

Antibacterial Efficacy of Different Toothpastes on Isolated Dental Caries Bacteria

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Abstract

The goal of the study was to assess the antibacterial activity of different commercially available toothpaste against the common oral bacterial species. Total 40 dental caries samples were collected from Chandigarh Tricity for the isolation of dental caries bacteria. The bacteria were isolated using serial dilution method and identified by studying various morphological and biochemical characteristics. The most diverse bacterial strains in the samples were tentatively found as *E. coli*, *Streptococcus*, *Staphylococcus*, *Micrococcus Klebsiella* and *Proteus* species. For the assessment of *in vitro* antibacterial activities, five toothpastes of different manufacturers were selected. All the tooth pastes exhibited inhibition against the isolates. Fluoride and triclosan containing T1 toothpaste showed maximum inhibition against the bacteria which was followed by T5 toothpaste. *E. coli* and *Micrococcus* sp. were

well inhibited by all toothpastes but the maximum zone of inhibition was exhibited by T1 (19, 18 mm respectively), followed by T5 (17, 12 mm respectively).

Keywords: Dental caries; toothpastes; bacterial isolates; antimicrobial activity

Introduction

Worldwide, it is predicted that 2.4 billion people grieve from caries of their permanent teeth while 486 million children grieve from caries of their prime teeth.^[1] Caries and other dental diseases like gingivitis and periodontitis are prevalent in the people of all the ages.^[2,3] In particular, the elderly those having exposed tooth roots, establish a special prone population.^[4] A broad and comprehensive study by National Oral Health Survey and Fluoride mapping India, 2004 demonstrated the occurrence of dental caries to be 80.2% among the population of 35-44 years old.^[5]

Many studies have demonstrated that dental caries is potentially caused by pathogenic microbial communities rather than a single pathogen.^[6] Oral cavity is intensely inhabited by microorganisms like bacteria, protozoa, viruses, fungi and archaea. Oral minute living organisms are the intricate bacterial microorganisms linked with human body.^[7,8] In mouth, the major microorganisms present are *Streptococci*, in particular *Streptococcus salivarius*, *S. oralis* and *S. mitis*.^[9,10] Facultative anaerobic gram-positive bacteria particularly *Capnocytophaga* species, *Actinomyces* species and *Streptococci* have been found to be dominant in plaque while gram-negative species included *Prevotella* species, *Veillonella* species, *Tannerella forsythia* and *Porphyromonas gingivalis*.^[11] The leading opportunistic pathogens causing dental caries is *Streptococcus mutans*.^[12] Recent advances based on metagenomic methods anticipated that the pathogenesis of dental disease encompasses multi microbial collaboration and imbalance.^[13]

Fluoride has antiplaque efficiency and it reduces the percentage of bacteria in oral cavity, there can improve the teeth health.^[14,15] It has been found that the growth of bacterial cells can be prevented by small concentrations of fluoride ions in the culturing medium.^[16] Other antimicrobial agents like triclosan, essential oils and zinc have been found to be efficient against specific oral bacteria and plaque.^[17,18] Triclosan is a chlorinated bisphenol that is attuned with other toothpaste constituents. It endorses the alteration of enzymes like lipoxygenase or

cyclooxygenase and thus possesses antibacterial effects.^[19,20] Toothpastes are used to decrease oral bacterial species by providing fluoride to the teeth. Therefore, this study was designed to determine various microbial species of teeth caries in patients and to demonstrate the proficiency of some available toothpastes, commonly used to prevent dental caries.

Material and methods

Collection of specimens from oral cavity

The dental caries samples were taken from different patients attending dental hospitals in different locations of Chandigarh using sterile cotton swab & transferred into 2 ml of sterile normal saline and labelled properly. The collected samples were transported to our laboratory and stored in refrigerator till further use.

Selection of toothpastes

Five different brands of toothpastes (including one herbal toothpaste) that are commonly used in the region were procured from the local market and the active ingredients of these dentifrices are given in Table 1. Solutions were made by mixing 2.0 gm of toothpastes in 2 ml of autoclaved distilled water and homogenized on a vortex mixer. Further dilutions in distilled water were made to obtain concentration ratios of 1:2, 1:4 and 1:8 and 1:16.

Table 1. Toothpastes used in this study and their constituents as written on the packages

Toothpastes	Ingredients listed
T1	Calcium carbonate, hydrated silica sorbitol, water, sodium laurylsulphate, flavor, sodium monofluorophosphate, sodium silicate, cellulose gum, potassium nitrate, benzyl alcohol, triclosan, sodium saccharin, CH45430, limonene, linalool
T2	Calcium carbonate, silica sorbitol, water, sodium saccharin, sodium lauryl sulfate, sodium monofluorophosphate, sodium silicate, limonene, benzyl alcohol, titanium dioxide, carrageenan, tetrapotassium pyrophosphate, sodium bicarbonate, flavor
T3	Aqua, sodium lauryl sulfate, sodium fluoride, potassium nitrate, xanthan gum, sorbitol, glycerin, PEG-6, hydrated silica, aroma, sodium monofluorophosphate, cocamidopropylbetaine, sodium saccharin, titanium dioxide, sodium hydroxide
T4	Potassium nitrate, glycerin, hydrated silica, sodium benzoate, cellulose gum, sodium saccharin, polyethylene glycol, sodium lauryl sulfate, flavour, sodium monofluorophosphate, titanium dioxide, purified water

T5	Akarkara (Anacycluspyretheum), Babool (Acacia arabica), Tomar (Xanthoxylumalatum), Neem (Azadirachtaindica), Pudina (Menthaspicata), Laung (Syzygiumaromaticum), Pippli (Piper sylvaticum), Vajradanti (Barleriapronitis), Bakul (Mimusopselengi), Vidang (Embeliaribes), Haldi (Curcuma longa), Pilu (Salvadorapersica), Majuphal (Quercusinfectoria), calcium carbonate, sodium benzoate
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Isolation of bacterial colonies

The different dilutions of caries samples were cultured by spread plate method on nutrient agar under aerobic condition at 37°C for 24 hours. On the basis of morphological and culture characteristics, most diverse bacterial colonies were further purified by streak plate method. A total of 80 colonies were selected on the basis of their morphology and further processed for its identification and evaluation of its sensitivity against the selected toothpastes.

Biochemical characterization and tentative identification of bacteria

The selected isolates were characterized and identified by gram staining^[21,22] and standard biochemical tests.^[23,24] Several biochemical assays such as citrate utilization test, indole test, urease test, oxidase test, coagulase test, catalase test and nitrate reduction test were carried out to categorise the bacterial isolates.^[25,26] In addition, methyl red test, gelatin liquefaction and fermentation of carbohydrates were performed according to described earlier.^[27]

Anti-bacterial assay of different toothpastes

To demonstrate antibacterial activity of different dilutions of toothpastes, agar well diffusion method was performed.^[28-30] In this process, Muller-Hinton agar plates were seeded with 0.1 mL of 24 h incubated liquid broths of each isolate. Up to four wells were bored in the agar of each plate by removing cuts with a sterile stainless steel cork-borer of 8 mm diameter. 0.1 mL of the toothpaste dilutions was added into each well. The plates were incubated at 37°C overnight. Growth inhibition areas appeared around the wells on agar plates were measured by scale to determine the activity against the tested isolate. The experiments were carried out in triplicate for each test organism.

Results

Identification of bacterial isolates and evaluation of different toothpastes

A total 40 dental plaque samples were swabbed from patient diagnosed with dental caries. Out of 80 isolates recovered and identified, prevalence of *Streptococcus* sp. was 25%, *Staphylococcus* 22%, *E. coli* 19%, *Micrococcus* sp. 15%, *Klebsiella* sp. 10% and *Proteus* sp. 9% (Table 2 and Figure 1). *Streptococcus* (gram-positive bacterium) is belonging to Streptococcaceae family, in the Lactobacillales order (lactic acid bacteria), in the phylum Firmicutes.^[31] *Streptococcus mutans* and *S. sobrinus* have been demonstrated as to play a vital role in the cause of dental caries^[32,33], because these can attach itself to the enamel.^[34] Previous studies have demonstrated that *Staphylococcus aureus* is not only an important basis of many infections but also has a strong link to teeth infections.^[35,36]

Table 2. Distribution of different isolates identified

Bacterial isolates	Distribution of isolates (out of 80)
<i>Streptococcus</i> sp.	25
<i>Staphylococcus</i> sp.	22
<i>E. coli</i>	19
<i>Micrococcus</i> sp.	15
<i>Klebsiella</i> sp.	10
<i>Proteus</i> sp.	9

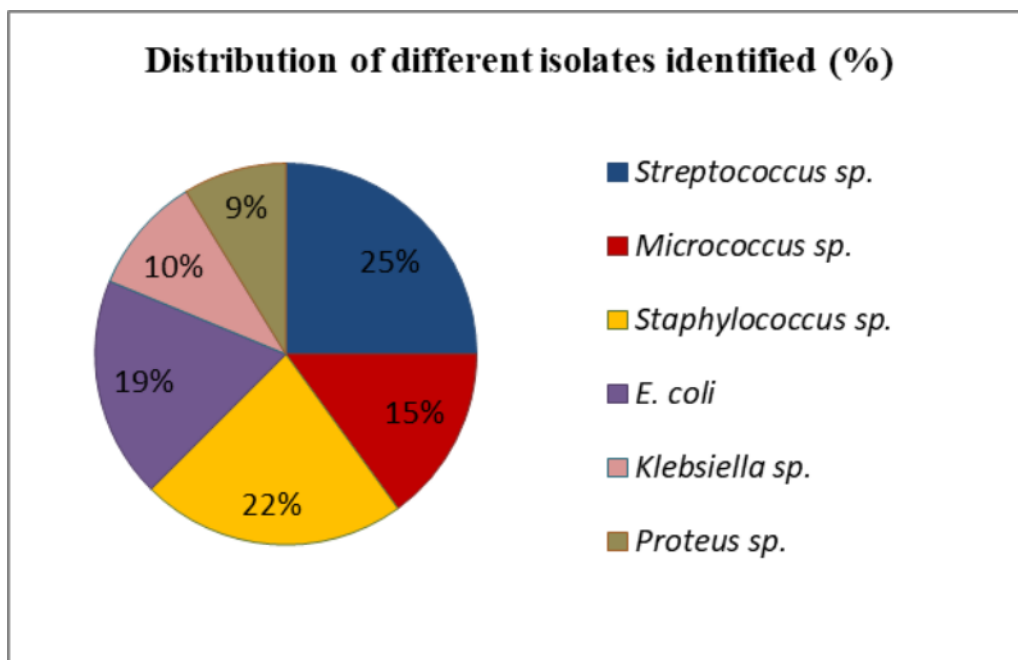


Figure 1. Percentage distribution of different isolates in the collected samples.

Antibacterial activity of toothpastes was analysed against isolated strains of *Streptococcus* sp, *Staphylococcus* sp, *E. coli*, *Micrococcus* sp, *Klebsiella* and *Proteus* sp by the method of agar well diffusion and the zone of clearance was measured in mm diameter (Table 3). Among fluoridated toothpastes T1 was more effective against *E. coli* and *Micrococcus* sp with maximum zone of inhibition of 18 and 19 mm diameter respectively. Herbal toothpaste T5 showed maximum zone of 17 and 16 mm diameter against *Micrococcus* and *Streptococcus* sp respectively (Figure 2 & 3).

Table 3. Antimicrobial activity (mm) of different toothpastes against the bacterial isolates

Bacterial Isolate	T1	T2	T3	T4	T5
<i>Streptococcus</i> sp.	15.5	12	11	9.5	16
<i>Micrococcus</i> sp.	19	12	11.5	10	17
<i>Staphylococcus</i> sp.	15.5	11	10	8	14
<i>E. coli</i>	18	11.5	11	9.5	12
<i>Klebsiella</i> sp.	14	10.5	10	8	11
<i>Proteus</i> sp.	11	10.5	10	8	10

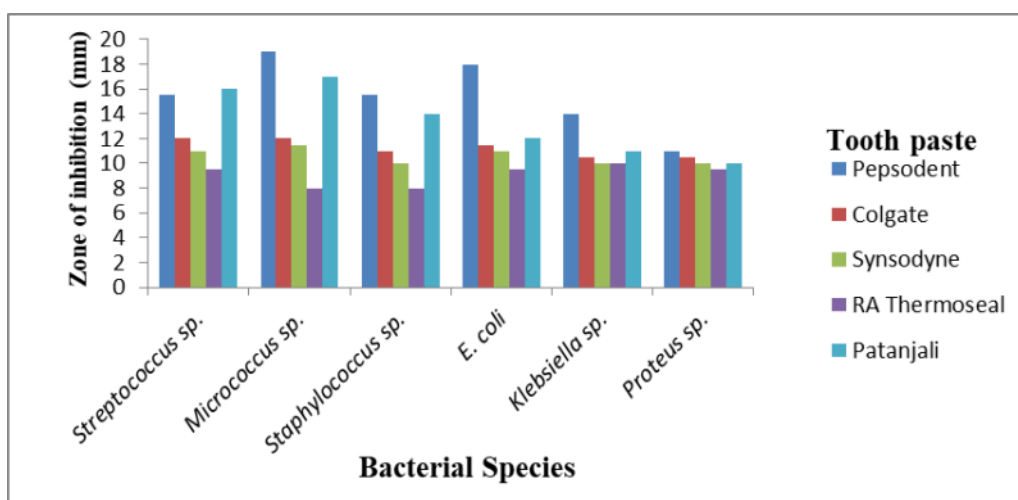


Figure 2. Graphical presentation of antimicrobial activity tooth pastes against the isolates.

(*E.coli*, *Staphylococcus* sp, *Micrococcus* sp, *Streptococcus* sp, *Klebsiella* sp and *Proteus* sp).

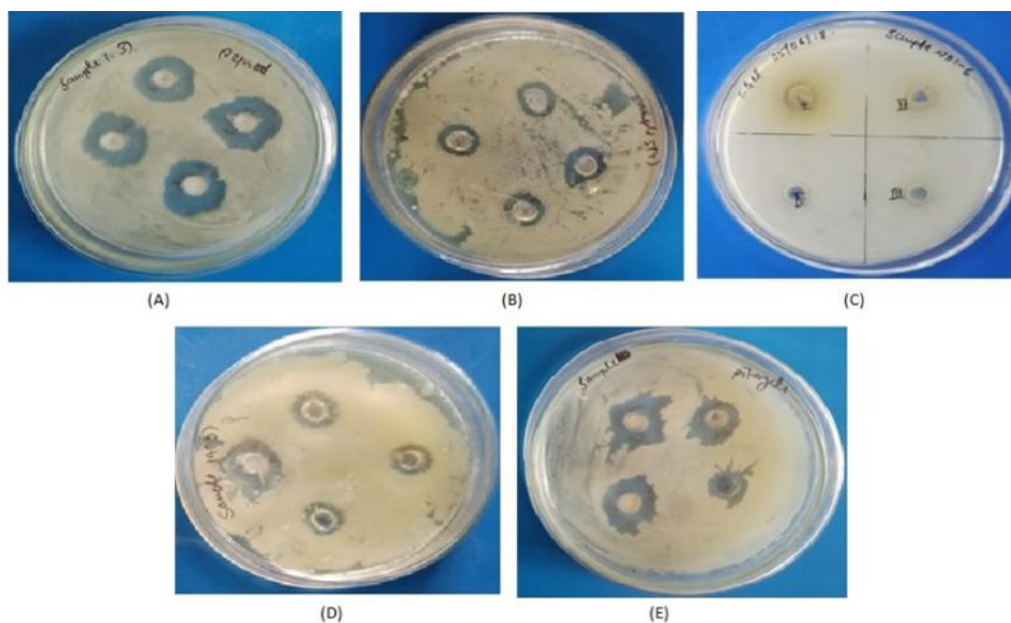


Figure 3. Zones of clearance or inhibition produced by toothpastes after 24 h incubation against the five isolates at five different concentrations. (A) *E. coli*, (B) *Staphylococcus* sp, (C) *Micrococcus* sp, (D) *Streptococcus* sp, (E) *Klebsiella* sp and (F) *Proteus* sp.

Discussion

Several studies have analysed the efficacy of toothpastes against oral and dental bacteria.^[37] The present study revealed that, dental care pastes presented wide variation in their efficiency against dental caries bacteria. Among all the inspected triclosan containing toothpastes, T1 arose as the most active, based on the substantial zone of bacterial inhibition produced against all the five tested isolates of dental caries samples. This might be due to the existence of chemical ingredients such as sodium monofluorophosphate and triclosan. Several studies using triclosan as inhibitor have demonstrated the good antimicrobial efficiency.^[38] The enzyme, enoyl-(acyl-carrier) reductase present in *Streptococcus* sp has been known to catalyse the last step in extension mechanism of fatty acid and triclosan has been demonstrated as repressor to the substrate (trans-2-enoyl-ACP) attachment to hamper the fatty acid production.^[39,40]

Next to triclosan and flourides, herbal products have been used magnificently for treating various diseases in Indian Ayurvedic system.^[41] Against *Streptococcus* sp, T5 toothpaste showed maximum significant antibacterial activity (16 mm). Also, *Micrococcus* was inhibited efficiently by this herbal product but slightly lesser than T1. Earlier studies have demonstrated the anti-plaque efficiency of herbal base toothpaste.^[42,43] Data in this study is in consistent with a latest research^[44] which analysed that natural plant based products to be less workable as compared to the constituents with triclosan.

On the flip side, herbal dentifrice, T5 showed better results as compared to T2, T4 and T3. The main ingredients of this toothpaste contain Pudina, Haldi, Neem, Turmeric, Pippli, Vajradanti, Laung and Babool. The existence of some secondary metabolites such as lectins, polyphenols, flavonoids and alkaloids in these constituents are known to be the exclusive cause of its antibacterial ability.^[45]

The present study has demonstrated the antibacterial efficiency of five different toothpastes against bacterial isolates form dental caries samples collected from Chandigarh Tricity, India. Among all the five toothpastes tested, T1 and T5 possessed good inhibition activity against the bacterial isolates; *Streptococcus*, *Staphylococcus*, *Micrococcus*, *E. coli* and *Klebsiella* species. Therefore, the toothpastes which have Triclosan and Fluoride as ingredients can be used to inhibit dental problems affected by these bacteria. Herbal toothpaste, T5 showed antimicrobial properties as it contained herbal products with secondary metabolites that has the ability to control dental infections; thus reducing the chances of fluoride toxicity.

Conflicts of interest

The authors have no conflicts of interest.

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