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# ASSESSMENT OF ANTIBACTERIAL EFFICACY OF DIFFERENT TOOTH PASTES ON DENTAL CARIES BACTERIA

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**ABSTRACT**
The research aimed to evaluate the efficacy of different tooth pastes on the growth of dental caries bacteria. A total of 20 dental plaques samples were collected for the isolation of dental caries bacteria around Marthandam area, Kanyakumari District, Tamil Nadu. The bacteria were isolated by using Basal salt medium and identified by studying cultural, morphological and biochemical tests. The isolated bacterial strains were *Streptococcus*, *Staphylococcus, Micrococcus* and *Lactobacillus* species. Five brands of toothpastes coded as T1-T5 were used for the assessment of antibacterial activity against the dental carries bacteria by agar well diffusion method. Among these, two brands such as T3 & T4 which contained Triclosan and Fluoride as ingredients possessed good inhibition activity against dental caries bacteria and T3 was found as more effective followed by T5, T2, T1 and T4.
**Keywords:** Dental caries, Bacterial dominance, Tooth paste, Triclosan and Antibacterial activity.


## INTRODUCTION

Dental problems are the most common cases in the common people associated mainly with dental hygiene practices and Dental caries have plagued human since the dawn of civilization and still constitutes one of the most common human infectious disease in different parts of the world[1]. It is an adherent deposit of bacteria and their products, which forms as a white greenish or even yellow film on all tooth surfaces[2] and causes destruction of enamel, dentin or cementum of teeth due to bacterial activities[3]. It was caused by certain acid producing bacteria[4], *Streptococcus mutans* and *Streptococcus sobrinus* are the major members of oral flora, frequently cause bacteremia and infective endocarditis[5]. The bacterial strains includes *Bacillus*, *Lactobacillus*, *Staphylococcus*, *Micrococcus*, *Streptococcus*, *Proteus* sp and *Pseudomonas* species were highly prevalent in dental caries[6].

Further, the efficacies of the toothpastes concerning their ingredients is not less important especially in developing countries where low grade products can be found in local markets and peoples are forced unknowingly to choose the products[7]. Toothpaste is a dentifrice which improves the aesthetic appearance and the health of the teeth. Dentifrices need to contain various antimicrobial agents in order to reduce, control and prevent different kinds of dental diseases[8]. It is important to determine the efficiency of antibacterial effect of different toothpastes brands which have different ingredient to reduce bacterial load in human mouth and contribute to dental health[9]. The present study was aimed to isolate dental caries bacteria and to access the efficacy of antibacterial activity of selected brands of toothpastes.

## EXPERIMENTAL

### Collection of clinical specimens and test samples

Dental caries bacteria were isolated from dental plaques of adult human patients around Marthandam area, Kanyakumari District, Tamil Nadu. A total of 20 plaque samples were picked up through forceps

and transferred into 2 ml of morphological saline. The collected specimens were immediately taken to the laboratory for bacterial isolation.
Tooth paste of five different brands (T1, T2,T3, T4 & T5) commonly used in Tamil Nadu, India were purchased from local super market, Marthandam, Tamil Nadu and were taken to the laboratory for analysis.

**Growth media for bacteria isolation**
Basal salt medium used for the isolation of dental caries bacteria from dental plaque samples contained (g/L): 1.0 g yeast extract, 0.3 g $(NH_4)2SO_4$, 0.14 g $MgSO_4 \cdot 7H_2O$, 0.2 g $CaCl_2 \cdot 2H_2O$, 0.1 g NaCl, 0.05 g $KH_2PO_4$, 0.05 g $K_2HPO_4$, 0.6 mg $H_3BO_3$, 0.17 mg $CoCl_2 \cdot 6H_2O$, 0.09 mg $CuCl_2 \cdot 2H_2O$, 0.1 mg $MnCl_2 \cdot 4H_2O$, 0.22 mg $ZnCl_2$, 10 g glucose[10]. The plaque samples in tubes were inoculated separately into 25 ml of Basal salt medium. The inoculated flasks were incubated at $35^0$C for 48 hrs. After incubation, freshly grown culture of 1 ml from each dental plaque was serially diluted up to $10^{-5}$ with distilled water. 100µl of diluted samples were spread over the Basal salt medium agar plates. The plates were incubated at 37°C for 72 hrs. After incubation, the morphologically different bacterial colonies were picked up and streaked into fresh basal salt agar plates.

**Identification of bacterial colonies**
The dental caries bacterial colonies isolated from dental plaques were identified by studying cultural, morphological characteristics and biochemical tests. Growth characteristics were studied on different media such as MacConkey agar, Mannital salt agar, Cetrimide agar and Eosine methylene blue agar. Morphology was studied with Gram's staining, spore staining and capsule staining. Then the biochemical characteristics includes catalase, oxidase and coagulase test were performed according to Bergey's Manual of Systematic Bacteriology[11].

**Antibacterial activity of toothpastes**
An assessment of tooth pastes for antibacterial activity was tested by agar well diffusion method. A stock solution was prepared by mixing 1 g of toothpaste in 10 ml of distilled water. The bacterial strains were grown in Nutrient broth medium at 37°C for 24 hrs and then 0.1 ml was spread on Nutrient agar plates. Wells of 6 mm in diameter were punched off into medium with a sterile cork borer and filled with 50 µl of toothpaste stock solutions in aseptic condition. All the plates were kept in a refrigerator to allow pre-diffusion of extract for 30 minutes. Further, the plates were incubated at 37°C for 24 hrs and antibacterial activities were evaluated by measuring the diameters of zones of inhibition.

## RESULTS AND DISCUSSION

The bacterial pathogens belongs to four genus such as *Streptococcus sp*, *Staphylococcus sp, Micrococcus sp* and *Lactobacillus sp* were isolated form dental caries samples. The bacteria were identified by studying cultural, morphological and biochemical characteristics (Table-1).

Table-1: Cultural, morphological and biochemical characteristics of bacteria

| S.No. | Characteristics | *Streptococcus sp* | *Staphylococcus sp* | *Micrococcus sp* | *Lactobacillus sp* |
|---|---|---|---|---|---|
| 1 | Gram staining | +ve | +ve | +ve | +ve |
| 2 | Morphology | Cocci | Cocci | Cocci | Rod |
| 3 | Motility | - ve | - ve | - ve | - ve |
| 4 | Capsule staining | + ve | - ve | - ve | - ve |
| 5 | Spore staining | - ve | - ve | - ve | - ve |
| 6 | Coagulase test | - ve | + ve | - ve | - |
| 7 | Growth on MacConkey agar | NLF | LF | LF | NLF |
| 8 | Growth on | - | Golden yellow | - | - |

| | | | | | |
|---|---|---|---|---|---|
| | Mannital salt agar | | pigment | | |
| 9 | Growth on Cetrimide agar | - | - | - | - |
| 10 | Growth on EMB agar | - | - | - | - |
| 11 | Growth on blood agar | α-hemolysis | ß-hemolysis | ß-hemolysis | - |
| 12 | Catalase test | - ve | + ve | + ve | - ve |
| 13 | Oxidase test | - ve | - ve | + ve | - ve |

'LF'= lactose fermentation; 'NLF'= Non lactose fermentation

Antibacterial activity of toothpastes were tested against three strains of *Streptococcus* sp, four strains of *Staphylococcus* sp, four strains of *Micrococcus* sp and three strains of *Lactobacillus* sp. After incubation, the zones of inhibition were measured with the help of numerical scale and the values in 'mm' were tabulated (Table-2).

Table-2: Zone of inhibition (mm) of toothpastes against dental caries bacteria

| S. No. | Strain No. | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|---|
| 1 | *Streptococcus sp1* | R | R | 10 | R | R |
| 2 | *Streptococcus sp2* | 12 | 27 | 30 | R | 27 |
| 3 | *Streptococcus sp3* | 10 | 24 | 28 | R | 24 |
| 4 | *Staphylococcus sp*1 | 10 | 20 | 22 | 10 | 24 |
| 5 | *Staphylococcus sp*2 | R | R | R | R | R |
| 6 | *Staphylococcus sp*3 | R | R | 20 | R | 11 |
| 7 | *Staphylococcus sp*4 | R | 18 | 22 | R | 17 |
| 8 | *Micrococcus sp*1 | R | R | R | R | R |
| 9 | *Micrococcus sp*2 | R | 26 | 24 | 12 | 20 |
| 10 | *Micrococcus sp*3 | 10 | 16 | 21 | R | 18 |
| 11 | *Micrococcus sp*4 | R | 12 | 14 | R | 12 |
| 12 | *Lactobacillus sp*1 | 16 | 12 | 16 | 10 | 14 |
| 13 | *Lactobacillus sp*2 | 10 | 14 | 17 | 10 | 13 |
| 14 | *Lactobacillus sp*3 | 10 | R | 16 | R | 15 |

'R'= Resistant

In this present study, T1 showed inhibition activity against *Streptococcus sp2* (12 mm), *Streptococcus sp3* (10 mm), *Staphylococcus sp*1 (10 mm), *Micrococcus sp3* (10 mm), *Lactobacillus sp*1 (16 mm), *Lactobacillus sp*2 (10 mm) and *Lactobacillus sp*3 (10 mm). T2 showed inhibition activity against *Streptococcus sp2* (27 mm), *Streptococcus sp3* (24 mm), *Staphylococcus sp*1 (20 mm), *Staphylococcus sp*4 (18 mm), *Micrococcus sp2* (26 mm), *Micrococcus sp3* (16 mm), *Micrococcus sp4* (12 mm), *Lactobacillus sp*1 (12 mm) and *Lactobacillus sp*2 (14 mm). T3 showed inhibition activity against *Streptococcus sp1* (10 mm), *Streptococcus sp2* (30 mm), *Streptococcus sp3* (28 mm), *Staphylococcus sp*1 (22 mm), *Staphylococcus sp*3 (20 mm), *Staphylococcus sp*4 (22 mm), *Micrococcus sp2* (24 mm), *Micrococcus sp3* (21 mm), *Micrococcus sp4* (14 mm), *Lactobacillus sp*1 (16 mm), *Lactobacillus sp*2 (17 mm), and *Lactobacillus sp*3 (16 mm). T4 showed inhibition activity against *Staphylococcus sp*1 (10 mm), *Micrococcus sp2* (12 mm), *Lactobacillus sp*1 (10 mm) and *Lactobacillus sp*2 (10 mm). T5 showed inhibition activity against *Streptococcus sp2* (27 mm), *Streptococcus sp3* (24 mm), *Staphylococcus sp*1 (24 mm), *Staphylococcus sp*3 (11 mm), *Staphylococcus sp*4 (17 mm), *Micrococcus sp2* (20 mm), *Micrococcus sp3* (18 mm), *Micrococcus sp4* (12 mm), *Lactobacillus sp*1 (14 mm), *Lactobacillus sp*2 (13 mm) and *Lactobacillus sp*3 (15 mm).

The inhibition percentages of toothpastes against dental caries bacteria were calculated. In this, T1, T2, T3, T4 and T5 were found as 50.00%, 64.29%, 85.71%, 28.57% and 78.57% respectively (Figure-1).

Overall, the inhibition activity against dental caries bacteria by T3 was more effective followed by T5, T2, T1 and T4.

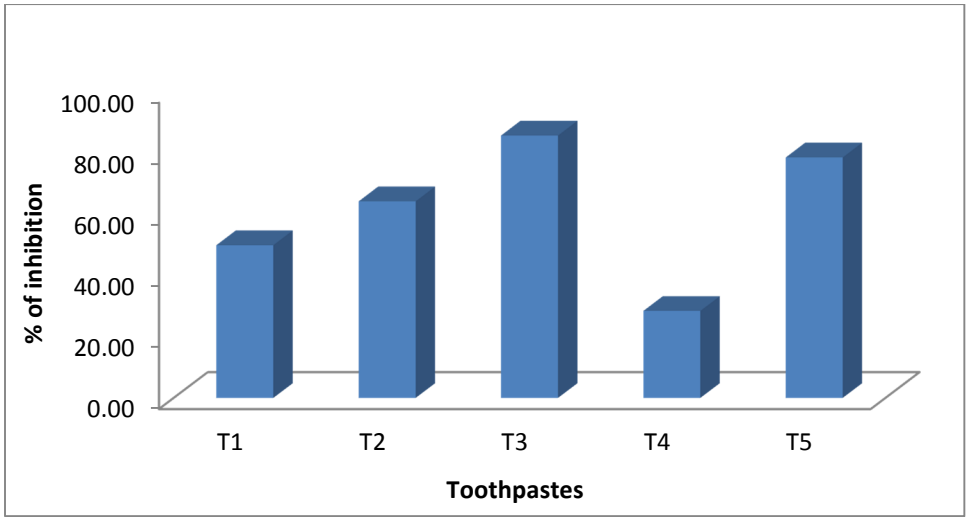


Fig.-1: Inhibition percentages of toothpastes against dental caries bacteria

Several clinical studies have demonstrated the efficacy of toothpastes against oral and gingival bacteria[12]. The results of this present study revealed that, dental care products exhibited wide variation in their effectiveness against dental caries bacteria and the toothpaste brand 3 (T3) establish superior inhibition activity against dental caries bacterial isolated from dental caries samples. Triclosan and Fluoride containing Toothpastes were found to be highly effective against the oral acidogenic bacteria[10, 13] and were recommending by the WHO, ADA & FDI[2]. Also, it should be borne in mind that the inhibition effect of toothpaste may not be directly compared with that of other toothpaste because different toothpaste constitutes different active ingredients and may diffuse at different rates[14].

## CONCLUSION

The present study has shown that the antibacterial efficacy of selected five brands of toothpaste against dental caries bacteria isolated form dental caries samples. Among five brands of toothpastes tested, two brands such as T3 & T4 possessed good inhibition activity against the tested bacterial strains such as *Streptococcus, Staphylococcus, Micrococcus* and *Lactobacillus* species. Therefore, the toothpastes which have Triclosan and Fluoride as ingredients can be used to control dental infections caused by these microorganisms.

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