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Cariogenic strains isolated and identified from dental plaque biofilm of caries active and caries free subjects.

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Abstract: **Total 500 sample of caries active and caries free mouth collected from dental unit and analysed for isolation of pathogenic strains. Isolates showed variation in their pattern of percentage recovery according to disease diagnose in each subject. 450 sample considered to be positive for complex communities of colonizing and acid producing bacteria. The sub gingival, supragingival, and different multiple site were selected to collect plaque sample of particular oral disease diagnosed in pateint. Streptococcus mutans bacteria was achieved higher in percentage of total positive cultures from plaque sample as compare to other streptoccoi group bacteria. The pattern of distribution of streptococci varied in different oral disease. Significant difference in pathogenic strain pattern recovered from male, Female and Children subject. Enterococci recovered more in female as compared to male subjects.**

Keywords:-- Dental Plaque Biofilm, Streptococcus mutans, Streptococcus sanguis, Streptoccous mitis, Streptococcus salvaris, enterococcus.

INTRODUCTION

Microbial biofilm are complex communities of bacteria and are common in the human body and in the environment. In recent years, dental plaque has been identified as a biofilm. The biofilm undergoes maturation, and the resulting pathogenic bacteria complex can lead to dental caries, gingivitis, and periodontitis. In addition, dental biofilm, especially sub gingival plaque in patients with periodontitis, has been associated with various systemic disease and disorders, including cardiovascular disease, diabetes mellitus, respiratory disease, and adverse pregnancy outcomes. Oral streptococci has both harmless and harmful bacteria."Mutans streptococci "are the most important bacteria associated with tooth decay. Streptococcus mutans, the microbial species most strongly associated with carious lesions, is naturally present in the human oral micro biota (Baron 1996).Our mouth consisting of microbial biofilm are complex communities of bacteria. Biofilm in the form of supragingival and subgingival plaque is the etiologic agent in dental caries and periodontal diseases. Over the past 50 years, the understanding and characterization of dental plaque have undergone significant evolution (Banaset al. 2007).

Dental caries is a transmissible infectious disease in which streptococcus mutans plays the major role. As in many infectious diseases, colonization by pathogens is required before the disease can occur. Streptococcus mutants are generally considered to be the principal etiological agent of dental caries (Russell 2008). There is a range of virulence factors important for the establishment of Streptococcus mutans in the complex microbial community of dental biofilm. It has been estimated that there are over more than 600 different species prevalent with distinct subsets predominating at different habitats (Dewhristet al. 2010). Streptococcus mutans species of oral streptococci live in the oral cavity. Each species has developed specific specialized properties for colonizing different oral sites and constantly changing conditions to fight competing bacteria and to withstand external challenges. Imbalances in the microbial biota can initiate oral disease. Under special condition, commensal streptococci can switch to opportunistic pathogens, initiating disease and damaging the host.

The ability of bacteria to survive and persist in a given environment will depend, in part, on their inherent genetic plasticity, which determines their ability to respond to fluctuating local environmental condition or stresses. The micro biota resident in the oral biofilm is subjected to many variable environmental stresses, including the availability or lack of nutrients, acidic pH and exposure to organic acids. Biofilm in the form of supragingival and subgingival plaque is the etiologic agent in dental caries and periodontal diseases. Therefore, control of the dental plaque biofilm is a major objective of dental professionals and critical to the maintenance of optimal oral health.

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MATERIALS AND METHODS

Dental plaque sample collection

500 dental plaque sample from caries active and caries free mouth were collected from daman (U.T) And Vapi city of Gujarat state area. A comprised study of 500 sample including 200 male, 200 female and 100 children of both sexes. Adults ranging from 18 to 60 years and children from 5 to 16 years. The nature of work followed in this study were fully explained to all participants and the study was conducted with written inform consent. The subject were screened using an exploratory survey and who volunteer in the study were interviewed using a questionnaire. Qualified subject were assayed whether they had any chronic disease or had not received antibiotic therapy for at least 7 weeks. Further, subjects were diagnosed with particular oral disease by trained dentist.The clinical examination were conducted by trained dentist to assess intra-examines reliability. The sub gingival, supragingival, and different multiple site were selected to collect plaque sample. Dental plaque sample were collected by using sterile tongue depressor to avoid contamination from other mouth parts and to aid a better vision of carious lesions.

Strain isolation and identification

Plaque sampling sited varied depending on the condition and disease diagnose in individual subjects. The plaque sample were collected from sub gingival and supragingival including region of mouth by using sterile disposable swab stick transferring sample to sterile tube container 1 ml sterile phosphate buffer saline. Sample were stored in cool place and then transported to laboratory. One hundred micro-litre of undiluted sample were spared on the surface of MS- agar plate using sterile swab. Culture were incubated an aerobically for 48 hrs at 37.C Count of more than 250 colonies (104 cell/ml) was considered as to positive sample (Friedrich 1981). Isolated strains were identified based on colony morphology, characteristics and biochemical test results.

RESULTS AND DISCUSSION

The sub gingival, supragingival, and different multiple site were selected to collect plaque sample of particular disease diagnose in pateint. Streptococcus mutans bacteria was achieved higher in percentage of total 450 positive cultures from 500 plaque sample as compare to other streptococcoi group bacteria. Isolation of Streptococcus species was done using selective enrichment technique including culturing of sample on MS-agar (Mitis-Salvarius agar), which promotes growth of streptococci and suppress other bacterial species(Carlsson 1967). Accordingly four hundred and fifty samplewere considered to be positive bacteria about (104 cells/ml) (Friedrich 1981).Isolates were first identified depending on their gram-staining, microscopic examination and catalase test. The streptococci are gram positive, individual cocci which are spherical or ovoid and are arranged in chains under light microscope and may be considered as catalase negative bacteria as indicated by identification scheme of Friedrich.

Accordingly, four hundred and fifty isolates were found to be true streptococci (showed positive results) and fifty isolates showed negative results. Isolates were also identified depending on the colonial shape and form on the surface of MS-agar media. Isolates could be varied between, hard coherent, ripple, raised colonies that were identified as Streptococcus mutans which was considerd as one of the most important etiological agent of dental caries (Loesche and W.J 1986), while other colonies showed characteristics of zooglleic form, which were firlmy attached to agar which were considerd as Streptococcus sanguis (Colman and Williams 1972) identification scheme. Other type of colonies produced a minute circular gum drop, pale-blue, large were considered as Streptococcus salivarius, the colonies that were small, flat, hard, blue in color with cupola centre were considered as Streptococcus mitis[hamada]. Colony showing Blue Black, shiny, and slightly raised colonies considerd as Enterococci.

Colonies were stained and assayed microscopicaly for their identification and characteristics. Further, by using KB005A Hi StrepTM Identification Kit biochemical analysis of strains wrere performed and data intrepreted (Table 1,2,3,4).Oral Streptococci differentiated by their ability to ferment certain sugars like mannitol, sorbitol and arginineand to adhere to smooth surface in the presence of sucrose (Hardie and Bowden 1976). Most isolated Streptococcal strains that ferment mannitol and sorbitol in addition to various other sugars, and synthesize adherent water- soluble glucan from sucrose, are considered Streptococcus mutans, they do not usually deaminate arginine to produce ammonia. Here we isolated Streptococcus mutans by considering there characteristics to ferment mannitol,sorbitol and raffinose sugar. Along with sugar, vogesproskauer' test and esculin hydrolysis positive test confirmsStreptococcusmutanswhereas, Streptococcus mitis does not ferment arginine about only 26-40% showsesculine hydrolysis test reactive. It is peroxidogenic, but shows negative test result foresculin, sorbitol, and mannitol fermentation test.

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Table 1: Morphological characteristics of isolates from dental plaque sample

Colony Characteristics	ISOLATED STRAINS				
	A1	A4	A5	A7	A8
Form of colony	Irregular	Circular	Circular	Circular	Circular
Translucency and opacity	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation of colony	Convex	Flat	Raised	convex	Raised
Surface of colony	Smooth	Hard	Smooth	Smooth	Smooth
Pigmentation	Pale Blue	Blue	Blue	Pale Blue	Blue-Black
Cell Shape	Cocas	Cocas	Cocas	Cocas	Cocas
Gram stain reaction	Positive	Positive	Positive	Positive	Positive
Spore stain	NO	NO	NO	NO	NO

Table: 2 Recovery of Strains on MS Agar from dental plaque sample (CFU %)

Clinical condition of Male subject	*S.mutans*	*S.salivarius*	*S.mitis*	*S.sanguis*	*Enterococci*
Caries Activ Mouth	3.2×10^{8}	2.0×10^{8}	1.6×10^{8}	2.0×10^{8}	1.6×10^{8}
Caries Free Mouth	1.7×10^{8}	1.6×10^{7}	1.2×10^{7}	1.0×10^{7}	1.8×10^{8}

Table: 3 Recovery of Strains on MS Agar from dental plaque sample (CFU %)

Clinical condition of Female subject	*S.mutans*	*S.salivarius*	*S.mitis*	*S.sanguis*	*Enterococci*
Caries Active Mouth	3.0×10^{8}	2.2×10^{8}	1.8×10^{8}	2.0×10^{8}	2.4×10^{8}
Caries Free Mouth	1.6×10^{8}	2.2×10^{7}	1.4×10^{7}	1.0×10^{7}	2.0×10^{8}

Table: 4 Recovery of Strains on MS Agar from dental plaque sample (CFU %)

Clinical condition of Children subject	*S.mutans*	*S.salivarius*	*S.mitis*	*S.sanguis*	*Enterococci*
Caries Active mouth	3.0×10^{8}	2.4×10^{8}	1.8×10^{8}	2.0×10^{8}	1.6×10^{8}
Caries Free Mouth	1.8×10^{8}	1.8×10^{7}	1.6×10^{7}	1.4×10^{7}	1.8×10^{8}

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Streptococcus mitis and Streptococcus sanguis shows similar characteristics for esculin hydrolysis and β-galactosidase activity test. From isolated strains, all four important pathogenic dental plaque strains shows sucrose utilization accept Streptococcus mutans. In Streptococcus mutans sucrose utilization is not detected appropriately. Streptococcus mutansand Streptococcus salivarius shows similar characteristics for V.P,Esculine, PYR, ONPG test along with arginine, glucose, lactose sugar utilization but differ only in their characteristics to utilize sorbitol and mannitol sugars, futher in this aspects detail analysis can bedone(Table5).

Table: 5 Biochemical Analysis of isolated strains from dental plaque

TEST	Isolated Strains				
	A 1	A4	A5	A7	A8
VogesProskauer's	+	-	+	-	-
Esculin hydrolysis	+	d	+	D	+
PYR	-	-	-	-	-
ONPG (β-galactosidase)	-	d	-	D	+
Arginine Utilization	-	-	-	+	+
Glucose	+	+	+	+	+
Lactose	+	+	+	+	+
Arabinose	ND	-	-	-	-
Sucrose	ND	+	+	+	+
Sorbitol	+	-	-	-	+
Mannitol	+	-	-	-	+
Raffinose	+	d	+	V	-

Note: Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.

0-10% = -
11-25%= [-]
26-75%= d
V = Variable reaction
76-89%= [+]
90-100%= +
ND = Not detected

On MS agar medium most streptococci show a characteristic colonial morphology which permits their provisional differentiation. Percentage of recovery of Streptococcus mutans were found more in caries active mouth as compare to caries free mouth. Level of Streptococcus mutans andStreptococccussalvaris were slightly similar as compare to other strains of streptococci. These isolated bacteria have been identified as the etiological agents of many oral mouth infection.

Dental plaque is such a complex microbial community growing as a biofilm on enamel surface . The etiology of both dental caries and various forms of periodontal disease has long been recognised to be bacterial accumulation and plaque composition (Marsh and Martin 1999).In previous studies it has been shown that ther are many species prevalent with distinct subsets predominating at different habitats (Dewhrist etal. 2010). In various studies attempts are made to define the Knowledge concerning streptococci strains from dental plaque sample. In this experiment we were able to isolate the pathogenic strains in oral plaque sample collected from various disease diagnose pattern in individual concern subject. Dental plaque is such a complex microbial community growing as a biofilm on enamel surface . From various disease diagnose pattern the isolated strains were calculated on basis of cfu and identified morphologicaly, microscopicaly, and performing biochemical test it was found that Streptococcus mutans were recovered higher in percentage as compare to other streptococci group bacteria. In previous studies there is a great deal of evidence has been accumulated which implicates Streptococcus mutans as a major etiological agent in the initiation of enamel caries (Jagtap and Karkera 2000). Here in our studies, we have isolated different strains of pathogenic streptococci which varied in difrent disease concern.

Pattern of isolation of streptococci varied in different diseases diagnose. On MS agar medium most streptococci show a characteristic colonial morphology which permits their provisional differentiation. Percentage of recovery of Streptococcus mutans were found more in caries active mouth as compare to caries free mouth. Level of Sterptococcus mutans and Streptococcus salvaris were

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slightly similar as compare to other strains of streptococci. These isolated bacteria have been identified as the etiological agents of many Oral Mouth infection (Table 6).

Table: 6 Bacterial profile of plaque sample from different disease diagnose and caries free mouth

Clinical Diagnose	Bacterial strains				
	S.mutans	*S.salivarius*	*S.mitis*	*S.sanguis*	*Enterococci*
Caries free mouth	+	+	++	±	++
Early onset of periodontitis	++	+	+	+	++
Dental Plaque	+++	++	++	+	+
Gingivitis	+++	+++	++	+	++
Burning mouth syndrome	+	+	+++	++	+
Aphthous stomatitis	+	++	++	++	+++

± **may be present**

+ **usually present at level < 10% of flora**

++ **levels<20%**

+++ **levels>20%**

In various studies attempts are made to define the Knowledge concerning streptococci strains from dental plaque sample. In this experiment we were able to isolate the pathogenic strains in oral plaque sample collected from various disease diagnose pattern in individual concern subject. Dental plaque is such a complex microbial community growing as a biofilm on enamel surface . The etiology of both dental caries and various forms of periodontal disease has long been recognised to be bacterial accumulation and plaque composition (Marsh and Martin 1999). From various disease diagnose pattern the isolated strains were calculated on basis of cfu and identified morphologicaly, microscopicaly, and performing biochemical test it was found that Streptococcus mutans were recovered higherin percentage as compare to other streptococci group bacteria. In previous studies there is a great deal of evidence has been accumulated which implicates Streptococcus mutans as a major etiological agent in the initiation of enamel caries (Jagtap and Karkera 2000). In previous studies it has been shown that there are many species prevalent with distinct subsets predominating at different habitats (Dewhrist etal.2010).

The streptococcus have developed a harmonious relationship with human body over the period of time and reported the single most abundant microflora in human microbiome structure (Cvitkovitch etal. 2003). Although , most of these organisms are commensal, several bacterial species, including those that cannot be grown in vitro, has been associated with either periodontal healthcare disease(Moore WEC etal. 2000;Socransky etal.2005).Various species of the geneus streptococcus , lactobacillus, lactococccus , enterococcus, staphylococcus, cornybacterium, veillonella and bacterioids are the prominent bacteria commonly found in the oral cavity (Rogers 2008;Wang etal 2012).

The nature of bacteria ability to produce acid and tolerate acid enviroment are important that enable them to exihibit high dental caries (Svensater etal.1997).Biochemical properties of the isolates were tested according to Berger's manual of systematic bacteriology (Bleiweis, etal. 1976). The following properties were determined : Catalase test, acid production from carbohydrates. Extensive taxanomic studies revealed that these organisms formed a fairly homogeneous group of non motile, catalase negative, gram positive streptococcal species (Carlsson , 1968). Oral streptococci differential bu their ability to ferment certain sugars (especialy mannitol and sorbitol) and to adhere to smooth surface in the presence of sucrose (Hardie and Bowden, 1976). Here in this experiment along with varius acid production property by using various sugar streptococci characterised by B-galactosidase acidity which found positive by Streptococcus fecalis in higher percetage as compare to streptococcus mitis. Further strains were characterised by Esculin hydrolysis, PYR enzyme activity and acetoin production by using KB005A HiStrepTM Identification Kit.

CONCLUSION:

In the present study, we were able to isolate and identify important cariogenic oral bacterial strains which belonged to the species Streptococcus and Enterococcus. Streptococcus mutans is known to be a major causative bacterium of dental caries in humans and is occasionaly isolated from different oral disease diagnosed in individual subjects.Streptococci

sanguis and enterococci recoverd more in caries free mouth as compare to Streptococci mutans. The percentage of Enterococci were found more in female as compare to male and children subject. Further research will be conducted to identify and study the cariogenicity, virulence, enviromental factors and Antibiotic activity on islolated pathogenic dental plaque strain.

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