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Comparative study of Hydroxyapatite and fluoride containing tooth paste on inhibition of dental carries

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Abstract

Though dental caries is the preventable disease but still grow and one of the most prevalent disease worldwide, particularly among the children. Several technique has been used to prevent or cure the dental caries like addition of the anti-bacterial in the toothpastes. Current study focused to evaluate the toothpaste containing fluoride and hydroxyapatite against the cariogenic bacteria isolated from the infected oral tooth. For this bacteria samples were collected from the dental plaque from the patients at dental clinics. Isolates were identified using biochemical tests and gram staining and antimicrobial activity was assessed through disc diffusion test. Result revealed that 6 species were identified (*S. aureus*, *A. viscosus*, *Actinomyces, Rothia dentocariosa*, *Veillonella* and *Scardovia).* Most dominated species was *S. aureus*followed by *A. viscosus.* When these isolated strains were tested against the toothpaste then it was found that toothpaste containing hydroxyapatite was more active against these targeted bacteria. besides this it was more active against the *S. aureus*on the other hands toothpaste containing fluoride was more active against *Rothia dentocariosa* and *Veillonella.* Overall toothpaste supplemented with hydroxyapatite was more efficient as compared to fluoride supplemented toothpaste.

Keywords: hydroxyapatite, Fluoride, toothpaste, dental caries, cariogenic bacteria

Introduction

Biofilm on the teeth is the result of bacterial pathogen in the oral cavity and must be controlled with the help of regular brush (twice a day). Regular brush can also prevent the dental caries formation and periodontal infections [1, 2]. In regular tooth paste detergents and fluoride are presents, which enhance the efficacy of tooth paste to control biofilm and in turn dental caries [3, 4]. Besides this several antimicrobial are also added which suggest the potential technique to prevent, control or reduce the risk of dental caries or other periodontics diseases [5]. However, ability of such agents in tooth paste has not yet been evaluated or tested effectively. Addition of antimicrobial agents in the tooth paste like herbal extracts, nanoparticles, fluorides and Hydroxyapatite etc. **[5,6].**

Hydroxyapatite (HAp) belong to the family apatites. Its general formula is $Ca_{10}(PO_4)_6(OH)_2$. it is widely distributed in the teeth and bone of the humans. That's why used in numerous medical applications for example coating, prostheses and implants [7,8]. Another important anion is fluoride which is the major chemical in preventing the dental caries. This anion is also added to the water, toothpastes, mouth washes and other oral supplements. The detail mechanism of anti caries property of fluoride ion is still debatable. However, one of the most well-known mechanism of fluoride is it increases the re-mineralization of the tooth which is demineralized by cariogenic bacteria [9, 10]. Current study aimed to evaluate toothpastes containing fluoride and hydroxyapatite against the cariogenic bacteria.

Materials and methods

Sampling and Identification of bacterial strains:

For the collection of the bacteria samples were collected from the patient plaque suffering from the dental caries with the help of sterilized apparatus. The collected sample were then incorporated into the sterilized medium containing trypicasein soy broth (TSB). Samples were transported to the laboratory of UOS and incubated for 24 hours at 37°. Colony morphology was observed under the microscope (color, edge and shape) and further identification was carried out through gram staining and biochemical tests such as indole test, Simon citrate test, maltose, catalase and Mannitol

Disc diffusion Method

Antimicrobial capabilities of the tooth paste containing Fluoride and Hydroxyapatite was determined with the help of disc diffusion test with different concentration (1:2, 1:4, 1:6, 1:8). In this technique the plates of nutrient agar were seeded with the 0.5ml of cultures (24h) of each bacterial strains. Seeded plates were allowed to dry for approximately one hour. A sterilized 8mm cork-borer was used to wells in the plate. After then 0.2mL of each dilution was poured into the wells while sterile distilled water was used as control. Plates were incubated for 24h at 37°C. Zone of inhibition was measured in mm. experiment were performed in triplicate.

Statistical analysis

Findings were Statistical Analyze by using a statistical package, SPSS windows version 15 by applying mean values using analysis of variance (ANOVA) with post-hoc least square differences (LSD) method. Data are shown in mean and standard error

Result

Some of the bacterial strains grown in the culture and their gram straining can be seen in figure 1. Besides this morphological observation result revealed that most of the colonies were red and cream in color, furthermore, 5 species were gram positive while only one species isolated were gram negative that is *Veillonella.* The most dominated bacteria were *S. aureus* followed by the *A. viscosus.* Isolated strains from the dental plaque and carries are listed in the table 1, while table 2shows the bacterial strains on the basis of biochemical tests and frequency of each strain is illustrated in the figure 2.

Table 1: Isolates along with their colony details.

S. no	Identified bacteria	color	Texture	Edged	Gram staining	Culture media	Incubation temperature/time
1	*S. aureus,*	White	smooth	entire edges	+	TSA	34/24
2	*A. viscosus*	Red	Smooth	Neat and regular	+	BAB	25/14
3	*Actinomyces*	red	Smooth	Entire	+	BHI	30/14

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4	*Rothia dentocariosa,*	cream	smooth	scalloped	+	ORSM	37/48
5	*Veillonella*	Grey green	smooth	Entire	-	BHI	37/5
6	*Scardovia*	cream	smooth	entire	+	BHI	37/4

Table 2: Biochemical Identification of Bacteria

S. no	Identified bacteria	Indole	Maltose	Simon citrate	Catalase	Mannitol
1	*S. aureus,*	-	+	-	-	+
2	*A. viscosus*	-	+	-	+	-
3	*Actinomyces naeslundii,*	-	+	-	-	+
4	*Rothia dentocariosa,*	-	+	-	+	-
5	*Veillonella,*	-	-	-	-	-
6	*Scardovia*	+	+	-	-	+

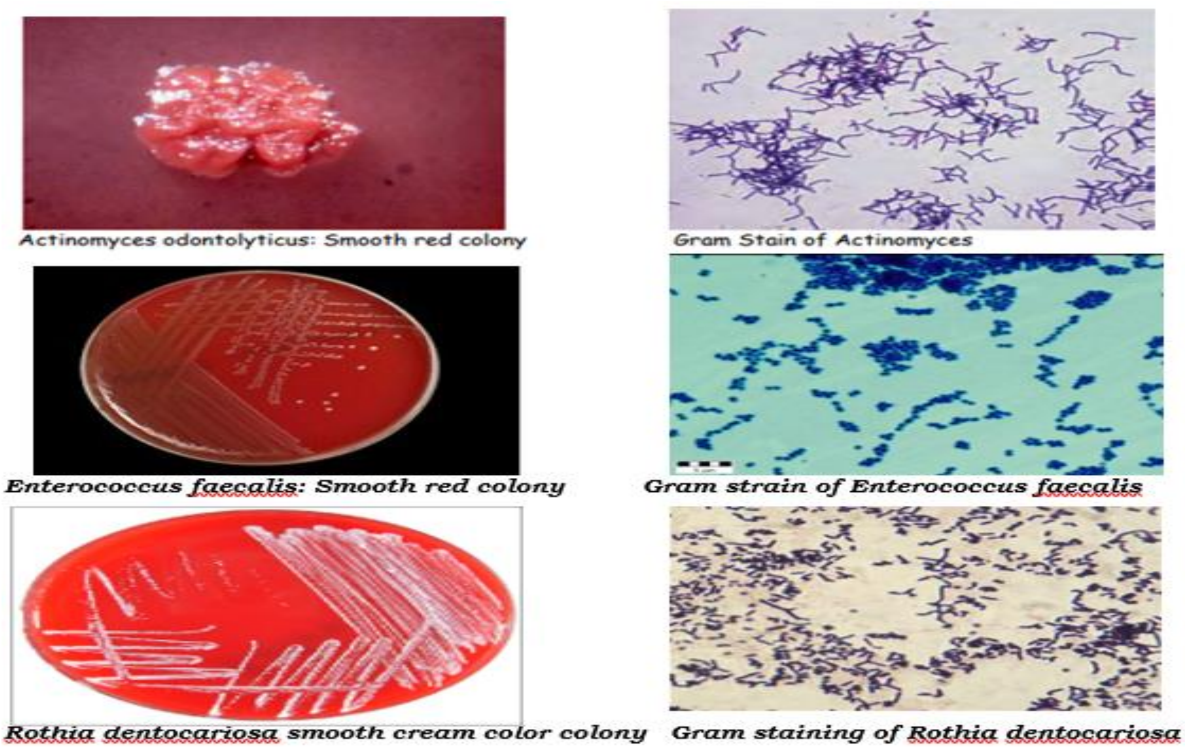

Figure 1: some of the bacterial isolates in their culture and gram staining result**.**

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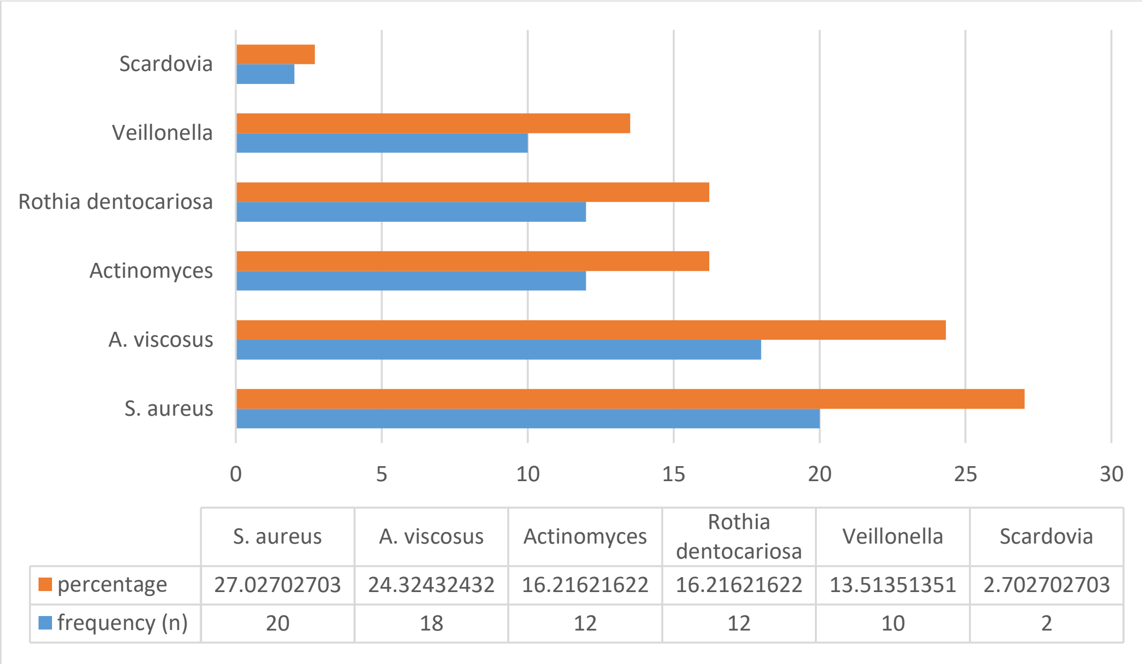

Figure 2: frequency and percentage of the isolated bacterial strains

When the tooth paste containing fluoride and Hydroxyapatite were tested against the isolated strain of the bacteria through disc diffusion test then it was revealed that tooth paste containing Hydroxyapatite inhibit most of the bacterial growth with mean value 29.19 as compared to fluoride containing tooth paste. Furthermore, dilution 1:4 and 1:8 shows excellent inhibition as compared to other dilution. Besides this, fluoride containing toothpaste was more active against *S. aureus* while Hydroxyapatite containing tooth paste was more active against the *S. aureus*, *A. viscosus* and *Actinomyces naeslundii* followed by the *Rothia dentocariosa*. Detail of the result can be seen in the table 3 while figure 3 illustrate the zone of inhibition

Table 3: Zone of inhibition with different dilution of the tooth paste

	Control	**1:2**	**1:4**	**1:6**	**1:8**	**Mean**	**C.Mean**
	Tooth paste containing fluoride						
S. aureus	00	33.2	32.0	20.5	24.6	27.5	20.66
A. viscosus	00	12.3	12.4	10.1	9.3	11.0	
Actinomyces naeslundii,	00	12.3	10.2	20.3	21.3	16.0	
Rothia dentocariosa,	00	30.2	20.3	21.0	31.2	25.6	
Veillonella	00	21.0	20.1	20.3	31.3	23.1	
Scardovia	00	20.3	20.1	21.05	20.5	20.4	
	Tooth paste containing Hydroxyapatite						
S. aureus	00	30.5	31.2	31.3	30.2	30.8	29.19
A. viscosus	00	30.5	32.1	28.0	31.3	30.4	
Actinomyces naeslundii,	00	30.5	31.0	30.2	29.9	30.4	
Rothia dentocariosa,	00	25.5	30.5	30.2	30.2	29.1	
Veillonella	00	23.5	30.4	28.9	26.7	27.3	
Scardovia	00	21.6	30.5	31.2	24.8	27.0	
		24.28	25.06	24.42	25.94	30.8	

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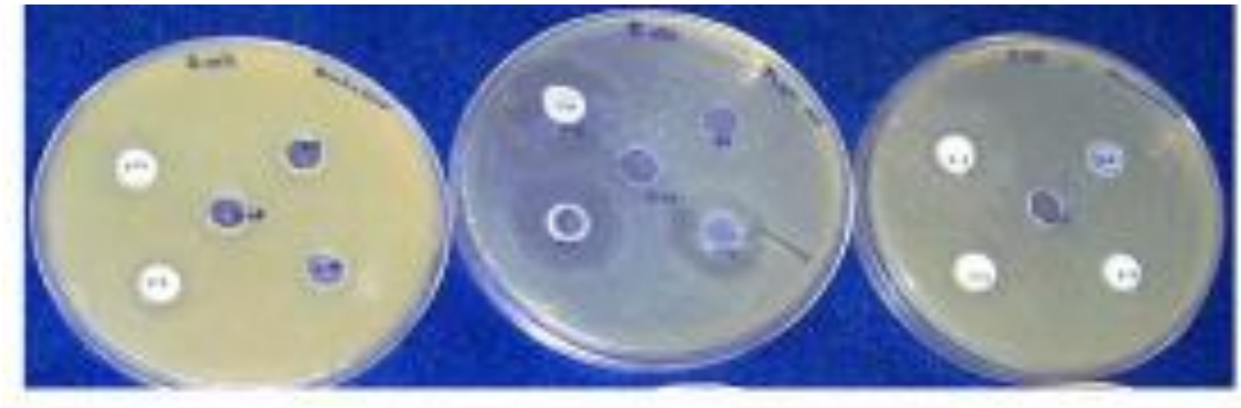

Figure 3: Zone of inhibition

Discussion

Keeping the oral ecosystem free of pathogenic bacteria is the key to prevent periodontal infections. Major etiological factor for dental infections are dental plaque. Formation of the dental plaque can be characterized from the limited number of pioneer bacterial species to the complex mature biofilm. This whole progression process begins with the adherence of the bacterial species to the salivary pellicle and then accumulation of the bacteria by growth. At the last surface of the tooth gets coated with dense and complex community of the microbes which ends up in the hard enamel tissue destruction [11] Current study aimed to assess the oral bacterial fauna of the patients suffering from the dental carries and to evaluate the antimicrobial activity of the fluoride and Hydroxyapatite containing tooth pastes. Result revealed that most of the colonies were red and cream in color, furthermore, 5 species were gram positive while only one species isolated were gram negative that is *Veillonella.* The most dominated bacteria were *S. aureus* followed by the *A. viscosus. Veillonella* has been isolated before from the oral cavity of human reported by several studies [12, 13, 14]. This species mainly occupies buccal mucosa, saliva and tongue. [15, 16] Furthermore, it also inhibits the biofilm present in the sub-gingival, specifically in those who suffered from the periodontal infections. [17, 18] Among species of *Veillonella, V. parvula* is mainly associated with dental caries particularly in the early childhood [19]. End product of these species that is lactic acid can be considered as warning sign for the onset of the periodontal infections. 46 Most of the *Veillonella* species utilize lactate as a source of energy and for growth. Lactate is produced by the growth of the Streptococcus species. Which in turn promote the growth of the Veillonella species. Because lactate produced by the Streptococcus species is utilized by the Veillonella species as source of energy [20].

When the tooth paste containing fluoride and Hydroxyapatite were tested against the isolated strain of the bacteria through disc diffusion test then it was revealed that tooth paste containing Hydroxyapatite inhibit most of the bacterial growth with mean value 29.19 as compared to fluoride containing tooth paste. Furthermore, dilution 1:4 and 1:8 shows excellent inhibition as compared to other dilution. Besides this, fluoride containing toothpaste was more active against *S. aureus* while Hydroxyapatite containing tooth paste was more active against the *S. aureus, A. viscosus* and *Actinomyces naeslundii* followed by the *Rothia dentocariosa.*

Most common method of evaluating the antimicrobial property of any substances is the Disk diffusion method. Though this method is one of the most popular one but still do not reflect 100% antimicrobial property of any substances [21]. Because activity of any tested substances can be greatly influence by the diffusion and solubility of the substances [22]. Several researchers work with fluoride and hydroxyapatite and tested it as antimicrobial agent such as hydroxyapatite was used by Feitosa et al [23], Resmim et al [24], Ragab et al [25]. They all conclude that Hydroxyapatite can be used as antimicrobial agent besides this several researchers suggest that fluoride can also use as antimicrobial agent such as Haraszthy et al [26]; Jafari et al [27], Lou et al [28]. Predoi et al [29] use hydroxyapatite and conclude that there was no antimicrobial activity shown by this chemical against S. aureus. This contrary result was obtained due to change in the environmental condition of the study. Because its activity can be greatly influenced by the surrounding [22].

Different type of methods has been used to determine the antimicrobial activity of the antimicrobial agents and the findings are greatly influenced by different factors like method using, tested microorganism and the solubility degree [30]. In case of hydroxyapatite, this chemical is insoluble particularly, in the medium that is alkaline [31]. For example, the Müeller–Hinton medium and phosphate-buffer saline (PBS), which have Ph of approximately 7.2 (alkaline). This ph profoundly influenced the solubility of the hydroxyapatite, in turn reduce the diffusion rate of this molecule. Besides this amount of the hydroxyapatite also effect the activity, such as thin film of the hydroxyapatite leads to insufficient interaction between ceramic material and organism. While in case of powdered hydroxyapatite has large surface area which also increase the interaction between bacteria, in turn increase the inhibition of the bacteria.

The cell wall of the bacteria S. aureus is made of the layer of peptidoglycan which is a thick layer, composed of amino acids and long chain of carbohydrates, giving the property of hydrophilic [32, 33]. In current study hydroxyapatite was observed more active against this gram positive which may be due to this property (hydrophilic). The hydrophilic nature of this bacteria is due to the presence of the hydroxyl group, which promote the interaction between the cell wall and anti-microbial agents [32]

Conclusion: Odor of the mouth and oral infections is indeed due to microflora, in order to avoid serious consequences one should keep this microflora to level that is not lethal. To keep this flora in specific level serval toothpaste are designed. Several toothpastes are supplemented with antimicrobial agents like fluoride and hydroxyapatite. These agents kill microbes either by disrupting the cell wall or inhibiting the essential enzymes. Current study conclude that hydroxyapatite is far better than fluoride. But this study was conducted in-vitro which means it should be conducted in vivo. Further, molecular level research should be conducted in order to know the basic and exact mechanism of its action.

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