kohanteb, J., & Dayaghi, M.,2007, Inhibitory activity of garlic (Allium sativum) extract on multidrug-resistant Streptococcus mutans. *Journal of the Indian Society of Pedodontics and Preventive Dentistry*, 25(4), 164–168.

[23] Glass, R. T.,1992, The infected toothbrush, the infected denture, and transmission of disease: *A review. Compendium*, 13(7), 592–598.

[24] Glass, R. T.,1992, Toothbrush types and retention of microorganisms: How to choose a biologically sound toothbrush. *Journal – Oklahoma Dental Association*, 82(3), 26–28.

[25] Gujjari, S. K., Gujjari, A. K., Patel, P. V., & Shubhashini, P. V.,2011, Comparative evaluation of ultraviolet and microwave sanitization techniques for toothbrush decontamination. *Journal of International Society of Preventive and Community Dentistry*, 1(1), 20–26.

[26] Ilić, D., Nikolić, V., Ćirić, A., Soković, M., Stanojković, T., Kundaković, T. et al.,2012, Cytotoxicity and antimicrobial activity of allicin and its transformation products. *Journal of Medicinal Plants Research*, 6, 59–65.

[27] Komiyama, E. Y., Back-Brito, G. N., Balducci, I., & Koga-Ito, C. Y.,2010, Evaluation of alternative methods for the disinfection of toothbrushes. *Brazilian Oral Research*, 24(1), 28–33.

[28] Lee, M. O.,2004, Bacteriological contamination of toothbrushes by dental plaque acidogenicity and related behaviors to toothbrush use. *J. Korean Soc. Dent Hyg.*, 4, 255–263.

[29] Lee, S. G., Kang, B. R., Kim, H. S., Park, H. H., Park, H. R., Yoon, S. K., & Nam, S. H.,2017,

Changes in the number of bacteria in a toothbrush according to the toothbrush management method. *Biomedical Research*, 28, 7306–7310.

[30] Makino, S. I., Cheun, H. I., Tabuchi, H., & Shirahata, T.,2000, Antibacterial activity of chaff vinegar and its practical application. *Journal of Veterinary Medical Science*, 62(8), 893–895.

[31] Mehta, A., Sequeira, P. S., & Bhat, G.,2007, Bacterial contamination and decontamination of toothbrushes after use. *New York State Dental Journal*, 73(3), 20–22.

[32] Mitrakul, K., Srisatjaluk, R. L., Srisukh, V., & Vongsawan, K.,2015, Efficacy of Citrus hystrix sprays in decontaminating Streptococcus mutans on childrens toothbrushes. *Scienceasia*, 41(1), 28–34.

[33] Nascimento, A. P., Watanabe, E., & Ito, I. Y.,2010, Toothbrush contamination by Candida spp. and efficacy of mouthrinse spray for their disinfection. *Mycopathologia*, *1*69(2), 133–138.

[34] Nelson-Filho, P., Isper, A. R., Assed, S., Faria, G., & Ito, I. Y.,2004, Effect of triclosan dentifrice on toothbrush contamination. *Pediatric Dentistry*, 26(1), 11–16.

[35] Pathak, A. K.,2012, Anti-candida activity of aqueous extracts of some herbals. *Indian J. Fund Appl. Life Sci.*, 2, 1–6.

[36] Peker, I., Akca, G., Sarikir, C., Alkurt, M. T., & Celik, I.,2014, Effectiveness of alternative methods for toothbrush disinfection: An in vitro study. *The Scientific World Journal*, 2014, 726190.

[37] Prabhakar, A. R., Ahuja, V., & Basappa, N.,2009, Effect of curry leaves, garlic, and tea tree oil on streptococcus mutans and lactobacilli in children: A clinical and microbiological study. *Bras Res Pediatr. Dent Int. Clin.*, 9, 259–263.

[38] Quirynen, M., de Soete, M., Pauwels, M., Goossens, K., Teughels, W., van Eldere, J., & van Steenberghe, D.,2001, Bacterial survival rate on tooth- and interdental brushes in relation to the use of toothpaste. *Journal of Clinical Periodontology*, 28(12), 1106–1114.

[39] Quirynen, M., De Soete, M., Pauwels, M., Gizani, S., Van Meerbeek, B., & van Steenberghe, D.,2003, Can toothpaste or a toothbursh with antibacterial tufts prevent toothbrush contamination? *Journal of Periodontology*, 74(3), 312–322.

[40] Salvia, A. C., Matilde, S., Rosa, F. C., Kimpara, E. T., Jorge, A. O., Balducci, I., & Koga-Ito, C. Y.,2013, Disinfection protocols to prevent cross-contamination between dental offices and prosthetic laboratories. *Journal of Infection and Public Health*, 6(5), 377–382.

[41] Sato, S., Pedrazzi, V., Guimarães Lara, E. H., Panzeri, H., Ferreira de Albuquerque, R. F., & Ito, I. Y.,2005, Antimicrobial spray for toothbrush disinfection: An in vivo evaluation. *Quintessence International*, 36(10), 812–816.

[42] Schlein, R. A., Kudlick, E. M., Reindorf, C. A., Gregory, J., & Royal, G. C.,1991, Toothbrushing and transient bacteremia in patients undergoing orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopedics*, 99(5), 466–472.

[43] Sconyers, J. R., Crawford, J. J., & Moriarty, J. D.,1973, Relationship of bacteremia to toothbrushing in patients with periodontitis. *Journal of the American Dental Association*, 87(3), 616–622.

[44] Shin, A. R., & Nam, S. H.,2018, Antimicrobial effects of various methods for the disinfection of contaminated toothbrushes. *Biomedical Research*, 29(13), 2880–2884.

[45] Silva, M. M., Vergani, C. E., Giampaolo, E. T., Neppelenbroek, K. H., Spolidorio, D. M., & Machado, A. L.,2006, Effectiveness of microwave irradiation on the disinfection of complete dentures. *International Journal of Prosthodontics*, 19(3), 288–293.

[46] Sogi, S. H., Subbareddy, V. V., & Kiran, S. N.,2002, Contamination of toothbrush at different time intervals and effectiveness of various disinfecting solutions in reducing the contamination of toothbrush. *Journal of the Indian Society of Pedodontics and Preventive Dentistry*, 20(3), 81–85.

[47] Tomar, P., Hongal, S., Saxena, V., Jain, M., Rana, K., & Ganavadiya, R.,2014, Evaluating sanitization of toothbrushes using ultraviolet rays and 0.2% chlorhexidine solution: A comparative clinical study. *Journal of Basic and Clinical Pharmacy*, 6(1), 12–18.

[48] Turner, L. A., McCombs, G. B., Hynes, W. L., & Tolle, S. L.,2009, A novel approach to controlling bacterial contamination on toothbrushes:

Chlorhexidine coating. *International Journal of Dental Hygiene*, 7(4), 241–245.

[49] Warren, D. P., Goldschmidt, M. C., Thompson, M. B., Adler-Storthz, K., & Keene, H. J.,2001, The effects of toothpastes on the residual microbial contamination of toothbrushes. *Journal of the American Dental Association*, 132(9), 1241–1245.

[50] Wetzel, W. E., Schaumburg, C., Ansari, F., Kroeger, T., & Sziegoleit, A.,2005, Microbial contamination of toothbrushes with different principles of filament anchoring. *Journal of the American Dental Association*, 136(6), 758–65.