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Research article

TOOTHBRUSH DISINFECTION –A MYTH OR REALITY? A COMPARATIVE EVALUATION OF 4% DISODIUM EDTA, 10% SODIUM PERBORATE IN THE DISINFECTION OF TOOTHBRUSHES: CLINICOMICROBIOLOGICAL STUDY

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ABSTRACT

Aim: The aim of this randomized clinical trial was to evaluate the bacterial survival rate on toothbrushes and efficacy of their decontamination by4% disodium ethyl diamine acetic acid [EDTA], 10% sodium perborate and compared with control. **Methods:** Thirty subjects with chronic periodontitis enrolled in 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 1 week and were placed with head down position in an 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 three groups and immersed in disinfectantslike4% disodium EDTA, 10% sodium perborate and their efficacy was evaluated by aerobic culture analysis. Chi – Square test was used for statistical analysis of the data. **Results:** Escherichia coli, Pseudomonas Aeroginosa, Streptococci and Klebsiella species were recovered from the samples. The results obtained showed that 4% Disodium EDTA 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:** After single brushing toothbrushes get contaminated by a wide array of bacteria's which a major cause of concern is. As contaminated toothbrush can reintroduce microorganisms into the oral cavity, it is therefore recommended for individuals to use solutions like 4% Disodium EDTA, which proved to be an effective disinfecting agent for decontaminating toothbrushes.

Keywords: Toothbrush, Microorganisms, Ethylenediaminetetraaceticacid, Sodium perborate.

INTRODUCTION

The most common oral hygiene aid used to improve the oral health of an individual is the toothbrush. After a single use, within thirty seconds to four minutes it gets contaminated by a wide array of bacteria, viruses, yeasts and fungi present both in oral cavity and storage area of toothbrushes.[1]These micro-organisms remain viable for periods ranging from 24 hours to 7 days. These contaminated toothbrushes might play a role in systemic and oral diseases. Injuries to oral tissues are aggravated by the use of contaminated toothbrushes when compared with sterile ones and may even cause septicaemia after brushingTransient bacteraemia can be induced by tooth brushing, increasing the potential risk of

transmission, which may be exacerbated in people with gingivitis and periodontiti[2,3].Knowledge of toothbrush contamination is yet void among the population and in the literature as well.Different brushing techniques have been described in the literature, but there is inadequate information about the 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 that can be easily implemented. Modern dentistry strongly emphasizes on prevention and bio security regarding how toothbrushes should be appropriately stored, used and disinfected. It is essential to decontaminate toothbrushes in order to eliminate pathogenic micro-organisms transmitted to used toothbrushes from oral cavity or from other toothbrushes and storage area[4].Soaking the toothbrush in alcohol was one of the first recommended procedures for toothbrush disinfection in 1920[5].Later in 1929 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 ultravioletlight [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 bio films on toothbrushes, reducing the viable count by more than 99%. The ability of Tetra sodium EDTA to neutralize both enveloped and nonenveloped viruses are 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.] Amongst the herbal agents literature has reported Neem [Azadirachtaindica] that has many medicinal properties and it has 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 chapter 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% tetra sodium EDTA, 10% sodium perborate in regard to bacterial contamination.

MATERIAL AND METHODS

Thirty patients (twenty males and ten 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, YashwantraoChavan Memorial and Rural Development Foundation's Dental College, Ahmednagar, Maharashtra, India. Ethical clearance for the study was approved by the Ethics Committee of YCMM & RDF'S University. 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 the protocol of the study was obtained from all the participants. A total of thirty Toothbrushes 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. The duration of the study was 1 week. At the beginning each participant was given the following oral hygiene instructions like brushing twice daily with the toothpaste 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 a sterilized end cutting nippers and the heat 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 (Hook and Tucker instruments LTD/England), ultrasonication for 30 seconds by using an ultrasonic device (England), followed by further vortex mixing for 15seconds[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 at37degree Celsius for 48hours 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 degree Celsius 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 4gm of powder of disodium EDTA in 100ml of sterile distilled water.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 three groups [Group I, II, III] and immersed in disinfectants for 20 minutes. Group I include 4% EDTA, Group II include sodium perborate, and Group III include control. 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 *Escherichia. 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 that were incubated on the various media are shown in Fig 1, 2, 3, 4. The comparison of decontamination effect [reduction in the number and percentage of micro-organisms] of different disinfectant solutions is displayed in Table 1.Table1 showed that there was no colony forming units per toothbrush in Group I, whereas Group II showed increased microbial counts of Escherichia coli followed by Klebsiella, Pseudomonas Aeroginosa, with no or least counts of streptococci. Group III showed increased microbial counts of Escherichia coli followed by Streptococci, Klebsiella and Pseudomonas Aeroginosa. The percentage of bacterial contamination is observed in Table 2 and Graph 1. The comparison between control group and Group II is displayed in Table3.The effect of disinfectants on microorganisms isolated from contaminated Toothbrushes is displayed in Table 4 and Graph 2. Statistically significant results were observed between Group I& Group II, and between Group I, Group III while no statistically significant results were obtained between Group I & Group II

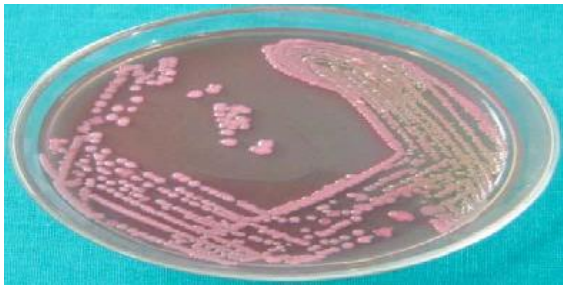

Fig 1: Growth of *E.coli* on MacConkey's agar

Fig 2: Growth of Pseudomonas Aeroginosa on MacConkey's agar.

Fig 3: Growths of Streptococci on Blood Agar.

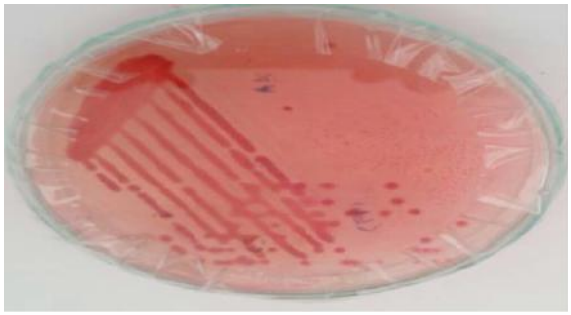

Fig: 4 Growth of Klebsiella on Blood Agar.

Table: 1 Colony forming units / toothbrush & the efficacy of disinfectant.

Disinfectant	4% Disodium EDTA	10%Sodium Perborate	Control
E.coli	00	58000	61500
P. Aeroginosa	00	1400	1500
Streptococci	00	00	9000
Klebsiella	00	1700	1300

*Median values [cfu/toothbrush] of four microbial species counts according to disinfectant used.

¥ Statistically significant reduction of microbial count with group I [$p \leq 0.01$]

Group I [4% disodium EDTA] 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 III [control] showed 0% reduction of the microbial load on toothbrushes.

Table 2– Percentage of bacterial contamination.

4%Disodium EDTA	10% Sodium Perborate	Control
00	4	10
00	40%	100%

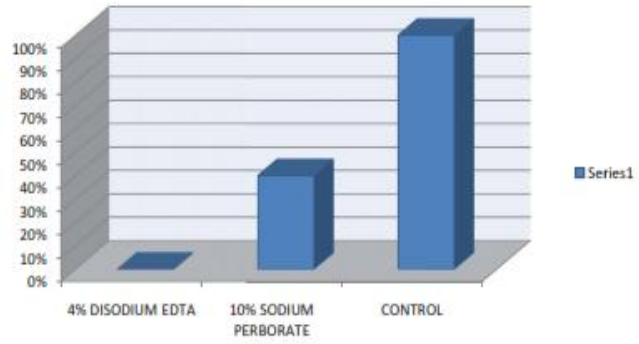

Fig 5: Showing percentage of bacterial contamination

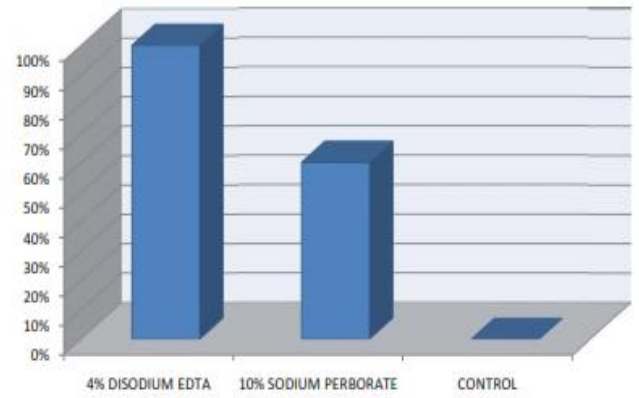

Fig 6 : Showing percentage of effectiveness of each disinfectant.

Table3: Comparison between control group and sodium perborate group.

Group III[Control]	**10**
Group II[10%Sodium Perborate]	**4**
Chi-Square test	**0.4**
P - Value	**0.50**
Significance	NS $^{\alpha}$

$^{\alpha}$Statistically Nonsignificant [$p \geq 0.001$]

Table 4: Effect of disinfectants on microorganisms isolated from contaminated toothbrushes**.**

Group	**Aerobic bacteria**	**Fungus**
Group I (4%DisodiumEDTA)	No growth	No growth
Group II(10% Sodium perborate)	*Escherichia coli* Streptococci Klebsiella species	No growth
Group III(Control)	Escherichia coli Pseudomonas species Streptococci Klebsiella species	No growth

DISCUSSION

Plaque is the etiologic agent in periodontal disease and the removal of plaque is the most important step toward a hygienic oral cavity. 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. They have also mentioned that bacterial contamination can be reduced by washing toothbrushes after use & drying in aerated condition[23].In the present study patients suffering from chronic periodontitis were selected to assess the bacterial contamination of toothbrushes. Cultivation of plaque microorganisms from sites of chronic periodontitisreveals high percentages of aerobic and anaerobic bacteria species as reported in 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 were present in the highest percentage was of Eserchiacoli and the last 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 etal 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 a cool environment, toothbrush may act as an enriched petri dish 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 Chlorhexidinegluconate[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].

Literature has suggested use of 3% Neem juice as an effective disinfectant in decontaminating the toothbrushes. Neem [Azadirachtaindica] is very popular for having medicinal properties. 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. This is may be due to presence of Polyphenol tannins present in the extract which could effectively bind to the surface associated bacterial proteins, resulting in bacterial aggregation thus 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 ultraviolet bulb or steam combined with 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 the 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 proved to be an effective disinfectant agent in reducing the microbial counts and detachment of biofilms from the contaminated toothbrushes. There is a need for disinfection methods that are rapidly effective, nontoxic and easily implemented. These studies thus indicate that Disodium EDTA solution has disinfection applications in the oral care field.

Clinical significance: Even though we have basic knowledge regarding disinfection procedures for our instruments & environment, certain things are practically not implemented such as decontamination of toothbrushes. In the 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.

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Conflict of Interest: Nil

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