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A CLINICAL STUDY ON ANTIBACTERIAL ACTIVITY OF TOOTHPASTES AGAINST DENTAL PLAQUE AND CARIES CAUSING BACTERIA

Bhagyashree T. Yele and Prof. Dharmvir A. Chouhan
P.G. Department of Microbiology, D. B. Science College, Gondia

Abstract
The present study was carried out to evaluate antibacterial activity of toothpastes, which were available in local market. A total 15 samples were collected for isolation of cariogenic bacteria. The bacterial strains were isolated by using selective Medias and identified on the basis of cultural, morphological, biochemical characteristics. The bacterial strains resistant to various choosen antibiotics subjected to action of toothpaste. Aqueous solutions of toothpastes were tested against dental caries bacteria. The distilled water suspension of toothpastes were found to has remarked antibacterial properties.in the current study total 7 brands of toothpastes used and labeled as T1-T7.The effectiveness of toothpaste was reported based on zone of growth inhibition against cariogenic bacteria. Toothpastes T3and T7 showin g broad spectrum of action against antibiotic resistant strains of Pseudomonas spp, Streptococcus spp., Staphylococcus aureus. And Lactobacillus spp.T1 is also effective against all selected strains of cariogenic bacteria. But T1 was comparatively less effective as compared to T3and T7. T3and T7 toothpastes are herbal. Other selected toothpaste T2, T4, T5 and T6 are ineffective against cariogenic strain of Streptococcus spp. This study shown herbal toothpastes have more antibacterial activity against selected strains of antibiotic resistant cariogenic bacteria like., To reduce the dental problem herbal based toothpastes should be recommended.

Keywords: *Cariogenic bacteria, dental plaque, dental Carie, Toothpaste.*

Corresponding author:
Prof. Dharmvir A. Chouhan
Assistant Professor and Head,
P. G. Department of Microbiology,
D. B. Science College, Gondia,
Mob: 7350330035,
Email.id: dharmvir1703@yahoo.co.in

QR code

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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]. According to WHO report in April 2012, globally 60-90% of children have dental cavities and about 100% of adult have dental cavities [2]. Dental caries can be defined as the localized destruction of susceptible dental hard tissues by acidic byproducts from bacterial fermentation of dietary carbohydrates [3]. Dental caries results from the interaction of specific bacteria with constituents of the diet within a biofilm termed "dental plaque" [4].

Dental caries causing Endogenous bacteria mainly *mutan streptococci* (*Streptococcus mutans* and *Streptococcus sobrinus*) and *Lactobacillus spp*. in the biofilm of dental plaque produce weak organic acids as metabolic by-products of fermentable carbohydrates. The acids would cause local pH to fall below a critical value resulting in demineralization of the tooth tissue [5]. The oral cavity contains a wide variety of oral bacteria, but only a few specific species of bacteria are believed to cause dental caries namely *Streptococcus mutans, Lactobacillus acidophilus, Actinomyces viscosus,* and *Nocardia spp. Streptococcus mutans* are most closely associated with caries [6,7]. Two major groups of bacteria which produces such acids are namely *Streptococcus mutans* and *Streptococcus sobrinus* and the *Lactobacillus species*. [8,9]. These strains survive well wherever they have niches to live in [10].

Other than microbial cause carbohydrate nutrition, the intermediate intake of carbohydrates, sweetened beverages and bad oral hygiene, high accidence of caries in the parents, low social status are the strongest risk factors in the children for dental carrie [11]. Sucrose is considered the most cariogenic dietary carbohydrate, because it is fermentable, and also serves as a substrate for the synthesis of extracellular (EPS) and intracellular (IPS) polysaccharides in dental plaque [12].

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 [13]. 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 [14].

Tetracycline is commonly used antibiotic in dental problem treatment as a prophylactic agent and for treatment of oral infections. The wide use of tetracycline had resulted in a major increase in the rate of tetracycline resistance among bacteria [15].

With the knowledge of curative properties of the medicinal plants against oral microorganisms and their incorporation in clinical practice we can aim to reduce if not remove this disease entity. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over the drugs [16].

MATERIALS AND METHOD:

Collection of Toothpaste

Toothpaste of 7 different brands (T1, T2, T3, T4, T5, T6, T7) which are easily available in local market Gondia, Maharashtra. Choose some herbal ingredient containing and some chemical ingredient containing toothpaste and were taken to the laboratory for analysis [17] taken half used toothpaste**.**

Sample collection

For isolation of Cariogenic bacteria total 15 plaque samples collected from dental plaque of human patients from Gondia city. Plaque samples taken from patient which are suffer from different stage of dental Carie and are belonging to different age group, ranging from 18-40 years. Sample were taken from dental plaque by sterilize toothpick and it transfer in 2 ml phosphate buffer saline (pH 6.8). The collected samples were immediately taken to the lab for further process [18].

Enrichment & Isolation of dental caries bacteria

For isolation of dental caries bacteria basal salt media was used. The plaque samples in tubes were inoculated separately in 10 ml basal salt medium tube. The inoculated tubes were incubated at 35^0C for 24 hrs. .After enrichment, 1 ml enriched sample was serially diluted up to 10^{-5} with phosphate buffer saline. 200 ul of each diluted sample was spread over the respective agar media plates like Baired parker agar, Mannitol salt agar, Yeast malt agar, Blood agar, Pseudomonas isolation agar, Cetrimide agar, Lactobacillus MRS Agar. The plates were incubated at 35^0C for 24 hrs. After

incubation plates observe for colonies with desired characteristics [17].

Maintenance of isolates

The distinct colony from each media plate is picked up and sub cultured on Nutrient agar slant. Incubated and maintained in laboratory.

Identification of bacterial Isolates

Each isolate is identified based on morphological examinations like Gram staining Motility, capsule staining, Endospore staining Biochemical characteristics like, Sugar fermentation test, IMViC test , Urease test , Catalase test, Oxidase test and Coagulase test were performed according to Bergey's Manual of Systematic Bacteriology [19].

Antibiotic sensitivity test

The bacterial isolates were subjected to analysis for susceptibility or resistance towards selected antibiotics. 9 different antibiotics selected which commonly used for treatment of dental Carie are selected. These antibiotics were Penicillin, Chloromphenicol, Ciprofloxacin, Erythromycin, Gentamycin, Kanamycin, Vancomycin, Methicillin, Tetracycline and Kirby-Bauer disc diffusion method is used [20]. After incubation zone size is recorded and calculated MAR Index.

To test antibacterial potential of toothpaste suspension

An assessment of toothpaste for antibacterial activity was tested by agar well diffusion method. A stock solution was prepared by mixing 1 gm of toothpaste in 10 ml of distilled water. Taken more resistance strain and make seeded agar plate. Using sterile 6 mm gel borer equidistant well was prepared on Muller Hinton agar plate. Each well filled with 100ul of each suspension of toothpaste. The plates were incubated further at 37^0 C for 24 hrs. The diameter of growth inhibition zone was measured and activity index was calculated [21].

RESULT AND DISCUSSION:

15 dental swab samples were tested for presence of cariogenic bacteria. out of these 5 samples were found to be positive for bacterial pathogen. These are Swab (C1), Swab (C2), Swab (C4), Swab (C11) and Swab (C13) Total 60 strains were isolated from these clinical specimens.

The pathogenic bacteria which responsible for dental Carie and dental plaque are *Pseudomonas spp. Streptococcus spp, Staphylococcus spp*. And *Lactobacillus spp* in current study occurrence of all the types of cariogenic bacteria is reported in dental plaque samples. These isolates reported in table 1.

Table .1 Occurrences of cariogenic bacteria

Sr no.	Specimen	No. of *Streptococcus* spp. Isolates	No. of *Staphylococcus* aureus Isolates	No. of *Pseudomonas* spp Isolates	No. of *Lactobacillus* spp. Isolates
1	Swab (C1)	6	3	2	1
2	Swab (C2)	6	3	2	1
3	Swab (C3)	0	0	0	0
4	Swab (C4)	6	3	2	1
5	Swab (C5)	0	0	0	0
6	Swab (C6)	0	0	0	0
7	Swab (C7)	0	0	0	0
8	Swab (C8)	0	0	0	0
9	Swab (C9)	0	0	0	0
10	Swab(C10)	0	0	0	0
11	Swab(C11)	6	3	2	1
12	Swab(C12)	0	0	0	0
13	Swab(C13)	6	3	2	1
14	Swab(C14)	0	0	0	0
15	Swab(C15)	0	0	0	0

Harshal (2009) has reported as dental caries with a prevalence of as high as 60% - 80% in children. Near 80% of the opportunistic bacteria isolated from plaque were found in saliva or tonsils, while 63% - 64% of the microbes present in saliva were present in dental plaques or tonsils [22]. This indicates organisms might be released from tooth surfaces into saliva and then colonizes on oral cavity surfaces such as the tonsils.

In our occurrence study out of 15 samples, 5 samples were positive for presence of cariogenic bacteria. From these 5 samples total 60 strains of cariogenic bacteria were isolated. Out of 60 isolates 30 were *Streptococcus spp.*, 15 were *Staphylococcus aureus*, 10 were *Pseudomonas aeruginosa* and 5 were *Lactobacillus spp.*.

In studies of Becker *et.al [23]* and Aas *et.al[24]*, they reported that genera associated with dental caries in both primary and permanent dentitions are *Streptococcus* including *S. mutans*, *S. sanguinis* and non-*S.mutans streptococci*, *Veillonella*, *Actinomyces*, *Bifidobacterium*, *Lactobacillus*, *Propionibacterium*, and *Atopobium*. In other study, has been shown that in plaque significantly associated with dental caries are the genera of *Streptococcus*, *Veillonella*, *Actinomyces*, *Granulicatella*, *Leptotrichia* and Thiomonas [25].

The isolated bacterial strains were identified by study of cultural, morphology and biochemical characteristics. These reported in table 2

Table-2: Morphological, cultural and biochemical characteristics of cariogenic bacteria

Sr.	Characteristics	*Staphylococcus aureus (Sa)*	*Streptococcus spp. (St)*	*Pseudomonas spp. (Ps)*	*Lactobacillus spp. (Lb)*
1	Gram staining	+ VE	+ VE	- VE	+VE
2	Morphology	Cocci	Cocci	Rod	Rod
3	Motility	- VE	- VE	+ VE	- VE
4	Capsule staining	- VE	+ VE	- VE	- VE
5	Indole test	-VE	-VE	-VE	-VE
6	MR test	+VE	+VE	-VE	+VE
7	VP test	+VE	-VE	-VE	+VE
8	Citrate utilization	+VE	-VE	+VE	+VE
9	Spore staining	- VE	- VE	- VE	- VE
10	Coagulase test	+ VE	- VE	- VE	-
11	Growth on MacConkey agar	LF	NLF	NLF	NLF
12	Growth on blood agar	βheamolysis	α-heamolysis	βheamolysis	-
13	Catalase test	+VE	-VE	+ VE	- VE
14	Urease test	+VE	-VE	-VE	+VE
15	Oxidase test	-VE	-VE	+ VE	- VE

+VE-Positive -VE-Negative LF-Lactose Fermenting NLF-Non Lactose Fermenting

All Ps isolates were Gram negative, motile, bacilli showing blue green color colonies on Cetrimide agar and pseudomonas isolation agar. These isolates were shown MR, VP negative test but shown culture citrate utilization potential. These strains were utilizing glucose as fermentable sugar but unable to utilized sugar like lactose and Mannitol. These isolates showing enzyme activity of Coagulase but liking enzyme activity of Catalase positive, Oxidase positive and Urease negative, and Indole negative. These isolates unable to produce H_2S. On the basis of these result all Ps isolates were identified as *Pseudomonas spp. [26]*.

All Sa isolates showed typical get black color colonies on Baired Parker agar and colonies with yellow hollow on Mannitol salt agar.cocci shaped, non-motile. They showed methyl red and Voges Proskauer test, Citrate, Urease positive. They were Oxidase and Indole negative but nitrate reductase, Catalase and Coagulase positive, they hydrolyze area but they did not utilized citrate and not produce H_2S gas. They ferment glucose Mannitol and lactose with production of acid only. On the basis of these results all Sa isolates were identified as *Staphylococcus aureu [27]*.

All St Isolates showed colonies with β- haemolysis on blood agar and colonies with cream color on YMA agar. They were methyl red positive, they were Gram positive, motile in nature they ferment glucose, lactose, Mannitol with production of acid only. They give Indole and VP negative, Citrate, Coagulase, Catalase, Urease, Oxidase negative On the basis of these results all St Isolates were identified as *Streptococcus spp. [28]*.

All Lb isolates showed colonies on Lacto bacillus MRS Agar large clear colonies after 24-72 hours incubation at 35^0c in an enhanced CO_2 environment. They showed methyl red and Voges Proskauer, Citrate test positive. They were Oxidase, Catalase and Indole negative. Urease positive, On the basis of these results all Sa isolates were identified as *Lactobacillus spp [29]*. Identification of these clinical isolates is summarized in Table 2.

These 60 isolates choosen for antibiotic susceptibility test. Out of these isolates total 8 isolate (2 isolate of *Streptococcusspp.*,2 isolate of *Staphylococcus aureus,* 2 isolate of *Pseudomonas spp.*, 2 isolate of Lactobacillus *spp.)* shown multiple drug resistance toward selected antibiotics.

Maripandi *et.al* (2011) reported that dental caries bacteria were resistant against Vancomycin, Chloromphenicol, Penicillin, Bacitracin and Streptomycin [30].

Antibacterial activity of toothpastes were tested against two strains of *Streptococcus* spp. (St6, St11), two strains of *Staphylococcus aureus.*(Sa5, Sa7), two strains of *Pseudomonas* spp. (Ps1, Ps6) and two strains of *Lactobacillus spp.* (Lb2, Lb4). (This shows more resistivity against all antibiotics) After incubation, the zones of inhibition were measured with the help of numerical scale and the values in 'mm' were tabulated (Table-3).

Table -3Antibacterial activity of tooth paste against cariogenic bacteria

Sr.No.	Toothpaste	Zone of Growth inhibition (mm)							
		Ps 1	Ps6	St 6	St11	Sa 5	Sa7	Lb2	Lb4
1.	T1	20	30	14	16	22	21	19	18
2.	T2	00	00	00	00	00	00	14	16
3.	T3	25	25	20	20	23	29	27	23
4.	T4	00	00	00	00	00	09	12	14
5.	T5	00	00	00	00	00	00	08	07
6.	T6	00	00	14	06	15	09	15	13
7.	T7	15	12	10	21	21	20	20	20
8	Control	00	00	00	00	00	00	00	00

Ps-*Pseudomonas spp., St-Streptococcus spp., Sa-Staphylococcus aureus, Lb-Lactobacillus spp.*

Several clinical studies have demonstrated the efficacy of toothpastes against oral and gingival bacteria [31]. The various toothpastes which have sodium fluoride and triclosan as the active ingredients marked as anti caries agents that prevent the formation of cavities in teeth. Fluoride works by strengthening the calcium phosphate in teeth enamel [32].

The main mechanism of fluoride cariostatic effect is described by Rolla *et al.* in 1993 [33] and noted that systemic fluoride was not effective due to very little fluoride incorporated into enamel through this approach. Topical fluoride application induces formation of fluorhy- droxyapatite on the enamel and on the root surface and give a more acid resistance. Sorbitol also acts as a sweetenter and makes more palatable. Silica is the ingredient that gives the toothpaste its abrasive quality. Toothpastes must be abrasive to remove plaque, stains and debris. It also does not scrape tooth enamel or damage gums[30] .Triclosan as a major chemical ingredient possess significant antibacterial activities [30].

In our study T1 toothpaste is effective against all the tested cariogenic pathogens. It is chemical based toothpaste. Sorbitol, hydrated silica, water, sodium lauryl sulphate, PEG-32, flavor, cellulose gum, Trisodium phosphate, Methyl paraben, Propyl paraben, Sodium saccharine, Zink citrate, Triclosan, Sodium fluoride, and C177891, are ingredients contain in it.(As on cartoon box of branded toothpaste).

T2 toothpaste is only effective against *Lactobacillus spp.* It is chemical based toothpaste. It contains the ingredients are Purified water, sorbitol, silica, Potassium nitrate, glycerin, polyethylene glycol-300, Sodium lauryl sulphate, flavor, sodium, saccharin, sodium hydroxide, titanium dioxide, xanthan gum, and cocamidopropyl betaine.

T3 toothpaste effective against choosen all strains of antibiotic resistant cariogenic bacteria. It is herbal based toothpaste, Asphatic bhasma, Tankana bhasma are main ingredients contain in it other

than glycerin, zinc citrate, and sodium benzoate and calcium carbonate are used.

T4 toothpaste effective against only *Lactobacillus* isolates. It is chemical based product ,containing Calcium carbonate, sorbitol, silica, water, sodium lauryl sulphonate,, flavor, containing clove oil, babul extract, cellulose gum, xanthan gum, sodium silicate, sodium saccharin,formaldehyde, foaming,non fluoride toothpaste.

T5 toothpaste effective only against Lactobacillus spp, it is chemical based toothpaste, sorbitol, hydrated silica, water, PEG-32, flavor, cellulose gum, cocamido-propyl betaine, sodium saccharin, zinc sulphate, MICA, Sodium hydroxide, C116255, C117200, C177891 and Eugenol.

Toothpaste T6 effective against, Sa (*Staphylococcus aureus*) and Lb (*Lactobacillus spp*.) isolates but not against Ps (*Pseudomonas spp*), it is chemical based toothpaste it contains sorbitol, hydrated silica, water, Sodium lauryl sulphate, PEG-32, flavor, cellulose gum, trisodium phosphate, methyl paraben, propyl paraben, sodium saccharine, zinc citrate, triclosan, sodium fluoride, C177891.

Toothpaste T7 effective against all isolates. It is herbal based toothpaste it contains the ingredients are calcium carbonate, sorbitol, silica, water, Sodium lauryl sulphate, flavor, miswak extract, cellulose gum, carrageenan, sodium silicate, PVM/MA, copolymer, sodium saccharin, sodium benzoate, C177891,triclosan, foaming, non-fluoride toothpaste.

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. [34]

CONCLUSION:

In present study reported that *Streptococcus* strain is a most prevalent pathogen in dental Carie and dental plaque. As well as *Pseudomonas*, *Staphylococcus*, *Lactobacillus* strains are also responsible for dental problem. These strain reported to bear multiple antibiotic resistance .In this current study, we observed that the antibacterial efficacy of selected seven brands of toothpaste against cariogenic bacteria which were resistant to various antibiotics. Two brands such as T3-T7 shown good antibacterial activity against tested bacterial strains.T1 Toothpaste is also effective against all selected strains but it least effective compared toT3 & T7. T3&T7 toothpastes were herbal based have components from various medicinal plants in its composition. T1, T2, T4, T5, T6 are chemical based toothpaste they are least effective against cariogenic bacterial strains. Therefore the toothpastes which have herbal composition is better than chemical based toothpaste for prevention and control of dental Carie and dental plaque. Mainly the dental caries bacteria are resistant to antibiotics therefore regular use of toothpaste is better for prevention rather than use of antibiotics.

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