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A COMPARATIVE EVALUATION OF ALTERNATIVE DISINFECTANTS TO DECONTAMINATE TOOTH BRUSH

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ABSTRACT: This study aimed to evaluate the effectiveness of alternative methods for toothbrush disinfection. **Methods** - 50 children of age grp 6-12yrs were included in the study. The new toothbrushes were given to children and were asked to brush in the morning and nigh for 1week under the supervision of parents. 2% chlorhexidine, 100% and 50% synthetic vinegar, 25% eucalyptus oil were tested. The head of both morning and night toothbrush were covered with sterile gauze and immediately transferred to microbiology department for microbial decontamination efficacy testing. **Results** :All disinfectant solutions used had equal antimicobial action for tooth brush disinfection. No difference in colonies or antimicrobial efficacy was seen between different disinfectants and morning and night toothbrush. **Conclusion-** These agents can be cost-effective, easily accessible, and comparatively effective for toothbrush disinfection. Because these agents are nontoxic, cost-effective and easily accessible, they may be appropriate for household use.

KEYWORDS: Toothbrush, decontamination, disinfectants

INTRODUCTION

Our understanding of microorganisms and their relationship to oral cavity has undergone a drastic change since the days of Leuwenhoek. From the days of speculation we have moved ontorecognize certain facts about microbes and their unique relationship with oral cavity. The oral cavity is known to be free of microorganisms at birth, later the oral cavity is invaded by a large variety of microbes. There is need to maintain the oral hygiene to keep away from all those harmful effect caused by these microorganisms.[1]

Oral hygiene is maintained by various techniques like Tooth brushing, tongue cleaning, flossing, mouth rinsing with disinfectant mouth washes. Tooth brushing is the most effective and commonly used method among them. 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. These microorganisms remain viable for periods ranging from 24 hours to 7 days. Contaminated tooth brush can re-introduce micro-organism into the oral cavity. These contaminated toothbrushes might play a role in systemic and oral diseases.[2, 3, 4]

In addition, toothbrushes are frequently stored in the bathroom or close to the toilet and sink and may be exposed to enteric bacteria dispersed by aerosols. Even small droplets from the toilet lead to the release of millions of bacteria into the atmosphere. Even contaminated finger contact contributes to toothbrush contamination. Several families generally store their toothbrushes in a common container which can lead to cross- infection.[3]

The ADA suggests soaking used toothbrushes in antimicrobial mouthrinses for patients in high-risk groups. Information about the use and handling of toothbrushes can be found in guidelines of the Centers for Disease Control (CDC). These guidelines suggest that people who are immunosuppressed may need to seek alternative means of oral hygiene, because toothbrushes can remain contaminated with potentially pathogenic organisms even after thorough rinsing with tap water. The CDC has proposed an especially high risk of cross-contamination in group and school settings, perhaps because of a lack of proper handling or storage of toothbrushes. This condition is specifically important for children, the elderly and high-risk patients, including immuno-suppressed individuals or those undergoing organ transplantation or chemotherapy.[2] Knowledge of toothbrush contamination is yet void among the population and in the literature as well because most clinicians still consider toothbrushes only as caries and plaque controlling devices. 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. We stress the fact that along with the brushing techniques, disinfection of toothbrush is also equally important for maintenance of health of oral tissues.

In the literature few methods have been investigated for toothbrush disinfection, but there is need for knowledge about different disinfection methods which acts rapidly, cost effective, non-toxic and which can be easily implemented.[5,6,7]

Aim:

To evaluate efficacy of Synthetic vinegar and Eucalyptus oil as disinfectants for tooth brush. Considering low cost and toxicity and aiming at viable home use application. Chlorhexidine was used as control.

Objective:

1. To evaluate contamination of tooth brush by micro-organisms after 7 days use.
2. To evaluate different disinfectants for decontamination of contaminated tooth brush.

Materials and methods:

A sample of 50 children within the age group of 6-12 years were enrolled in the study from the outpatient department of preventive and pediatric dentistry Kothamangalam. Informed consent regarding the benefits and the protocol of the study was obtained from all the participants. They were provided with new tooth brush and toothpaste for both morning and night brushing**(Fig1).** Tooth brush 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 Fonnes technique for a time period of two to five minutes. All the study participants were asked to brush for under the guidance of their parents. 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. Toothbrushes were collected from all study participants after 1 week.The head of the toothbrush was covered with sterile gauze and immediately transferred to microbiology department for microbial decontamination efficacy testing. Transmission of *Streptococcus mutans* occurs even through dentifrices. To avoid any such factors confounding the result of this study, each subject was given an individual toothbrush and toothpaste **(Fig 2) .**

Method of Collecting the Data:

Inclusion criteria:

- Participantsaged between 6-12yrs.
- Individuals having a DMFT score of less than 3.
- Participants who are not suffering from any known systemic diseases.
- Eligible participants who reside in the hostel accommodation inside the institution campus.
- Selected individuals who fulfill the eligibility criteria and agree to participate and give informed consent.

Exclusion criteria:

- Participants below 6 years and above 12 years.
- Individuals having severe dental caries and DMFT score of more than 3.
- Participants undergoing orthodontic treatment or with extensive intraoral prosthesis.
- Individuals using antibiotics or antiseptic mouthwashes for atleast 3 months prior to the study and during the study.
- Individuals who do not agree to be included in the study and fail to provide the written consent.

Each toothbrush was tested against Normal saline, Chlorhexidine [0.12%] ,50% and 100% Vinegar, 25% eucalyptus oil. Bristles were cut with a sterile B. P blade and weighed and divided into five equal parts**(Fig.3).** Each equally weighed part was transferred into a sterile test tubes and 2 mL of disinfectant added. Each solution was vortexed for 5 minutes **(Fig.4).** 20µl of each solution was then plated on Brain Heart Infusion agar plate with an L-spreader to estimate Colony forming units**(Fig.5).** The agar plates were incubated at 37^0C for 24hrs. Colonies were counted using digital counter**(Fig.6).** Agar plates showing colony forming unit**(Fig.7).**

Results:

The data collected were analyzed using analysis of variance (ANOVA) test. The difference between all the disinfectant solutions was compared. The bacterial count of all morning and night samples is listed in **Table.1** and **Table.2** respectively. Mean bacterial count against disinfectant solutions were calculated for both morning and night brushing and listed in **Table 3** and **Table 4** respectively. The results of Anova test used to compare the difference between all the disinfectant solutions for the morning and night brushing are listed in Table 5 and Table 6 respectively. Bristles placed in saline had maximum colonies indicating used tooth brush was contaminated. The bristles placed in 2% chlorhexidine showed minimal bacterial count. This was followed by 100% vinegar, 25% eucalyptus oil and 50% vinegar. Our study did not show any statistically significant differences between morning and night brushing. **Graph 1** and **Graph 2** showing morning and night results respectively. Even though Chlorhexidine had minimal Colonies, the differences in Colonies among all the disinfectants were not statistically significant.

Inference – All disinfectant solutions used in the study have equal antimicrobial action for tooth brush disinfection. No difference in colonies or antimicrobial efficacy was seen between morning and night

Fig.1. Individual toothbrush.

Fig. 2. Individual tooth paste

Fig.3. Bristles were cut and weighed

Fig.4. Disinfectant added and vortexed

Fig.5. plated on Brain Heart Infusion agar plate with an L-spreader

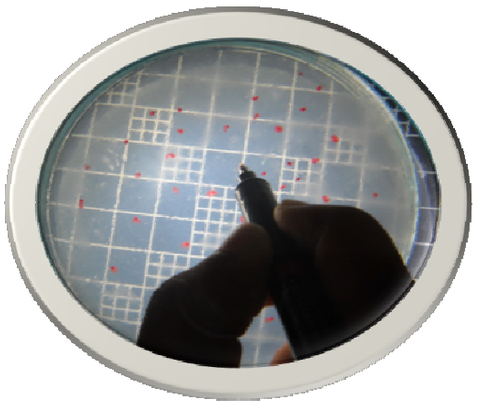


Fig.6.Digital estimate of Colony forming units

Fig.7. Agar plates showing colony forming unit

Table1: Values of microbial colonies of used toothbrush bristles during day time

S.no.	Time	saline	Chlorhexidine	25%Eucalyptus oil	50% vinegar	100% vinegar
1	Day	4	0	44	9	33
2	Day	35	1	23	1120	7
3	Day	130	0	43	32	18
4	Day	1280	0	8	146	6
5	Day	20	0	48	40	10
6	Day	2	2	0	32	0
7	Day	4	0	1	3	1
8	Day	4	1	0	220	0
9	Day	3	0	2	145	75
10	Day	21	0	2	1135	2
11	Day	2200	3	6	48	10
12	Day	2100	1	2000	243	6
13	Day	1	2	0	9	12
14	Day	3	0	2	3	12
15	Day	3	0	0	33	0

Table 2: Values of microbial colonies of used toothbrush bristles during night time

S.no.	Time	saline	Chlorhexidine	25%Eucalyptus oil	50% vinegar	100% vinegar
1	Night	9	3	0	1252	121
2	Night	43	0	15	580	0
3	Night	2100	0	45	500	33
4	Night	7	1	4	10	14
5	Night	20	0	125	122	12
6	Night	2	0	5	270	33
7	Night	10	1	40	642	0
8	Night	1	2	2	564	1
9	Night	5	3	4	24	17
10	Night	1	5	3	80	10
11	Night	2210	0	9	400	286
12	Night	2200	0	0	342	2100
13	Night	1	3	2100	12	0
14	Night	5	2	114	128	0
15	Night	10	0	0	86	5

Table 3: Mean value microbial colonies of used toothbrush bristles during day time with disinfectants used in study

Group	Mean	N	Std. Deviation
Saline	387.333	15	786.3587
Chlorhexidine	0.6667	15	0.9759
100% Eucalyptus	145.267	15	513.4056
100% Vinegar	12.8	15	19.2621
50% Vinegar	214.533	15	378.8392
Total	152.12	75	463.3303

Table4: Mean value of microbial colonies of used toothbrush bristles during night time with disinfectants used in study

Night Time Count			
Group	**Mean**	**N**	**Std. Deviation**
Saline	441.6	15	894.889
Chlorhexidine	1.3333	15	1.58865
100% Eucalyptus	164.4	15	537.003
100% Vinegar	175.467	15	537.69
50% Vinegar	334.133	15	338.614
Total	223.387	75	552.954

P<0.05- significant

Table5: Results of Anova test showing difference between all the disinfectant solutions for the morning brushing-ANOVA

			Sum of Squares	df	Mean Square	F	Sig.
Day time Count group	Between Grops	(Combined)	1524238	4	381059.5	1.857	0.128
	Within Groups		14361710	70	205167.3		
	Total		15885948	74			

P<0.05- significant

Table 6: Results of Anova test showing difference between all the disinfectant solutions for the morning brushing-ANOVA

			Sum of Squares	df	Mean Square	F	Sig.
Night Time Count * group	Between Groups	(Combined)	1724479.79	4	431120	1.444	0.229
	Within Groups		20901602	70	298594		
	Total		22626081.8	74			

P<0.05- significant

Discussion

The literature has shown that tooth brushes can be a reservoir for the direct transmission of microorganisms, as well as a source for inoculation or reintroduction of microorganisms from infected to non-infected tissues.[7] Injuries to oral tissues are aggravated by the use of contaminated toothbrushes when compared with sterile ones and may even cause septicemia after brushing. Transient bacteremia can be induced by tooth brushing, increasing the potential risk of transmission, which may be exacerbated in people with gingivitis and periodontitis.[8,9]

As early as 1920, **Cobb**[10] was the first investigator to report the recurrence of infection in mouth in patient using

contaminated toothbrush. When patient was advised to soak the toothbrush in alcohol before and after using it patient recovered from disease.

Glass and Shapiro[11] observed that changing the toothbrush at short intervals, helped patient achieve elimination of inflammatory disease symptoms, suggestive that toothbrush acted as a reservoir for microorganisms capable of producing diseases.

Dayoub[12] stated that the number of micro-organisms in the toothbrushes kept in aerated conditions was lower than in the toothbrushes stored in plastic bags.

Caudry[13]reported that a wet environment increases bacterial growth and cross contamination. 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.

Sogi etal[14] reported 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.[3]

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.

Several studies conducted in past used different disinfection techniques like UV radiation microwave irradiation, boiling water, chemical agents like hydrogen peroxide, cetylpyridinium chloride, chlorhexidine, etc., had shown reduction in microbial count on toothbrush bristles suggesting need for toothbrush disinfection.[10,11]

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.[12,13,15]

In the absence of such products in our markets alternative methods needs to be studied.In our study we used 50% and 100% synthetic vinegar, 25% eucalyptus oil and chlorhexidine was used as control.

CHX is a cationic bisbiguanide with a broad antibacterial spectrum (Gram-negative and Gram-positive), some virus and antifungal activities and with low mammalian toxicity was first described in 1954.

It is a benchmark control in various studies Chlorhexidine destroys the integrity of cell membrane, penetrates the cell and precipitates the cytoplasmic proteins leading to bacterial cell destruction. It is also biocompatible with oral tissues and is widely acknowledged as an extremely effective antiplaque and antigingivitis agent. Chlorhexidine is not sporicidal so cannot be used as an intermediate-level disinfectant.

Filho et al[15] studied toothbrush disinfection among children using 0.12% chlorhexidine. Reported total destruction of microorganisms.In studies conducted by **Bhat *et al*. and**[16]**NanjundaSwamy*et al*.,**[17]chlorhexidine produced 100% reduction of the *Streptococcus mutans* count.[1] The study conducted by **Suma Sogi *et al***.[18] found that chlorhexidine produces 88% bacterial reduction in toothbrushes after 14 days.

Effectiveness of 0.2% on 0.12% CHX for disinfection was proved to be identical. Hence, 0.2% concentration is used in this study which is most commonly used concentration in mouthwashes.[19,20,21,22]

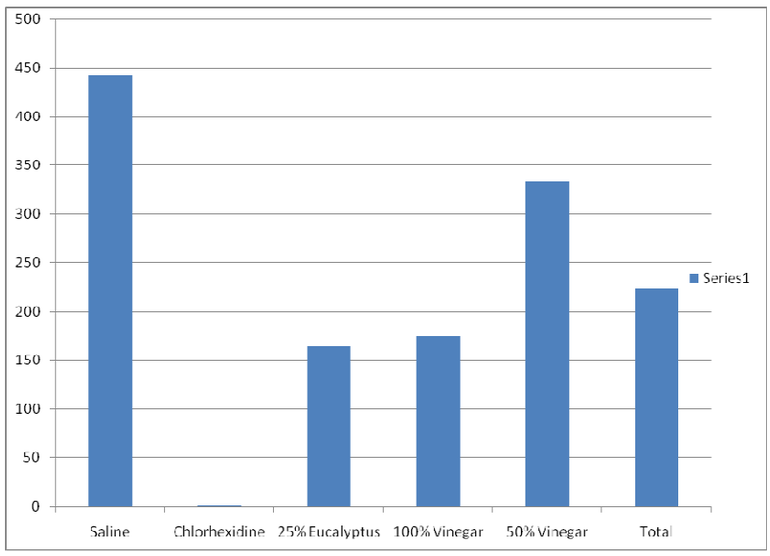
Eucalyptus Oil is an essential oil which is known to have antibacterial and germicidal activity. In vitro studies showed that eucalyptus extracts is very effective against cavities, dental plaque, gingivitis and other dental infections owing to its germicidal properties. This is why eucalyptus essential oil is so commonly found as an active ingredient in mouthwash, toothpaste, and other dental hygiene product.

The study by **Nagata.h et al**[23] showed that the use of eucalyptus extract chewing gum may promote periodontal health. Eucalyptus extract chewing gum had a significant effect on Plaque accumulation (PLA), gingival index (GI), bleeding on probing (BOP), periodontal probing depth (PD), and clinical attachment level (CAL).

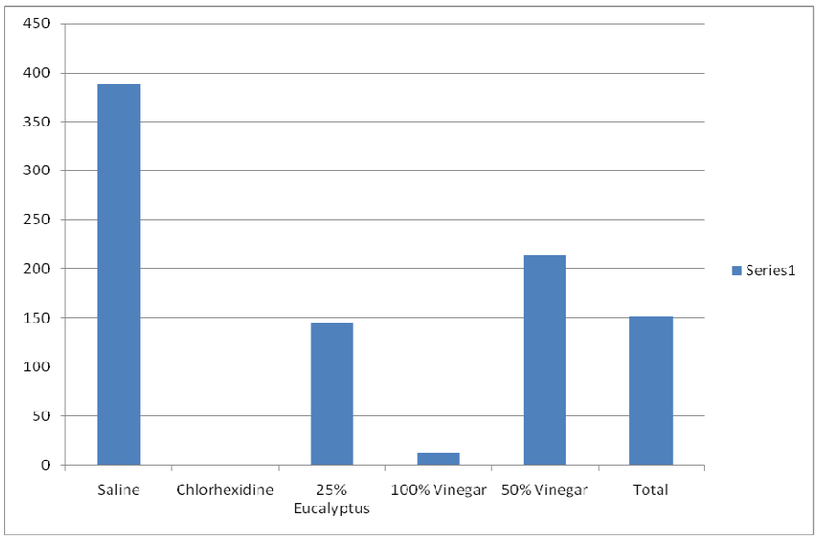
Essential oils containing mouthwash showed comparable results with that of chlorhexidine gluconate. Essential oils cause bacterial cell wall destruction, their enzymatic inhibition and extraction of bacterial polysaccharide. Their antiplanktonic effect is better than their antibiofilm activity, and these mouthwashes could be considered as a good choice for the prevention of systemic bacterial dissemination.

Studies have shown that mouthwashes containing essential oils and alcohol, such as Listerine, have the best antibiofilm activity and could be used to prevent plaque formation after periodontal treatment.[24] In our study 25% eucalyptus oil was used as one of the disinfectants and it showed significant antimicrobial activity comparable to chlorhexidine. Vinegar is nontoxic, cost-effective, easy to access, and appropriate for household use. This agent is however new in dentistry. Only a few studies have been reported about the use of vinegar in dentistry. Vinegar was frequently used in 50% and 100% concentrations to disinfect acrylic resins.

Graph 1: Mean day time count of microbial colonies of used toothbrush bristles with disinfectants used in study



Graph 2: Mean night time count of microbial colonies of used toothbrush bristles with disinfectants used in study



Silva et al.[25] reported that 100% white vinegar as a good antimicrobial against *C. albicans*and *S. aureus* for acrylic resins. In contrast, **Komiyama et al.**[20] stated 50% white vinegar to be effective in toothbrush disinfection for *S. aureus*, *S. mutans*, and *Streptococcus pyogenes*, but not *C. albicans.* In our study 100% Vinegar was as effective as chlorhexidine which was in accordance to study done by **Da Silva et al. and Salvia et al**.[25, 26] in which he showed 100% vinegar is as effective as 1% NaOCl and 2% chlorhexidine digluconate. Similarly **Yildirim- Biceret et al**[24] found it to be the most effective agent against tested organisms. 50% vinegar although significant could not achieve antimicrobial activity as high as 100% vinegar.

Raj VB et al.[27] in his study showed that vinegar was the most effective decontamination agent followed by lime and salt water.

This difference could be related to the kind of white vinegar used and consequently the existing amount of acetic acid in it. Further studies determining all of the effects, including the biocompatibility or toxic effects of synthetic vinegar, may increase clinicians awareness about its antimicrobial capacity.

CONCLUSION

Even though we have basic knowledge regarding disinfection procedures certain things are practically not implemented such as disinfection of toothbrushes. Toothbrush disinfection has not been considered by oral health professionals, and the subject has not received enough attention in the literature. 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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