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Isolation of Bacteria Causing Dental Caries and Study of Effect of Toothpastes, Mouthwash, and Essential Oils on the Obtained Isolates

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Abstract: Caries is a chronic disease that progresses slowly and is characterized by localized and irreversible destruction of the tooth. Total 5 samples were collected from the patient suffering from dental caries. Samples were screened for isolation of micro-organisms on primary screening media like nutrient agar and secondary screening media like blood agar. Total 32 isolates were obtained by primary screening from which 16 isolates were able to form zones of inhibition on secondary media (blood agar). One of preventive method against dental caries is by maintaining the oral hygiene by using toothpaste. Thus effectiveness of toothpaste, mouthwash, and essential oils was determined by zone of inhibition on obtained isolates that caused dental caries. The sensitivity performed with different toothpastes showed that the toothpastes were active against the inhibition of bacteria.

Keywords: Dental caries, Bacteria, Toothpastes, Mouthwash, Essential oils.

I. INTRODUCTION

Dental caries also known as cavities, is a most common dental problem in the world. Caries result from the interaction of some specific bacteria with the dietary constituents of biofilm known as “dental plaque”. Is a chronic disease, that damages the hard tissues of teeth and is formed from the accumulation of plaque on teeth surfaces formed by acid producing bacteria from fermentable carbohydrates.

Bacteria interact with carbohydrates that can be fermented in the long run, forming acids thereby lowering pH below critical and resulting in demineralization of hard tissue of teeth [1]. Known dental caries microorganisms are Streptococcus, Lactobacillus, Staphylococcus. The most common cause of dental caries is Streptococcus mutans. It acts as an initiator of dental caries, while Lactobacillus spp, contributes to the developmental process and the continuation of caries. Recently it has been reported that Veillonella, Bifidobacterium, Propionibacterium, Actinomyces spp., and Atopobium spp. bacteria also play important role in the development and continuation of dental caries [1].

Natural product have been recently investigated more thoroughly as promising agents for the prevention of oral diseases, especially dental caries. The increasing resistance to available antimicrobial has attracted the attention of the scientific community regarding a search for new-cost effective drugs of natural or synthetic origin. Essential oil in general demonstrate antimicrobial activity against cariogenic microbes [2].

Several chemical preventive agents have beneficial effects in the control of plaque and to reduce or prevent oral disease. Hence, various chemical formulations were tried in dentifrices. The plant derived essential oils may be an effective alternative to overcome microbial resistance [3].

Essential oils (EOs) are important for their detected antimicrobial activity including *S. mutans*. They are complex, volatile, natural compounds formed by aromatic plants as secondary metabolites. They are known for their bactericidal, virucidal, fungicidal, sedative, anti-inflammatory, analgesic, spasmolytic, and locally anesthetic properties [3].

II. MATERIALS AND METHODS

A. Collection of samples

Samples were collected from the infected area of the tooth through a sterile cotton wool swab and transferred to a eppendorf tube containing phosphate buffer and kept at 4°C until used for further analysis. Samples were collected from “Pushp Dental Clinic” Gundlav and “Abhinandan Dental Clinic” Valsad. In all total 5 samples were collected.

B. Isolation Of Bacteria Causing Dental Caries

Primary screening of bacteria causing dental caries Sample obtained were allow to attain room temperature before examination. One loopful of sample was streaked on sterile nutrient agar plate and the plates were incubated at 37°C for 24 hours. After the incubation period, the plates were observed for colonial growth of different isolates. The isolates were purified by repeating the streaking for 2-3 times on nutrient agar plate and were maintained on slants at 4°C.

C. Secondary Screening Of Bacteria Causing Dental Caries

Organism which were able to grow on nutrient agar plate were further subjected to blood agar medium for secondary screening. The isolates were streaked on sterile blood agar plate and were incubated at 37°C for 24 hours. After incubation, the plates were observed for clear zones of inhibition around each colony obtained on plates.

D. Study of Colonial Characteristics

The bacteria isolated from secondary screening were characterized on basis of morphology, gram staining, and colony characteristics.

E. Study Of Effect Of Toothpastes On Obtained Isolates

Suspension of different isolates were made in distilled water and were streaked on sterile Muller-Hinton agar plate with the help of sterile swab. Wells were made with the help of sterile cup borer (6 mm diameter) The wells were loaded with different toothpastes and essential oils to be assayed (undiluted) and plates were incubated at 37°C for 24 hours. After the incubation period, the plates were observed for zone of inhibition around the wells which were loaded with toothpaste. The size of zones were measured with the help of zone reader, to analysed weather the organism is sensitive or resistance towards the toothpaste used. Same way , this procedure was performed for mouthwash and essential oils.

III. RESULTS AND DISCUSSION

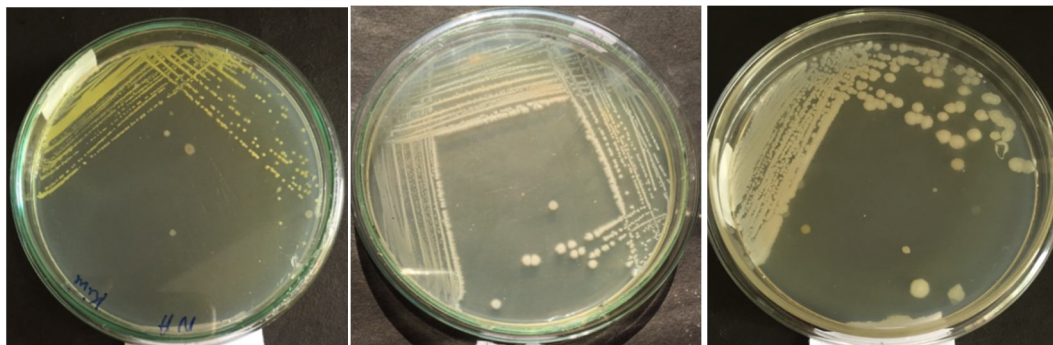


Figure 1. Isolation of bacteria causing dental caries on nutrient agar plates by primary screening.

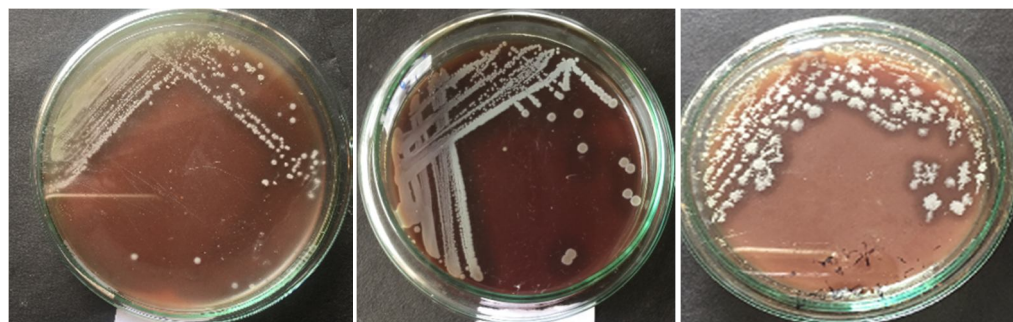


Figure 2. Isolation of bacteria causing dental caries on blood agar plate by secondary screening.

From the 5 pus samples collected from dental clinics, 32 bacterial isolates were obtained during primary screening on nutrient agar plate . Out of 32 isolates obtained, 16 isolates were obtained during secondary screening on blood agar medium . Out of 16 isolates obtained, 2 isolates were gram positive cocci in cluster, 9 were gram positive short rods, 2 were gram positive long rod and 3 were gram negative rods.



Fig 3. Effect of toothpastes , mouthwash, and essential oils on isolates.

A. Result of Activity Assay of Chemical-Based Toothpastes Circulating in the Market.

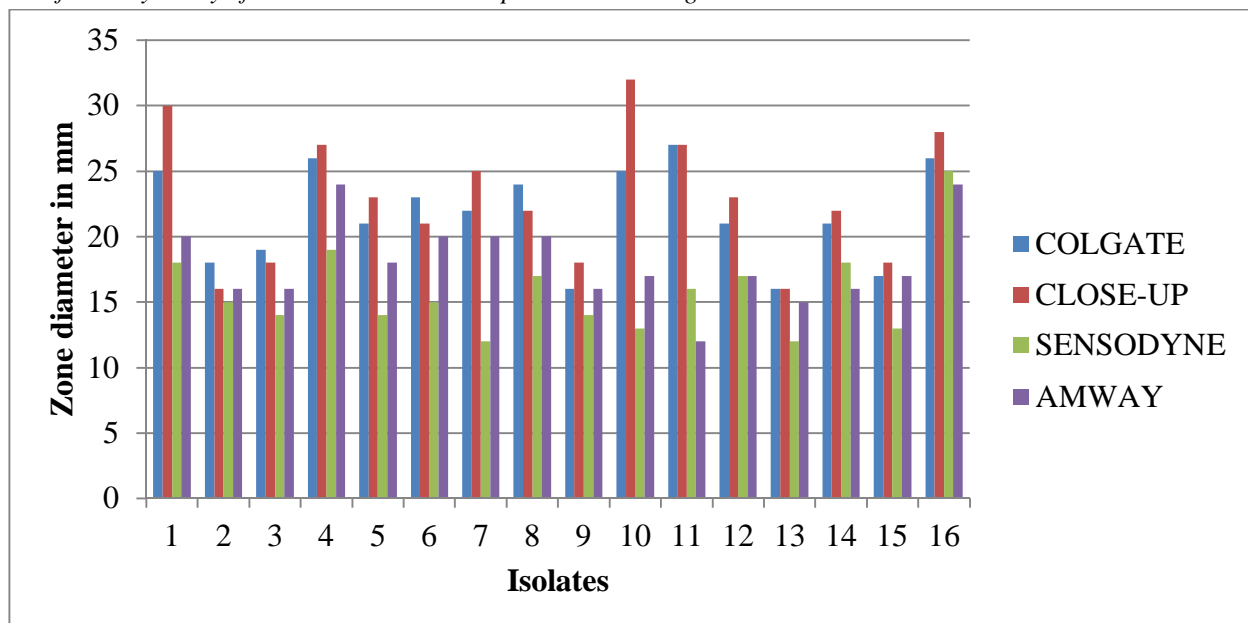


Fig 1. Effect of chemical-based toothpastes on obtained isolates.

The result of activity assay of 4 chemical-based toothpastes circulating in the market can be seen in fig 1. The zone of inhibition against the isolate results by the toothpastes close-up was more effective followed by Colgate , Amway, Sensodyne. Isolate no. 10 had the largest zone of inhibition with a diameter of 32 mm against the toothpaste Close-up. Sensodyne was least effective against the isolated bacteria. Among the various tooth paste which have sodium fluoride and triclosan as the active ingredients marked as anticaries agents that prevent the formation of cavities in teeth [4]. Fluoride works by strengthening the calcium phosphate in teeth enamel. Topical fluoride application induces formation of fluorhydroxyapatite on the enamel and on the root surface and give a more acid resistance. Sorbitol also acts as a sweetener 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 [1].

In all total 16 isolates were tested against 4 different and popular band of toothpaste like Colgate, Close-up, Sensodyne, and Amway, which are chemical based toothpaste readily available in market. Results suggest that isolate no. 10 was inhibited by the production of zone of inhibition of 32 mm diameter which is largest zone obtain when compare to other isolates with the toothpastes Close-up followed by isolate no. 1,16,4,11,7,5,12,8,14,6,3,9,15 while isolate no. 2 and 13 were least affected by Close-up. Similarly, Colgate was giving highest zone of inhibition against isolate no. 11 at 27 mm diameter followed by 4,16,1,10,8,6,7,5,12,14,3,2,15 with least for isolate no. 9 and 13 at 17 mm diameter. Amway was giving highest zone of inhibition against isolate no. 4 and 16 at 24 mm diameter followed by 1,6,7,8,5,10,12,15,2,3,9,14,13 and with least for isolate no. 11 at 12 mm diameter. Sensodyne was giving highest zone of inhibition against isolate no. 16 at 25 mm diameter followed by isolate no. 4,1,14,8,12,11,2,6,3,5,9,10,15 and with least for isolate no. 7 and 13 at 12 mm diameter.

B. Result Of Activity Assay Of Herbal Toothpastes Circulating In The Market

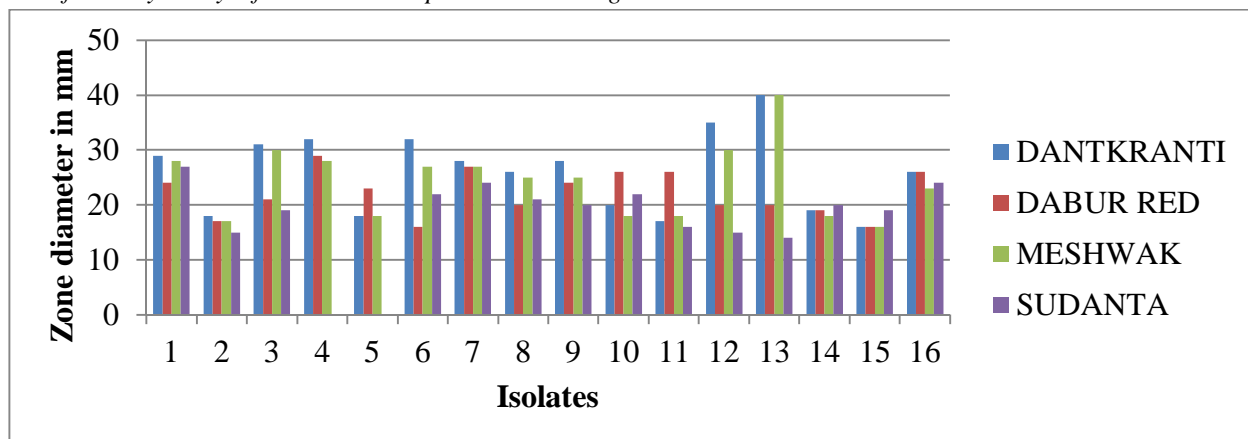


Fig 4. Effect of herbal toothpastes on obtained isolates.

In all total 16 isolates were tested against 4 different and popular band of toothpaste like Dantkanti, Meshwak, Dabur-red, and Sudanta which are herbal toothpastes readily available in market. Results suggest that isolate no. 13 was inhibited by the production of zone of inhibition of 40 mm diameter which is largest zone obtain when compare to other isolates with the toothpastes Dantkanti and Meshwak followed by isolate no. 1 while isolate no. 15 were least affected. Similarly, Dabur red was giving highest zone of inhibition against isolate no. 4 at 29 mm diameter with least for isolate no. 6 and 15 at 16 mm diameter.

Sudanta was giving highest zone of inhibition against isolates no. 1 at 27 mm with least for isolate 13 at 14 mm diameter. Isolate no. 4 and 5 were resistant to toothpaste sudanta.

Furthermore, the efficacies of the tooth pastes regarding their chemical composition is not important especially in developing countries like India where low grade products can be found in local markets and consumers are forced unknowingly to choose the products. In our study herbal paste which contained herbal extract of different medicinal plants

(Anacyclus pyrethrum, Azadirachta indica, Acacia arabica, Xanthoxylum alatum, Metha spicata, Syzygium aromaticum, Piper sylvaticum, Barleria prioritis, Mimusops elengi, Embelia ribes, Curcuma longa, Salvadora persica, Quercus infectoria, Cinnamomum camphora, Zingiber officinale, gairic powder) and did not contained triclosan and fluo- rides are more effective against bacteria. [5].

Clove has a string antibacterial property, fight against toothache and bacterial problems and its astringent property helps in keeping gums healthy.

Cinnamon provides good flavour and is anti-inflammatory.

Bakul and mayaphal helps in making gums and teeth strong.

Black peper keeps away bad breath and gum problems.

Shunthi contains sesquiterpenes, which have anti-inflammatory and antibacterial properties.

C. Result of Activity Assay of Mouthwashes Prescribed by the Doctors.

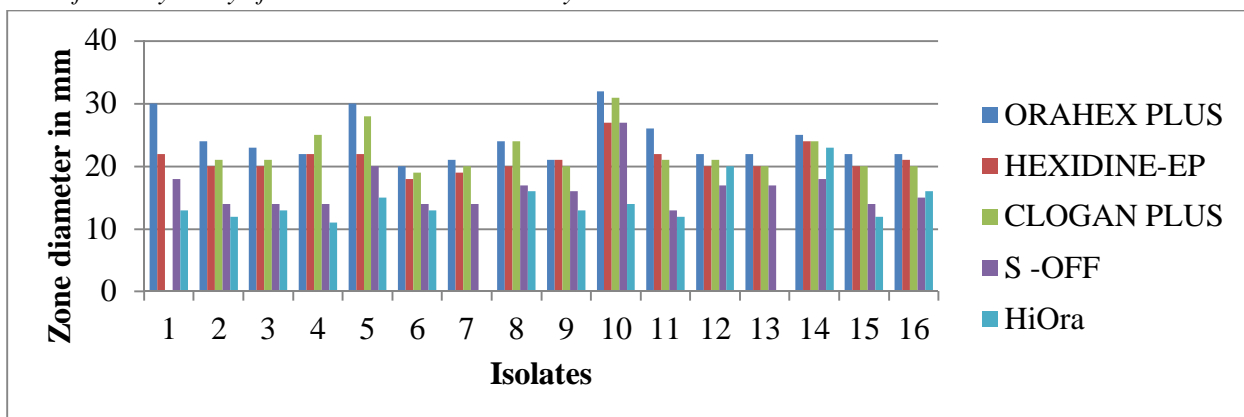


Fig 5. Effect of mouthwashes against isolates obtained.

In all total 16 isolates were tested against 4 different and popular band of toothpaste like orahex plus, hexidine- EP, clogan plus, S-OFF, HiOra which are mouthwashes readily available in market. Results suggest that isolate no. 10 was inhibited by the production of zone of inhibition of 32 mm diameter which is largest zone obtain when compare to other isolates with the mothwash orahex plus followed by isolate no. 1, 11,14,2,8,3,4,12,13,15,16,9, while isolate no. 6 were least affected at 20 mm diameter . Similarly, Hexidine-EP was giving highest zone of inhibition against isolate no. 10 at 27 mm diameter followed by isolate no. 14,1,4,5,11,16,9,2,3,8,12,13,15,7, and with least for isolate no. 6 at 18 mm diameter.

Clogan plus was giving highest zone of inhibition against isolates no. 10 at 31 mm followed by 5,4,8,14,2,3,11,12,7,9,13,15,16 and with least for isolate no. 6 at 19 mm diameter. Isolate no. 1 were resistant to mouthwash Clogan plus. S-OFF was giving highest zone of inhibition against isolate no. 10 at 27 mm diameter followed by isolate no. 5,1,14,8,12,13,9,16,2,3,4,6,7,15, and with least for isolate no. 11 at 13 mm diameter. HiOra was giving highest zone of inhibition against isolate no. 14 at 23 mm diameter followed by isolate no. 12,8,16,5,10,1,3,6,9,2,11,15 and with least for isolate no. 4 at 11 mm diametr. Isolate no. 7 and were resistant to mouthwash HiOra.

All mouthwashes examined contain one or more different active agents into their ingredient. Most of them contains chlorhexidine gluconate, sodium fluoride, zinc chloride, potassium nitrate, triclosan but in different concentration . The use of mouthwash containing above compositions is for prevention of plaque in absence of brushing, prevention and treatment of gingivitis, treatment of oral candidiasis, controlling secondary infections for aphthous ulcers , aid in treatments of mouth and throat infections. The use of chlorhaxidine gluconate are known to inhibit the accumulation of dental plaque. The mechanism of action of these diverse agents are not completely known, but one site of activity is at the cell surface by interference with carbohydrate transport into micro-organism . Phenolic compound such as thymol also have the ability to inhibit the accumulation of dental plaque, however, it has been reported as potentially irritant [6]. Mouthwash solutions usually encompass antimicrobial activity that ensures their work in eliminating harmful periodontal bacteria, which aids in preventing future dental carries, gingivitis and periodontitis [7].

D. Result Of Activity Assay Of Essential Oils

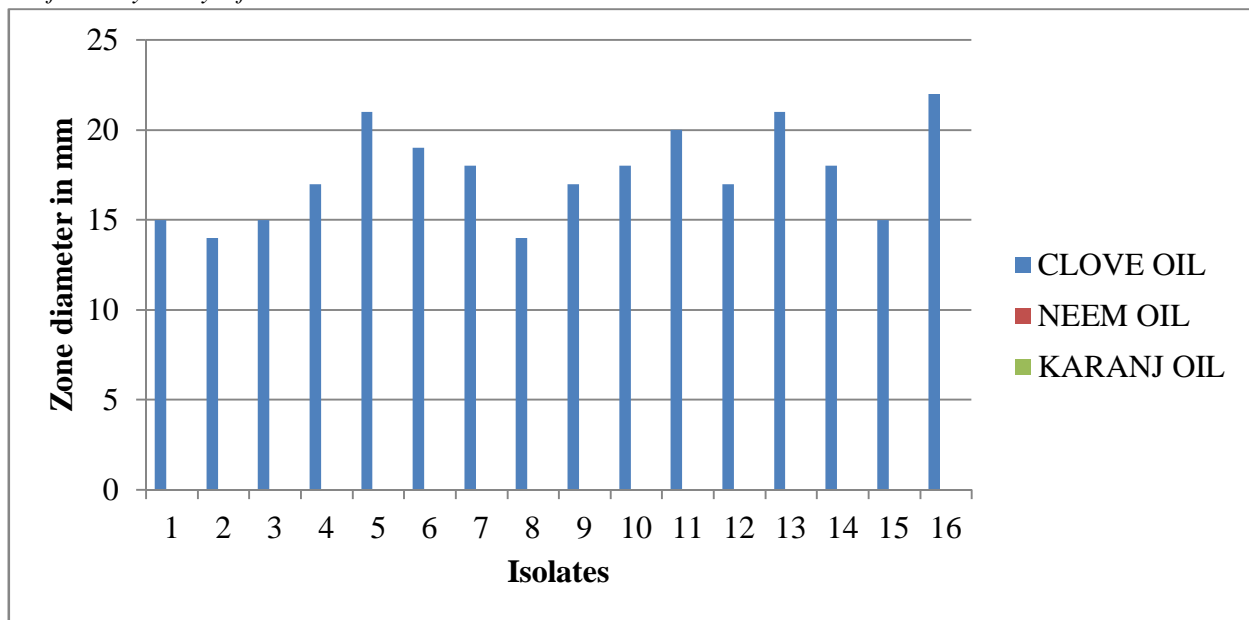


Fig 6. Effect of essential oils against ontained isolates.

In all total 16 isolates were tested against 3 different and popular essentialoil like neem oil, clove oil and karanj oil which are readily available in market. Results suggest that isolate no. 16 was inhibited by the production of zone of inhibition of 22 mm diameter which is largest zone obtain when compare to other isolates with the clove oil followed by isolate no. 5,13,11,6,7,14,4,9,12,1,3,15, while isolate no. 2 and 8 were least affected at 14 mm diameter. All the bacterial isolates was resistance against neem and karanj oil, as no inhibition zone was observed. Clove oil is also widely used in dentistry. The proven properties of antifungal, antioxidant, and antibacterial properties make it a commonly added ingredient in eugenol-based dental products [4].

IV. CONCLUSIONS

Almost all the bacterial isolates found were known to have sufficient potential to cause tooth decay which suggests the need to develop good oral hygiene practices. From toothpastes- close-up, Dantkanti and meshwak, from mouthwash- Orahex plus and clogan plus, from essential oils –clove oil was found to be most effective against dental caries prevention.

V. ACKNOWLEDGMENT

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