

Effectiveness of Antimicrobial Solutions on *Streptococcus mutans* in used Toothbrushes

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ABSTRACT

Purpose: The objective was to determine the effectiveness of antimicrobial solutions on *Streptococcus mutans* in used tooth brushes.

Methods: Sixty children used their toothbrushes twice a day, for seven consecutive days. The toothbrush bristles were then immersed into antimicrobial solutions for 12 hours: Group I-3% neem; group II-5% turmeric; group III-0.5% cetylpyridinium chloride, group IV-0.2% chlorhexidine gluconate. They were then placed into test tubes containing the resultant suspensions were three-fold diluted. Aliquots of 0.1 ml were plated in Mitis Salivarius Agar (enriched with sucrose) using dilution and plating method. Incubation was done in an anaerobic jar for 72 hours at 37°C.

Results: The results obtained showed that there was 88% reduction in the *Streptococcus mutans* in the 3% neem group, 86% reduction in the *Streptococcus mutans* in the 0.5% cetylpyridinium chloride group, 78% reduction in the *Streptococcus mutans* in the 5% turmeric group, 65% reduction in the *Streptococcus mutans* in the 0.2% chlorhexidine gluconate group. The difference between them was found to be statistically significant ($p < 0.001$).

Conclusion: Bacterial contamination of toothbrushes was a major cause of concern. All the antimicrobial solutions used in this study proved to be effective toothbrush decontaminants. The efficacy of 3% neem was highest in our study followed by 0.5% cetylpyridinium chloride, 5% turmeric and 0.2% chlorhexidine gluconate.

Keywords: Toothbrush, Contamination, Antimicrobial solutions, *Streptococcus mutans*, Neem, Turmeric, Cetylpyridinium chloride, Chlorhexidine gluconate.

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INTRODUCTION

At birth, the oral cavity is free of microorganisms, as the fetus develops its own sterile conditions. The transmission of *Streptococcus mutans* was even found to start from the fourth month of a predentate child, either directly from the saliva of the mother or other family members or indirectly through fomites, such as spoons, cups, toys or contaminated toothbrushes.^{1,2} It has been reported that *Streptococcus*

mutans count outnumbered many other organisms in development of caries, plaque and biofilm.^{3,4} Efficient removal of dental plaque is thus essential for maintaining oral health.⁵ The most common oral hygiene aid used to improve the oral health of an individual is toothbrush. They are manufactured free of microorganisms. After a single use, within 30 seconds to 4 minutes it gets contaminated by a wide array of bacteria, viruses, yeasts and fungi present both in the oral cavity and also the external environment.⁶ These microorganisms remain viable for periods ranging from 24 hours to 7 days. These contaminated toothbrushes might play a role in systemic and oral diseases.^{7,8} These microorganisms may multiply not only from the oral cavity, but also from the environment where the toothbrushes are stored.^{1,9} Modern dentistry strongly emphasizes prevention and biosecurity regarding how toothbrushes should be appropriately stored, disinfected and changed at regular intervals. Nevertheless, studies have investigated the microbial contamination of toothbrush bristles after use and the most effective disinfection methods.^{1,2,8-16} However, there are no studies regarding the effect of turmeric in reducing toothbrush contamination.

The purposes of this study were to evaluate:

1. The contamination of toothbrushes by *Streptococcus mutans*.
2. To compare the efficacy of four antimicrobial solutions, i.e. 3% neem, 5% turmeric, 0.5% cetylpyridinium chloride and 0.2% chlorhexidine gluconate in the disinfection of these toothbrushes through a clinical trial.

METHODOLOGY

Inclusion Criteria

- Subjects having DMFT score = 0
- Subjects aged 18 to 25 years.

Exclusion Criteria

- Subjects who did not give consent.
- Subjects using antibiotic medications, mouthwashes, chewing gums, tobacco at the time of the study or 15 days prior to it.
- Subjects having any oral or systemic disease.
- Subjects undergoing any dental treatment.

Schedule for the Study

The study duration was predecided as the experimental trial was conducted for 1 week (7 days).

Selection of Study Subjects

A total of 60 dental students aged 18 to 25 years (30 males and 30 females) were selected using simple random sampling from Rajarajeswari Dental College and Hospital. Informed consent was obtained from the participants and ethical clearance from the institution.

Blinding Procedure

It was a double-blind clinical trial. The study subjects remained blind to further procedures after collection of toothbrushes. The microbiologist and statistician remained blind regarding the disinfectant solutions the toothbrush bristles. The toothbrushes were precoded from numbers 1 to 60 prior to providing them to the study participants.

Instructions to the Subjects before the Study Procedure

At the beginning of a week each participant was given a Colgate toothbrush and toothpaste with the following oral hygiene instructions

- Brush twice in a day
- Method: Modified Bass method
- Time required: 2 to 3 minutes
- The toothbrushes should be exclusively used by the participant and not to be shared with anyone
- The toothbrush was to be placed upright in a rack and should be kept isolated.

STUDY PROCEDURE

Sterility Control

Five new toothbrushes which were freshly opened from the packets were subjected to microbial analysis to check for *S. mutans* colonies on bristles. This was done to ensure that the new toothbrushes were free from contamination before its use by study subjects.

Preparation of Test Solutions

Phosphate Buffered Saline (PBS)

Commercially available.

3% Neem

100 gm of neem sticks were cut, blended and stored in sterile screw-capped bottle and allowed to soak for 2 to 4 hours at room temperature. Distillation was done with ten parts of water, and 60% distillate were collected, cooled and filtered. Later, 300 ml of extract were dissolved in 1000 ml of deionized distilled water.

5% Turmeric

100 gm of turmeric rhizomes were cut, blended and stored in sterile screw-capped bottle and allowed to soak for 2 to 4 hours and it was centrifuged at 2000 rpm for 20 minutes. The supernatant was passed through filter paper and 500 ml of extract were dissolved in 1000 ml of deionized distilled water.

0.2% Chlorhexidine

Commercially available.

0.5% Cetylpyridinium Chloride

0.5 gm was added in 100 ml of deionized distilled water.

METHODOLOGY

At the end of 1 week, the toothbrushes were collected in sterile plastic bags. They were sent for further processing to Al-Ameen Microbiology Lab, Bengaluru. Under aseptic conditions, the head of each toothbrush was decapitated and was immersed in presterilized test tubes containing 10 ml PBS having pH 7.4. Totally, 10 tufts of bristles of each toothbrush were cut in aseptic conditions and 2 tufts of those were immersed in each antimicrobial solution for 12 hours. The five solutions were PBS (control), 3% neem, 5% turmeric, 0.5% cetylpyridinium chloride and 0.2% chlorhexidine gluconate (Fig. 1). All 300 test tubes were subjected to vortexing for 15 seconds using cyclomixer.

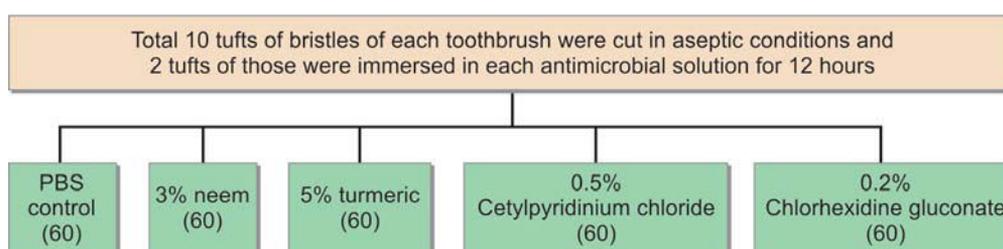


Fig. 1: Antimicrobial solutions

The resultant suspensions were three-fold diluted. Aliquots of 0.1 ml were plated in Mitis Salivarius Agar (enriched with sucrose) using dilution and plating method. Incubation was done in an anaerobic jar for 72 hours at 37°C. Colonies were counted using colony counter and then expressed as number of CFU/mL of saliva. The actual number of colonies were multiplied with 1×10^3 as the samples were diluted 1000 times.

Statistical Analysis

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean \pm SD (min-max) and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance. Student t-test (paired) has been used as the data is dependent set or measured on different materials on same subjects. The statistical software used was SPSS 15.0.

RESULTS

The results obtained showed that there was 88% reduction in the *Streptococcus mutans* in the 3% neem group, 86% reduction in the *Streptococcus mutans* in the 0.5% cetylpyridinium chloride group, 78% reduction in the *Streptococcus mutans* in the 5% turmeric group, 65% reduction in the *Streptococcus mutans* in the 0.2% chlorhexidine gluconate group. The difference between them was found to be statistically significant ($p < 0.001$) (Fig. 2).

The mean \pm SD observed in control group was 31.03 ± 3.16 , the mean \pm SD observed in 3% neem group was 27.30 ± 2.79 ($p < 0.001$), the mean \pm SD observed in 0.5% cetylpyridinium chloride group was 26.68 ± 3.36 ($p < 0.001$), the mean \pm SD observed in 5% turmeric group was 24.20 ± 2.79 ($p < 0.001$), the mean \pm SD observed in 0.2% chlorhexi-

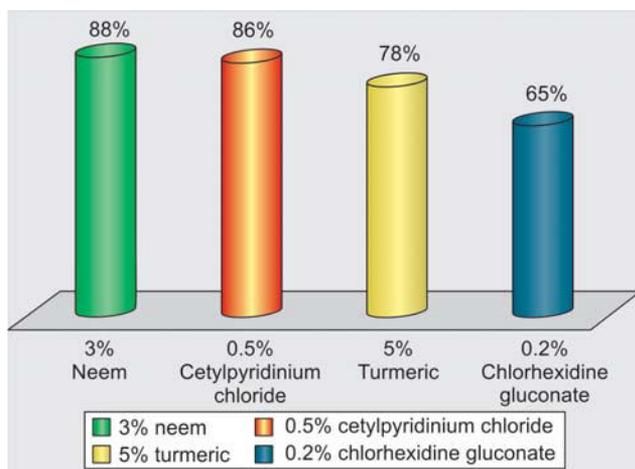


Fig. 2: Reduction in percentage of the *Streptococcus mutans* CFU/mL among the antimicrobial agents

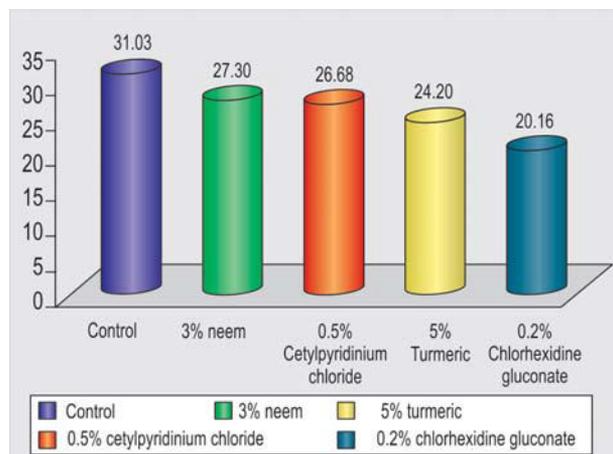


Fig. 3: Comparison of the *Streptococcus mutans* CFU/ml of the antimicrobial agents with control

dine gluconate group was 20.16 ± 3.31 ($p < 0.001$) (Fig. 3) (Table 1).

It was observed that when the results were compared genderwise, the mean obtained in the 3% neem group was 25.10 in male and 27.30 in female ($p = 0.010$) and in the 0.2% chlorhexidine gluconate was 18.77 in male and 20.02 in female ($p = 0.040$), thus, showing a significant difference; however, in the 0.5% cetylpyridinium chloride in males it was 24.43 and 26.68 in females ($p = 0.093$) and in 5% turmeric 24.10 it was for males and 24.40 for females ($p = 0.533$); however, the difference was not statistically significant (Table 2).

DISCUSSION

Storage conditions of toothbrushes are an important factor for bacterial survival. Dayoub et al in 1977¹⁷ reported that the number of microorganisms in the toothbrushes kept in aerated conditions was lower than in toothbrushes stored in plastic bags. Several authors have reported that bacterial contamination can be reduced by washing toothbrushes after use, and drying in aerated conditions.² Caudry et al in 1995¹⁸ reported that a wet environment increases bacterial growth and cross contamination. Therefore, as time increases between one toothbrushing and another, more micro-organism development can occur in the toothbrushes stored in a wet/moisture environment.²

The toothbrushes from the participants were collected after 7 days similar to other studies.^{2,19} Biofilm on the old toothbrush bristles were also observed despite the time of use and storage conditions. Time necessary for colonization is contradictory varying from 1 to 30 days.²

Neem (*Azadirachta indica*) is very popular for having medicinal property. Balappanavar et al in 2009,¹ in their study had shown that 3% neem extracts can reduce upto 86% *Streptococcus mutans* in toothbrushes which is

Table 1: Comparison of the *Streptococcus mutans* CFU/ml of the antimicrobial agents with control

Group	Min-max (range of colony count in CFU/ml)	Mean \pm SD	p-value from control
Control	25.0-36.0	31.03 \pm 3.16	—
3% neem	5.00-19.00	27.30 \pm 2.79	<0.001
0.5% cetylpyridinium chloride	4.00-19.00	26.68 \pm 3.36	<0.001
5% turmeric	14.00-27.00	24.20 \pm 2.79	<0.001
0.2% chlorhexidine gluconate	21.00-35.00	20.16 \pm 3.31	<0.001

Table 2: Genderwise comparison of effect of different antimicrobial solutions on *S. mutans* compared with control

Comparison	Difference		p-value
	Male	Female	
Control vs 3% neem	25.10	27.30	0.010
Control vs 0.5% cetylpyridinium chloride	24.43	26.68	0.093
Control vs 5% turmeric	24.10	24.40	0.533
Control vs 0.2% chlorhexidine gluconate	18.77	20.02	0.040

agreeable with our results of 88% reduction of *Streptococcus mutans*. This may be due to the presence of polyphenolic tannins present in the extract which could effectively bind to the surface associated bacterial proteins, resulting in bacterial aggregation thus effectively reduces the *Streptococcus mutans* count.²⁰⁻²⁴

Cetylpyridinium chloride (CPC) is a cationic surface active agent. Its detrimental effect is due to its ability of disruption of organisms, membrane functions, leakage of cytoplasmic materials and finally collapse of the intracellular equilibrium.^{11,25,26} It has shown excellent reduction of 86% in *Streptococcus mutans* in our study; these results are in concurrence with studies of, Sandra et al in Canada 2004¹¹ who had used CPC sprays and also Caudry et al in USA in 1995,¹⁹ who used Cepacol[®] an antiseptic containing CPC against microorganisms in toothbrush bristles.

Turmeric/Xanthorizol (XTZ) has proved to be a useful antimicrobial to reduce the *S. mutans* count of 78% in our study thus coinciding with results of J Hwang in 2000²⁷ and also Y Rukayadi and J Hwang in Japan in 2006²⁸ and Kim JE et al in 2008²⁹ who demonstrated reduction in *S. mutans* growth in oral bacterial biofilms.

Chlorhexidine gluconate is also a cationic agent that exhibits broad spectrum antimicrobial effect and is a benchmark in various studies, in ours we found 65% comparable to Balappanavar et al in Belgaum in 2009.¹ Previous studies reported that 0.2% chlorhexidine gluconate has shown to inhibit *Streptococcus mutans* counts very effectively in contaminated toothbrushes.^{1,10,12-14,19,28,29}

COMPARISON OF MEAN REDUCTION OF STREPTOCOCCUS MUTANS CFU BETWEEN DIFFERENT TOOTHBRUSH DISINFECTANTS

The American Dental Association recommends a routine change of toothbrushes every 3 months.² Glass³⁰ specifically

recommended that healthy patients replace their toothbrush every 2 weeks. Patients who are sick should change their toothbrushes at the beginning of an illness, when they first feel better, and when they are completely well. Chemotherapy or immune-suppressed patients should change their toothbrushes every 3 days, and persons submitted to major surgery should change their toothbrushes every day. Many patients, however, reported psychological, economic, and environmental barriers to changing their toothbrushes so frequently. Establishing an easy and effective method for disinfecting a toothbrush would be an important and economical way to prevent the continuation of reinfection of oral diseases.²

CONCLUSION

- Bacterial contamination of toothbrushes is a major cause of concern
- The present study revealed the antimicrobial activity of two tested chemicals and herbal products on *Streptococcus mutans*
- The efficacy of 3% neem was highest in our study followed by 0.5% cetylpyridinium chloride, 5% turmeric and 0.2% chlorhexidine gluconate
- A detailed study is required to know the effect of these chemicals on other transmissible microorganisms in contaminated toothbrushes.

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