Bacterial Contamination of Toothbrushes and their Disinfection by 4% Edta, 10% Sodium Perborate and 3% Neem Juice: A Clinico - Microbiology Study

Nilofer Sultan Sheikh*, Neelima S. Rajhans, Nikesh Moolya, Nilkanth Mhaske, Gabrieala Jude Frenandes and H.M. Sudeep

Department of Periodontics and Implantology, YCMM & RDF's Dental College, Ahmednagar, India

Abstract: *Aim:* The aim of this randomized clinical trial was to evaluate the bacterial survival rate on toothbrushes and efficacy of their decontamination by 4% disodium ethyl diamine tetra acetic acid [EDTA], 10% sodium perborate and 3% neem juice.

Methods: Forty subjects with chronic periodontitis enrolled to this randomized controlled clinical trial were provided with autoclaved toothbrushes which were free from microorganisms. Brushing instructions were given to each participant. Toothbrushes were collected from all study participants after one week and were placed with head down position in autoclaved test tube containing sterile peptone water. Toothbrushes collected were sent for aerobic culture in laboratory for growth of micro-organisms. Incubation was done for 24 hours at 37^0C.The toothbrushes were then divided into four groups and immersed in disinfectants like 4% disodium EDTA, 10% sodium perborate and 3% neem juice. Their efficacy was evaluated by aerobic culture analysis and chi – square test was used for statistical analysis of the data.

Results: The results obtained showed that 4% disodium EDTA and 3% neem juice showed 100% efficacy, whereas 10% Sodium perborate showed 40% effectiveness in decontaminating the toothbrushes. Distilled water as a control showed least effectiveness in cleaning toothbrushes.

Conclusion: The present data appeared to indicate that 4% Disodium EDTA and 3 % Neem juice are equally effective but 3% Neem juice may be considered non-toxic, cost effective and quite feasible for disinfection of toothbrushes.

Keywords: Toothbrush, ethylenediaminetetraacetic acid, neem, sodium perborate.

INTRODUCTION

The most common oral hygiene aid to improve oral health of an individual is the toothbrush. After a single use, within thirty seconds to four minutes it gets contaminated by bacteria, viruses, yeasts and fungi present both in oral cavity and storage area of toothbrushes [1]. These micro-organisms remain viable from 24 hours to 7 days and these contaminated toothbrushes might play a role in systemic and oral diseases. Injuries to oral tissues are aggravated by use of contaminated toothbrushes when compared with sterile ones and may even cause septicaemia after brushing. Transient bacteraemia can be induced by tooth brushing, increasing the potential risk of transmission, which may be exacerbated in people with gingivitis and periodontitis [2, 3]. Different brushing techniques have been described in literature, but there is inadequate information about maintenance of toothbrushes to avoid their contamination with micro-organisms. Hence there is a need for disinfection methods that are rapidly effective, non toxic and can be easily implemented. It is essential to decontaminate toothbrushes in order to eliminate pathogenic micro-organisms transmitted to use toothbrushes from oral cavity or from other toothbrushes and storage area [4]. Soaking toothbrush in alcohol was one of the first recommended procedures for toothbrush disinfection [5]. Kauffmann [6] listed some methods for sanitation and drying of toothbrushes such as sunlight and table salt to absorb their moisture and to keep the brush in a closed container with a preparation containing formaldehyde for its disinfection. Other methods included the use of ultra violet light [7], immersion in a disinfecting solution [8, 9] and spraying of antimicrobial solution on bristles [10-12]. Tetra sodium EDTA has been reported to be effective in killing mature biofilms on toothbrushes, reducing viable count by more than 99%. The ability of tetra sodium EDTA to neutralize both enveloped and nonenveloped viruses is also important in relation to minimizing the cross–infection risks associated with toothbrushes [13, 14]. Sodium perborates are the group of oxidants that possess a high spectrum of activity and are environment friendly [15, 16]. Neem is known by the botanical name Azadirachta indica. Latinized name of neem is derived from the Persian. Azad means “free”, Dirakht means “tree”, i – Hind means “Of Indian origin”. Hence it is named as “The free tree of India”. The neem tree is an incredible plant and has been declared the “Tree of the 21st Century”; by the United Nations. The US National

*Address correspondence to this author at the Department of Periodontics and Implantology, YCMM & RDF's Dental College, Ahmednagar, India; Tel: 07798816942; E-mail: dr.nilu18sheikh@gmail.com

E-ISSN: 2311-8695/14

Academy of science published a report in 1992 entitled "Neem" has been regarded as - a tree for solving global problems [35]. Neem [Azadirachta indica] has many medicinal properties and it is been used in India since ancient times as the preferred medicine for treating teeth and gum diseases. It has therapeutic activities such as antiulcer, antiseptic, insecticidal, astringent and for cleaning teeth in gingivitis and periodontitis [17, 18]. The purpose of this study was to evaluate the bacterial survival rate on toothbrushes and to assess the efficacy of their decontamination by immersing them in different disinfectants such as 4% DP sodium EDTA, 10% sodium perborate and 3% neem juice in regard to bacterial contamination.

MATERIAL AND METHODS

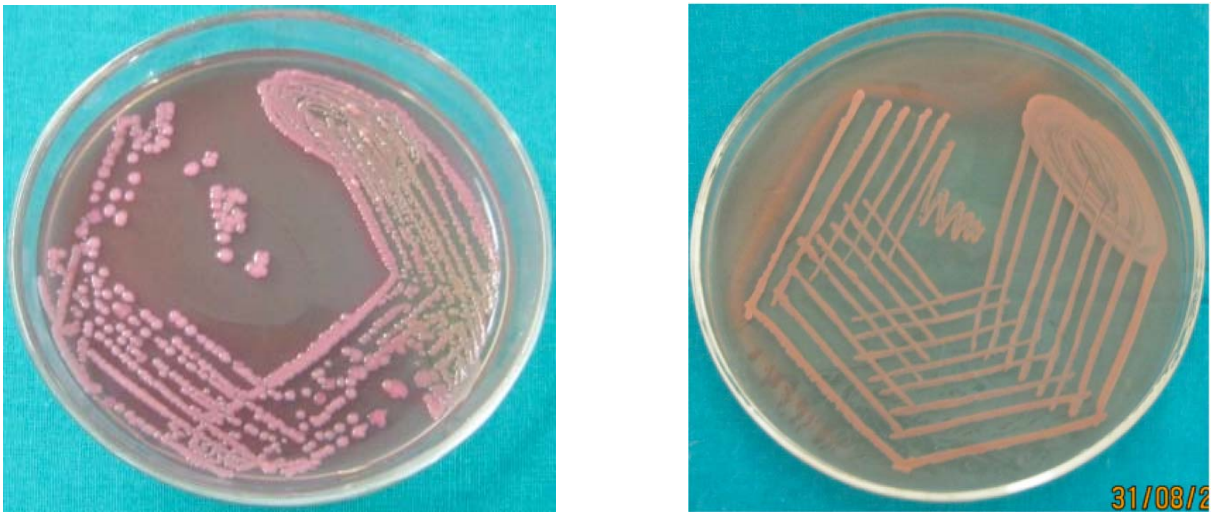
Forty patients (twenty two males and eighteen females) aged more than 35 years suffering from chronic periodontitis having an attachment loss of 3-5 mm were randomly selected from the outpatient Department of Periodontology, Yashwantrao Chavan Memorial and Rural Development Foundation's Dental College, Ahmednagar, Maharashtra, India. Subjects using antibiotics, mouthwashes, chewing gums, tobacco and subjects with oral or systemic disease or undergoing any dental treatment were excluded from the study. Informed consent regarding the benefits and protocol of the study was obtained from all the study participants. A total of forty tooth brushes and toothpastes procured from ICPA Pharmaceuticals, Mumbai, India were autoclaved and given to each participant to ensure that the new toothbrushes were free from contamination before its use by study subjects. At the beginning each participant was given the following oral hygiene instructions like brushing twice daily with the tooth paste by Modified Bass technique for a time period of two to five minutes. All the study participants were instructed to use the toothbrush exclusively and not to share it with anyone. The toothbrushes were placed upright in a rack and were kept isolated [17]. At the end of one week, the toothbrushes were collected from all study participants and stored in the test tubes containing sterile peptone water up to the level of the head of the toothbrush and closed with autoclaved cotton rolls. Each toothbrush was decapitated using sterilized end cutting nippers and the head transferred to a tube containing 10 ml of sterile phosphate buffered saline (P.B.S) [19]. The contents were then subjected to vigorous mixing for 60 seconds, ultrasonication for 30 seconds by using an ultrasonic device followed by further vortex mixing for 15 seconds [1]. Ten fold dilutions in (P.B.S) were then prepared for each toothbrush head and 0.1% of the appropriate dilutions were spread on duplicate of blood agar, nutrient agar and Mac Conkey's agar media with a sterilized spreader. The plates were incubated aerobically at 37 ^{o}C for 48 hours and assessed for bacterial growth [20, 21]. Test tubes containing Sabouraud's dextrose agar media slant were sub cultured by stroking with nichrome loop and incubated at 27 ^{o}C for 48-72 hours to assess fungal growth [4]. The different patterns of colonies of micro-organisms were identified by observing their colony morphology, gram staining and biochemical reactions.

PREPARATION OF DISINFECTANT SOLUTIONS

4% disodium EDTA was obtained by diluting 4 gm of powder of disodium EDTA in 100 ml of sterile distilled water. To obtain 3% neem juice 100gm of soft neem sticks were cut, blended and stored in sterile screw capped bottle and allowed to soak for 2-4 hours at room temperature. Distillation was done with ten parts of water and 60% distillate were collected, cooled & filtered. Later, 300ml of extract were dissolved in 1000ml of deionized distilled water [17]. 10% sodium perborate was prepared by diluting 10 gm of powder of sodium perborate in 100ml of sterile water. Commercially available distilled water served as the control group. The tooth brush heads were divided into four groups [Group I, II, III and IV] and immersed in disinfectants like 4% EDTA, 10% sodium perborate and 3% neem juice for 20 min. Control groups of 10 toothbrushes contaminated with the tested microorganisms were immersed into sterile deionized water instead of the disinfectant solution. After the immersion period, the toothbrushes were transferred to tubes containing sterile distilled water for 2 seconds to eliminate the excess of the disinfectant. Then the solutions were discarded and toothbrushes were kept in the containers, with the head of the toothbrushes facing outwards for air drying [4]. The collected data was analysed statistically and Chi square test was used at the 5% significance level.

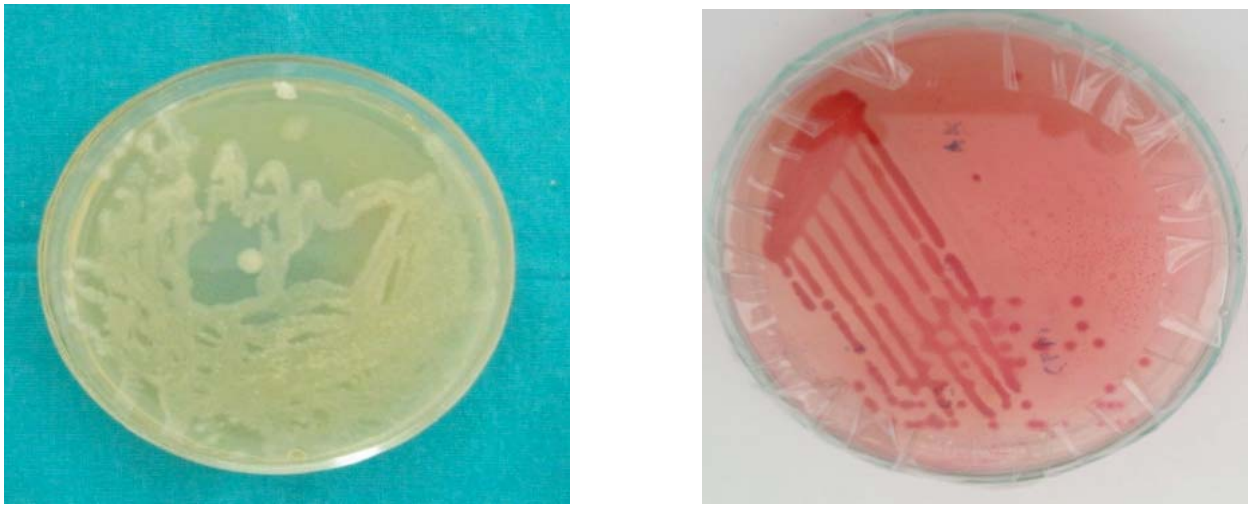
RESULTS

In the present study, the toothbrushes showed contamination with *Escherica coli*, Pseudomonas Aeroginosa, Streptococci, and Klebsiella. Maximum species of micro-organisms that were found in sample were of *E. coli* followed by streptococci, Klebsiella & Pseudomonas Aeroginosa. No fungal growth was found in any of the samples. The types of microorganisms isolated from the toothbrushes and incubated on the various medias are displayed in



a b

Figure 1: a: Growth of *Escherichia coli* on macconkey's agar. **b:** Growth of pseudomonas aeroginosa on macconkey's agar.



a b

Figure 2: a: Growth of streptococci on blood agar. **b:** Growth of klebsiella on blood agar.

Table 1: Colony Forming Units/Toothbrush and the Efficacy of Each Disinfectant

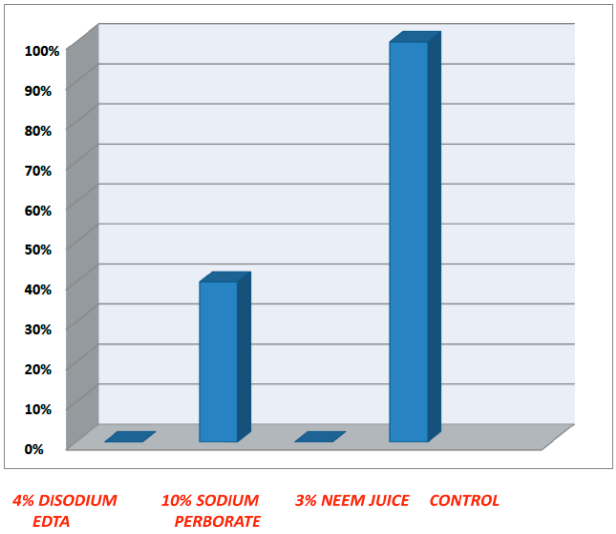
Disinfectant	*E. coli*	*Pseudomonas aeroginosa*	*Streptococci*	*Klebsiella*
4% Disodium EDTA	00	00	00	00
10% Sodium Erborate	58000	1400	00	1700
3% Neem Juice	00	00	00	00
Control	61500	1500	9000	3000

Figures **1a**, **b** and **2a**, **b**. The comparison of decontamination effect [reduction in the number and percentage of micro-organisms] of different disinfectant solutions is displayed in Table **1**. Table **1** showed that there were no colony forming units per toothbrush in Group I and Group III but the colony forming units per toothbrush in Group II and IV were almost comparable. The percentage of bacterial contamination is observed in Table **2** and Graph **1**. The comparison between control group and Group II is displayed in Table **3**. The effect of disinfectants on microorganisms isolated from contaminated Toothbrushes is displayed in Table **4** and

Table 2: Percentage of Bacterial Contamination

Control	3% Neem Juice	4% Disodium EDTA	10% Sodium Perborate
10	00	00	4
100%	00	00	40%

Graph **2**. Statistically significant results were observed between Group I & Group II, Group II & Group III and between Group I, Group II & Group III while no statistically significant results were obtained between Group II & Group IV.



Graph 1: Percentage of bacterial contamination.

Group I [4% disodium EDTA] and Group III [3% Neem juice] showed 100% results by showing no growth of micro-organisms on any of the toothbrushes.

Group II [10% sodium perborate] showed only 40% reduction in the microbial load on toothbrushes.

Group IV [control] showed 0% reduction of the microbial load on toothbrushes.

DISCUSSION

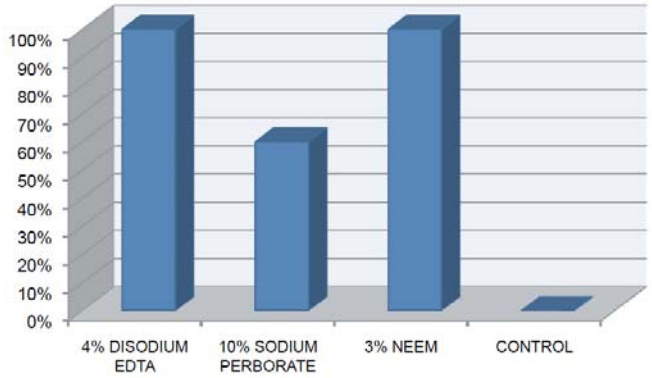
Plaque is the etiologic agent in periodontal disease and the removal of plaque is the most important step towards optimum oral hygiene. Removal of plaque is performed with various oral hygiene devices, of which toothbrush is the commonly used one. After brushing and also during storage, the toothbrush may get contaminated with some microbes. So storage condition of toothbrushes is an important factor for bacterial survival [22]. Dayoub reported that the number of micro-organisms in the toothbrushes kept in aerated conditions was lower than in the toothbrushes stored in plastic bags. Bacterial contamination can be reduced by washing toothbrushes after use and drying in aerated condition [23]. In the present study patients suffering from chronic periodontitis were selected to assess the bacterial contamination of tooth brushes. Cultivation of plaque microorganisms from sites of chronic periodontitis reveals high percentages of aerobic and anaerobic bacteria species as reported in

Table 3: Comparison between Control Group and 10% Sodium Perborate Group

Group IV [Control]	Group II [10% Sodium Perborate]	CHI-Square Test	p-Value	Significance
10	4	0.4	0.50	NS

Table 4: Effect of Disinfectants on Microorganisms Isolated from Contaminated Toothbrushes

Group	Aerobic bacteria	Fungus
Group I(4% Disodium EDTA)	No growth	No growth
Group II(10% Sodium perborate)	*Escherichia coli* *Streptococci* *Klebsiella species*	No growth
Group III(3% Neem juice)	No growth	No growth
Group IV(Control)	*Escherichia coli* *Pseudomonas species* *Streptococci* *Klebsiella species*	No growth



Graph 2: Percentage of effectiveness of each disinfectant.

various studies [24, 25]. The results obtained in this study showed that the micro-organisms isolated were *Escherica coli*, *Pseudomonas aeroginosa*, *Streptococci*, and *Klebsiella*. The species that was present in the highest percentage was of *Eserchia coli* and the least species was of *Pseudomonas aeroginosa*. There was no fungal growth in any of the toothbrushes, which is somewhat similar to the study done by Sogi *et al.* where 30% growth of micro-organisms was seen after first day of usage of toothbrush which increased to 100% by the end of twenty eight days. The isolated microorganisms were *staphylococcus pyogenes*, *klebsiella*, *E. coli*, *Proteus species* and beta – haemolytic *Streptococcus faecalis* [26] whereas another study by Grewal and Kaur reported 40% of growth of microorganisms after first day of usage, which reached to 100% by the end of 1 month that was maintained up to 3 months. The microorganisms isolated were *Klebsiella*, *E. coli* and *Streptococcus faecalis* [27]. Caudry reported that a wet environment increases bacterial growth and cross contamination [8].As the number of days increases, the number of micro-organisms will also increase in the toothbrush bio film. Just like growth media which have properties of nutrients, moisture and storing in cool environment, toothbrush may act as an enriched petridish on a stick which may lead to bacterial growth [28]. Taji identified *Candida*, *Corynebacterium*, *Pseudomonas* and *coli* forms in used toothbrushes [1].

Other studies concluded that these microorganisms may survive for more than 6 hours after utilization of the toothbrush. These authors correlated these results with the possibility of cross-infection, which is of great importance, particularly among children and immunocompromised patients, and reinforced the role of the daily disinfection of toothbrushes [29, 30].

According to Devine *et al*. [13] there is a need for disinfection methods that are rapidly effective, cost-effective, and nontoxic that can be easily implemented. However, most of the proposed methods, such as Chlorhexidine gluconate [9, 11] tetra sodium EDTA and UV sanitization [13, 29] fail mainly in terms of cost-effectiveness and ease of implementation.

The results of the present study regarding the high effectiveness of EDTA are in accordance with previous results, and the total absence of viable microorganisms was observed after immersion for 20 minutes. 4% disodium EDTA has also shown 100% efficacy in decontaminating toothbrushes. It has been suggested that it severely damages permeability barriers in the microbial species. EDTA damage is caused by removal of either Ca++ or Mg++ ions or both from bacterial cell envelop [13]. In the present study 10% sodium perborate failed to reduce any microbial contamination on toothbrushes. Sodium perborate-based tablets are indicated for the cleansing of prostheses and orthodontic appliances associated with mechanical action [11]. Some authors have observed the antimicrobial activity of these products on prostheses [31, 32]. Harrison *et al.* and McCabe *et al*. observed that sodium perborate-based tablets contributed significantly to the treatment of prosthetic stomatitis [32, 33].

3% Neem juice also showed 100% efficacy in decontaminating the toothbrushes. Neem [Azadirachta indica] is very popular for having medicinal properties. It has been proved that 3% Neem extracts can reduce up to 86% streptococcus mutans in toothbrushes [18]. Another study conducted by Padma K Bhatt etal showed 88% reduction of streptococcus mutans in toothbrushes. Polyphenol tannins present in the extract of neem juice could effectively bind to the surface associated bacterial proteins, resulting in bacterial aggregation which effectively reduces the bacterial count [17]. The design of the toothbrush in terms of filament anchoring may have an effect on the retention of microorganisms on the toothbrush [33]. These days there are toothbrush sanitizer or germ terminator and antibacterial storage systems that use an ultra violet bulb or steam combined by a proprietary automatic drying process to kill 99.99 % of the microorganisms present on toothbrushes [7]. In the absence of such products in our markets the method used to minimize contamination is by soaking the toothbrush in an antimicrobial solution like EDTA and Neem, rinsing the bristles thoroughly after each use, and storing in an upright position which will help drain the water and dry the brush faster. Although evaluation of the efficiency of toothbrush disinfectants is recognized by means of

the methodology used in this study, it is necessary for this analysis to be complemented by other tests, such as evaluation of the action of disinfectants against specific anaerobic microorganisms found in periodontal disease. It is also necessary to use a larger and consequently more representative sample of the studied population, with the purpose of seeking more significant and more scientifically reliable results. It is suggested that future studies should be conducted to evaluate the cleaning capacity of different disinfectants used at present, in different concentrations and exposure times and use the best disinfectant to maintain toothbrushes for a long term basis.

CONCLUSION

Based on the results, it can be concluded that 4% disodium EDTA and 3% Neem juice are equally effective but 3% Neem juice may be considered effective, non-toxic, cost-effective & easily available for disinfection of toothbrushes.

CLINICAL SIGNIFICANCE

Even though we have basic knowledge regarding disinfection procedures for our instruments and environment, certain things are practically not implemented such as decontamination of toothbrushes. In medical field, some of the diseases might have been unnoticed which could be transmitted through contaminated toothbrushes. Therefore, there is a necessity to concentrate on disinfection of toothbrushes thereby preventing infections, re-infections or cross infections.

ACKNOWLEDGEMENT

The authors would like to thank ICPA Pharmaceuticals, Mumbai, India for the donation of the toothbrushes used in the study and Department of Microbiology, YCMM and RDF Dental College, Ahmednagar, India for their microbiological analysis used in the study.

REFERENCES

[1] Taji SS, Rogers AH. The microbial contamination of toothbrushes: A pilot study. Aust Dent J Apr 1998; 43(2): 128-30. http://dx.doi.org/10.1111/j.1834-7819.1998.tb06101.x

[2] Glass RT. The infected toothbrush, the infected denture, and transmission of disease: a review. Compendium 1992; 13(7): 592-8.

[3] Nelson-Filho P, Faria G, da Silva RA, 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): 152-158.

[4] Konidala U, Nuvvul S, Mohapatra A, Nirmala S. Efficacy of various disinfectants on microbially contaminated toothbrushes due to brushing. Contem Clin Dent 2011; 2: 302-7. http://dx.doi.org/10.4103/0976-237X.91793

[5] Cobb CM. Toothbrushes as a cause of repeated infections of the mouth. Boston Med Surg J 1920; 183: 263-264. Cited by Sato S, Ito IY, Lara EH, Panzeri H, Al- Buquerque Junior RF, Pedrazz V. Bacterial survival rate an toothbrushes and their decontamination with antimicrobial Solutions. J Appli Oral Sci 2004; 12(2): 99-103.

[6] Kauffman JH. A study of toothbrush II. Dent Cosmos 1929; 71: 132- 140. Cited by Sato S, Ito IY, Lara EH, Panzeri H, Al-Buquerque Junior RF, Pedrazz V. Bacterial survival rate an toothbrushes and their decontamination with antimicrobial solutions. J Appli Oral Sci 2004; 12 (2): 99-103.

[7] Glass RT, Gensen HG. The effectiveness of a u-v toothbrush sanitizing device in reducing the number of bacteria, yeast and viruses on toothbrushes. J Okla Dent Assoc 1994; 84: 24-28.

[8] Caudry SD, Klitorinos A, Chan ECS. Contaminated toothbrushes and their disinfection. J Can Dent Assoc 1995; 61: 511-516.

[9] Nelson F, Macari S, Faria G, Assed S, Ito IY. Microbial contamination of toothbrushes and their decontamination. Pediat Dent J 2000; 22: 381-384.

[10] Meier S, Collier C, Scaletta MG, Stephen J, Kimbrough R, Kettering JD. An *in vitro* investigation of the efficacy of CPC for use in toothbrush decontamination. J Dent Hyg 1996; 70: 161-165.

[11] Neal PR, Rippin JW. The efficacy of a toothbrush disinfectant spray - an *in vitro* study. J Dent 2003; 31: 153-157. http://dx.doi.org/10.1016/S0300-5712(02)00081-7

[12] Sato S, Ito IY, Lara EH, Panzeri H, Al- Buquerque Junior RF, Pedrazz V. Bacterial survival rate on toothbrushes and their decontamination with antimicrobial solutions. J Appli Oral Sci 2004; 12(2): 99-103. http://dx.doi.org/10.1590/S1678-77572004000200003

[13] Devine DA, Percival RS, Wood DJ, Tuthill TJ, Kite P, Killington RA etal. Inhibition of biofilms associated with dentures and toothbrushes by tetrasodium EDTA. J Appl Microbiol 2007; 103(6): 2516-24. http://dx.doi.org/10.1111/j.1365-2672.2007.03491.x

[14] Percival SL, Kite P, Eastwood K, Murga R, Carr J, Arduino M, Donlan RM. Tetrasodium EDTA as a novel central venous catheter lock solution against biofilm. Infect Control Hospital Epidemiol 2005; 26: 515-519. http://dx.doi.org/10.1086/502577

[15] Mc Donnel. G & Russell A. Antiseptics and Disinfectants: Activity, action and resistance. Clinical Microbiology Reviews 1999; 12: 147-179.

[16] Russell AD & Russell NJ. Biocides: Activity, action and resistance. A Symposium of the Society for General Microbiology 1995; 53: 327-365.

[17] Bhat PK, Badiyani BK, Sarkar S, Chengappa S, Bhaskar NN. Effectiveness of antimicrobial solutions on streptococcus mutans in used toothbrushes. World J Dent 2012; 3(1): 1-5.

[18] Balappanavar AY, Nagesh L, Ankola AV, etal. Antimicrobial efficacy of various disinfecting solutions in reducing the contamination of toothbrush: A comparative study. Oral Health Prev Dent 2009; 7(2): 137-45.

[19] Poxton IR, Brown R. PH measurements and buffers, Oxidation-Reduction potentials, Suspension fluids and preparation of glassware. In: Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney Practical Medical Microbiology. 14th ed. Longman Singapore Publishers. New York, USA 1996; p. 838.

[20] Cruck shank R, Duguid TP, Marmion BP, Swain RHA. Medical Microbiology Vol 11. 12th ed. Churchill, Livingstone. England 1975; p. 154.

[21] Al-Talib RA, Alnaimi RJ, Mustafa EA. The Microbial Contamination of Toothbrushes and Their Disinfection by Antimicrobial Solution. Al-Rafidain Dent J 2008; 8(2): 144-150.

[22] Pai V. Effect of a single-use toothbrush on plaque microflora. Indian J Dent Re 2009; 20: 404-6. http://dx.doi.org/10.4103/0970-9290.59431

[23] Dayoub MB, Rusilko D, Gross A. Microbial contamination of toothbrushes. J Dent Res 1977; 56: 706. http://dx.doi.org/10.1177/00220345770560063501

[24] Doyle RJ, Rosenberg M, Drake D: Hydrophobicity of oral bacteria, In: Doyle RJ, Rosenberg M (eds): Microbial Cell Surface Hydrophobicity. Washington, DC, American Society for Microbiology, 1990.

[25] Dzink JL, Tanner AC, Haffajee AD, *et al*. Gram-negative species associated with active destructive periodontal lesions. J Clin Periodontol 1985; 12: 648. http://dx.doi.org/10.1111/j.1600-051X.1985.tb00936.x

[26] Suma Sogi HP, Subba Reddy VV, Sashi Kiran ND. Contamination of toothbrush at different time intervals & effectiveness of various disinfecting solutions in reducing the contamination of toothbrush. J Indian Soc Pedo Prev Dent 2002; 20: 81-5.

[27] Grewal N, Kaur S. A study of toothbrush contamination at different time intervals & comparativeness of various disinfecting solutions in reducing toothbrush contamination. J Indian Soc Pedo Prev Dent 1996; 14: 10-3.

[28] Silva DA, Kothari T, Shah R, Sahetya S. Toothbrush disinfection: Is your toothbrush making you sick? Indian Dent Assoc 2007; 1: 88-91.

[29] Berger JR, Drukartz MJ, Tenenbaum MD. The efficacy of two UV toothbrush sanitization devices. A pilot study. NY State Dent J 2008; 74(1): 50-2.

[30] Muller HP, Barrieshi-Nusair KM, Könönem E, Yang M. Effect of triclosan copolymer-containing toothpaste on the association between plaque and gingival bleeding: a randomized controlled clinical trial. J Clin Periodontol 2006; 33(11): 811-8. http://dx.doi.org/10.1111/j.1600-051X.2006.00993.x

[31] Da Silva FC, Kimpara ET, Mancini MN, Balducci I, Jorge AO, Koga-Ito CY. Effectiveness of six different disinfectants on removing five microbial species and effects on the topographic characteristics of acrylic resin. J Prosthod 2008; 17(8): 627-33. http://dx.doi.org/10.1111/j.1532-849X.2008.00358.x

[32] Harrison Z, Johnson A, Douglas CWI. An *in vitro* study into the effect of a limited range of denture cleaners on surface roughness and removal of Candida albicans from conventional heat-cured acrylic resin denture base material. J Oral Rehabil 2004; 31(5): 460-7. http://dx.doi.org/10.1111/j.1365-2842.2004.01250.x

[33] McCabe JF, Murray ID, Kelly PJ. The efficacy of denture cleansers. Eur J Prosthodont Rest Dent 1995; 3(5): 203-7.

[34] Shurrab MY. Antimicrobial efficiency of some antiseptic products on endodontic microflora isolated from gangrenous pulp issue. J Contemp Dent Pract 2006; 7(4): 53-63.

[35] Venugopalan Santosh, Kumar Visweswaram Navaratnam, Neem [Azadirachta indica]: Prehistory to contemporary medicinal uses to mankind; Asian Pac J trop biomed 2013; 3(7): 505-514.

Received on 30-06-2014 Accepted on 28-07-2014 Published on 22-12-2014

DOI: http://dx.doi.org/10.12974/2311-8695.2014.02.02.3