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Evaluation of *Streptococcus mutans* in saliva of caries free and caries active individuals in gulbarga city

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ABSTRACT

Background and objectives: The prevalence of dental caries in developed countries declining day by day, at the same time the incidence of dental caries is increasing in the developing countries even progress in hygiene and lifestyle. It is well established phenomenon that among the oral group of mutans streptococci, *Streptococcus mutans* is strongly associated with development of dental caries. Recent development suggested that not only the presence of *S.mutans* in the dental plaque and in saliva counts, but also, the number of cariogenic strains of *S.mutans* has tremendous affects on caries. Our study in Gulbarga city mainly emphasized, on the rate of isolation of *S.mutans* in the non stimulated saliva. METHODS: A total of 102 subjects, with or without dental caries and are with or without habitual to chewing or smoking tobacco were examined. All the isolates obtained, were further processed for Rapid biochemical identification tests kit and antimicrobial susceptibility tests was determined by according to clinical laboratory standards. RESULTS: A total of (50%) of individuals showed the presence of *S.mutans* in saliva samples. The isolation rate of *S.mutans* in the saliva samples from the individuals with caries was high of 57.14% then without caries (34.37%). However the highest incidence of *S.mutans* was observed in the saliva samples collected from the without caries of non-habitual individuals (75%) then with caries of habitual (54.16%). On the contrary in habitual individuals with caries showed highest percentage of (63.63%) then in the habitual without caries. Conclusion: Drastic reduction in saliva secretion and oral microbial flora including *S.mutans* suggests that chewing inhibits the dental plaque formation thus decreases the caries formation.

KEYWORDS

Streptococcus mutans;
Caries;
Habitual.

INTRODUCTION

Dental caries is a gradual decay and disintegration of soft or bony tissue or a tooth. Caries is one of the most common infections diseases of mankind. Dental caries goes on increasing day by day, irrespective of progress in human life style, living standards and per-capita. Rising incidence of dental caries may be due to change in the diet compositions, kind of diet and the changes in the environment of oral cavity, due to habits. The environment of the oral cavity and dental structure and diet influence the initiation of colonization of caries forming bacterial growth. Many

Regular Paper

bacterial species are involved in the dental caries formation. However, for the initiation of caries, oral Viridans streptococci play an important role. The oral Viridians streptococci are divided into 4 groups, based on chemotaxonomic and genotypes. These are Anginosis, Mitis, Mutans and Salvarius. Among these four groups of streptococci, the mutans group are strongly associated with dental caries. The mutans group of streptococci are equipped with high acid producing capacity and capable of producing extra cellular polysaccrides. The first observation to link the mutans streptococci to dental caries was made by Fitzgerald and Keyes[1] by converting the caries resistant hamsters to caries susceptible by feeding the diet rich in sucrose and introducing the streptococci into the hamsters fed with sucrose rich diet. Zinner inoculated the hamsters with human streptococci to observe the same type of caries[2]. Krasse concluded that the mutans streptocococi were capable of inducing caries[3]. The mutans group of oral streptocococi consists of seven species viz: *S.cricetus, S.rattus, S.mutans, S.sobrinus, S.downei, S.mucaccae* and *S.ferus*. Among these *S.mutans* and *S.sobrinus* are most frequently isolated with human dental plaque and closely associated with dental caries[4].

Streptococcus mutans was first described by Clarke[5] in 1924 and showed the association of these with dental caries disease. Even *S.mutans* is generally accepted as one of the principle etiological agents of caries[6], the epidemiological studies revealed that *S.mutans* and *S.sobrinus* is present in the oral cavity. The high colonization rate of *S. mutans* may be due to the presence of special bacterial determinants which help in the colonization[7]. Even though high number of *S.mutans* presence does not help in the formation of dental caries unless the host has fermentative dietary patterns[8]. The relationship between the caries activity in susceptible host and the higher synthesis of some virulence factors by different strains of *S. mutans* has been demonstrated[9]. In the western world, the prevalence of caries disease has declined but 5-20% of the population in the age group of 1.5 to 7 years age group remains at high risk[10-12]. In developing countries, the ratio of dental caries is rising may be due to the change in the food habit and life style. In our work, we have concentrated on the isolation rate (incidence) of oral *S.mutans* in the saliva of individuals with or without dental caries and also in the individuals with or without habits in Gulbarga city.

MATERIALS AND METHODS

Subjects and trial conditions

The study subjects comprised of total 102 subjects, the caries active-caries free individual volunteers attending the outpatient department (OPD) of different dental college and hospitals of Gulbarga city, served as the source of sample for the study, the caries and without caries individuals were selected, in such a way that they belonged to different sex, age and socio-economic background.

Sampling method

Samples of unstimulated saliva were collected from different individuals attending OPDs of Nijlingappa Dental College and Hospital, Gulbarga and Al-Bader Dental College and Hospital, Gulbarga. The saliva (2-3 ml) was collected without stimulant in sterile plastic capped bottles. These collected samples were immediately transported to the laboratory and processed for the screening of *S. mutans* using highly specific culture medium.

Specific medium used for the isolation of *S.mutan*

The specific media used for the screening and isolation of *Streptococcus mutans* is mitis-salivarius bacitracin agar medium (MSB).

Composition of mitis-salivarius bacitracin agar medium

Standard formula	gms/litre
Casein enzymatic	15.00
Hydrolyate peptic	5.00
Digest of animal tissue	1.00
Dextrose	50.00
Sucrose	50.00
Dipotassium phosphate	4.00
Typhan blue	0.075
Crystal violet	0.0008
Agar	15.00

Final pH - 7.0 +_ 0.2

The saliva samples were inoculated on the Mitis-salivarius bacitracin agar media, (MSB) and incubated aerobically at 37^0C for 48 hours. The colony showing distinct cultural characteristics of *S.mutans* was selected and further used for the performance of biochemical tests. Rapid biochemical identification kit (from Hi-Media) was used along with conventional biochemical

Regular Paper

tests like fermentation of mannitol and sorbitol, hydrolysis of arginine, esculin and Voges Proskeur. Based on the cultural, biochemical and morphological characteristics the isolates were identified up to species level.

The rapid biochemical identification kit was purchased from the Hi media Pvt. Ltd., Mumbai and other biochemical test were prepared and performed according to the procedure described in the standard microbiology lab manuals[13].

Antibiotic susceptibility testing

All the *S.mutans* isolates were subjected to antibiotic susceptibility testing against the 11 frequently using antibiotics in this area and elsewhere against the treatment of dental caries. All the 11 antibiotics discs were purchased from the Hi media Pvt. Ltd., Mumbai. The method adopted for the antibiotic susceptibility testing is the Kirby Bauers agar disc diffusion assay using Muller-Hinton agar. The discs were dispensed on the pre-inoculated agar plates with testing *S.mutans* isolates and were incubated anaerobically at 37^0C using anaerobic jar. The zone of inhibition was measured with zone measuring scale provided by Hi-media. The inhibition zone of size was interpreted according to the national committee of clinical laboratory standards criteria[14].

RESULTS

In the present study a total 102, subjects belonging to different genders and age groups with or without dental caries and also with or without habits were included (TABLE 1). Over all incidence of *S.mutans* was 50 % and the isolation rate of *S.mutans* in the samples collected for the individuals with caries was higher of 57.14% (40/70) as compared to from the samples collecting for the individuals without caries (34.37% 11/32). *S.mutans* (50%) isolation rate was higher in the non-habitual individuals with (54.16%; 26/48) or without (68.75%; 11/16) caries. On the contrary in habitual individual's persons with caries showed highest percentage incidence of *S.mutans* (63.63%), when compared none in the habitual and without caries individuals. This clearly indicates that in habitual person's drastic reduction in the saliva secretion and oral microbial flora including *S.mutans* in the saliva. Gender wise isolation rate of *S.mutans* was observed to be more in females (59.45%) than males (44.61%). However in the non habitual with or without caries, isolation rate was almost similar pattern among the both sexes except in without caries non habitual females (77.8%) showed highest *S.mutans* isolation rate in their saliva as compared to their counter part (57.14%) as well as to (53.6%) non-habitual females with caries. On the contrary non habitual males with or without caries harbors almost similar *S.mutans* (55% and 57% respectively) in their saliva samples.

All the 51 isolates of *S.mutans* were subjected to antibiotic susceptibility testing using 11 frequently used antibiotics in dental caries infection due to *S.mutans*. The resistance pattern of *Streptococcus mutans* isolates to 11 antibiotics are shown in TABLE 2. The highest resistance of 96% was observed against Penicillin G and followed by Ciprofloxin (78.4%), Chlroamphenicol (64.7%) and Amoxicillin and Vancomycin (58.82%) and lowest of 31% of *S.mutans* showed resistance against Streptomycin. However, the resistance pattern of *S.mutans* isolated either from with caries or without

TABLE 1: Rapid biochemical identification test

Voges proskaurs test	Esculin hydrolysis	PYR	ONPG β-galoctosidase	Arginase dihydrolase	Glucose	Ribose	Arabinoase	Sucrose	Sorbitol	Mannitol	Raffinose
+	+	-	-	-	+	-	-	+	+	+	+

*Streptococcus mutans positive biochemical test

TABLE 2: *S.Mutans* isolates in saliva (Habitual) and (non – habitual) with and without caries

		Number of samples		Total	Incidence of *S.mutans*		Total
		Male	Female		Male	Female	
With caries	Habitual	22	0	22	14 (63.63%)	0	14(63.63%)
	Non-habitual	20	28	48	11(55.00%)	15(53.57%)	26(54.16%)
	Total	42	28	70	25(59.52%)	15(53.57%)	40(57.34%)
Without caries	Habitual	16	0	16	0	0	0
	Non-habitual	7	9	16	4(57.14%)	7(77.77%)	11(68.75%)
	Total	23	9	32	4(17.39%)	7(77.77%)	11(34.37%)
Total		65	37	102	29(44.61%)	22(59.45%)	51(50.00%)

Regular Paper

TABLE 3: Antibiogram resistance patterns expressed by *S.mutans* strains

Sl. no.	No. of antibiotics	With carries (%)					Without caries (%)					Grand total
		Habitual		Non habitual		Total	Habitual		Non habitual		Total	
		Male	Female	Male	Female		Male	Female	Male	Female		
1.	Amoxicillin (Ax)	6/14 (42.85)	0	8/11 (72.1)	9/15 (60.0)	23/40 (57.5)	-	-	3/4 (75.0)	4/7 (57.4)	7/11 (63.63)	30/51 (58.82%)
2	Ampicillin (Ap)	9/14 (64.28)	0	6/11 (54.54)	7/15 (46.66)	22/40 (55.00)	-	-	2/4 (50.00)	4/7 (57.4)	6/11 (54.54)	28/51 (54.90%)
3	Chloramphenicol (Ck)	9/14 (64.28)	0	6/11 (54.54)	11/15 (73.33)	26/40 (65.00)	-	-	¾ (75.00)	4/7 (57.4)	7/11 (63.63)	33/51 (64.7%)
4	Ciprofloxcin (Cl)	3/14 (21.42)	0	3/11 (27.27)	3/15 (20.00)	9/40 (25.5)	-	-	2/4 (50.00)	1/7 (14.00)	3/11 (27.25)	40/51 (78.43%)
5	Erythromycin (Er)	5/14 (35.7)	0	4/11 (36.36)	7/15 (46.66)	16/40 (25.5)	-	-	¼ (25.00)	3/47 (42.8)	4/11 (36.36)	20/51 (39.21%)
6	Gentamicin (Gm)	6/14 (42.85)	0	4/11 (36.36)	6/15 (40.00)	16/40 (40.00)	-	-	¾ (75.00)	1/7 (14.00)	4/11 (36.36)	20/51 (39.11%)
7	Levofloxcin (Lv)	5/14 (35.7)	0	4/11 (36.36)	6/15 (40.00)	15/40 (37.5)	-	-	2/4 (50.00)	3/7 (42.8)	5/11 (45.45)	20/51 (39.21%)
8	Pencillin - G (P)	14/14 (100.0)	0	11/11 (100.0)	15/15 (100.0)	40/40 (100.0)	-	-	2/4 (50.00)	7/7 (100.0)	9/11 (81.81)	49/51 (96.07%)
9	Streptomycin (St)	3/14 (21.02)	0	3/11 (27.27)	6/15 (40.00)	12/40 (30.00)	-	-	¼ (25.00)	3/7 (42.00)	4/11 (36.36)	16/51 (31.37%)
10	Tetracycline (Te)	5/14 (35.00)	0	5/11 (45.45)	6/15 (40.00)	16/40 (40.00)	-	-	2/4 (50.00)	2/7 (28.00)	4/11 (36.36)	20/51 (39.21%)
11	Vancomycin (Vm)	9/14 (64.4)	0	6/11 (54.54)	8/15 (72.72)	23/40 (57.50)	-	-	¾ (75.00)	4/7 (63.63)	7/11 (63.63)	30/51 (58.82%)

TABLE 4: Multidrug resistance among *S.mutans*

Sl. no.	No. of antibiotics	With carries (%)				Total	Without caries (%)				Total	Grand total
		Habitual		Non habitual			Habitual		Non habitual			
		Male	Female	Male	Female		Male	Female	Male	Female		
1.	Amoxicillin (Ax)	0	-	0	0	-	-	-	0	0	0	0
2	Ampicillin (Ap)	0	-	0	0	-	-	-	1	0	1(9.09%)	1(1.96%)
3	Chloramphenicol (Ck)	0	-	1	0	1(2.5%)	-	-	0	0	0	1(1.96%)
4	Ciprofloxcin (Cl)	3	-	1	6	10(25%)	-	-	0	1	1(9.09%)	11(21.50%)
5	Erythromycin (Er)	3	-	3	2	8(20%)	-	-	1	1	2(18.18%)	10(19.60%)
6	Gentamicin (Gm)	4	-	4	4	12(30%)	-	-	1	3	4(36.36%)	16(31.37%)
7	Levofloxcin (Lv)	3	-	1	1	5(12.5%)	-	-	1	2	3(27.27%)	8(15.68%)
8	Pencillin - G (P)	1	-	1	2	4(10%)	-	-	0	0	0	4(7.8%)
9	Streptomycin (St)	0	-	0	0	-	-	-	0	0	0	0
10	Tetracycline (Te)	0	-	0	0	-	-	-	0	0	0	0
11	Vancomycin (Vm)	0	-	0	0	-	-	-	0	0	0	0

caries individuals was observed to be almost similar. It is interesting to note that isolates from the without caries individuals showed high percentage of resistance against Vancomycin and low to Penicillin-G when compared to the isolates from with caries individuals (63.63% versus 57.50%) and 81% versus 100% respectively). Multidrug resistance pattern of *S.mutans* isolates from with caries and without caries individuals is shown in the TABLE 3. Resistance to minimum of two antibiotics and maximum of 8 antibiotics was observed. Maximum of nearly one third of isolates were resistance to 6 antibiotics followed by 4 (21.50%) antibiotics, 5 (19.60%) and 7 (15.68%) and lowest of 2% isolates were resistance against 3 antibiotics. However 7 to 8 % of isolates were resistance to as good as 8 antibiotics. None of the isolates was either susceptible to all antibiotics or resistance to antibiotics tested in this study.

Gender wise isolation rate of *S.mutans* was observed to be 15% more in females (59.45%) than male (44.61%). However in the non habitual with or without caries, isolation rate was almost similar pattern among the both sexes except in without caries non habitual females (77.8%) showed highest *S.mutans* isolation rate

Regular Paper

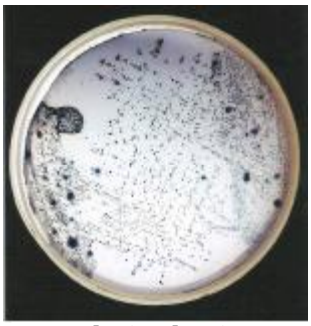

Figure 1: Colonies showing *S.mutans*

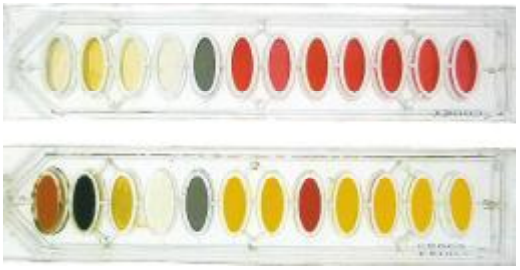

Figure 2: Rapid biochemical identification kit

Figure 3: Antibiotic susceptibility test

in their saliva as compared to their counter part and (57.14%) as well as to non habitual females (53.6%) with caries. On the contrary non habitual males with or without caries harbours almost similar *S.mutans* (55% and 57% respectively) in their saliva samples collected.

The above results revealed that overall fifty percent of the salivary samples from different subjects yielded *S.mutans*. Of these 2/3 subjects were having caries. The saliva samples collected from the habitual; but without caries yielded low *S.mutans*. On the contrary, to the saliva samples collected from the habitual with caries. The rate of *S.mutans* isolates in the saliva collected from the subjects with caries suggested that the frequencies of *S.mutans* in caries subjects are more than that of without caries subjects.

DISCUSSION

In our study, we found that the rate of isolation of *S.mutans* from saliva was found to be 50% out of 102 subjects examined. The rate of *S.mutans* in saliva samples from the individuals with caries was high of 57.14% as compared to without caries (34.37%). A most interesting finding was the abundance of mutans streptococci in a population with negligible dental caries. Observations have earlier been made of mutans streptococci in population of this extreme caries level[15,16]. Another possible explanation of the presence of *S. mutans* in a low caries population may be that the strain occurring at low disease levels should be other than those found at high disease levels[17,18]. Caries is a multifactorial disease, is caused by bacterial deposits on the tooth surface. Both bacteria and diet plays an important role in the caries process. As the present investigation revealed that, in the habitual individuals with caries showed highest percentage of *S.mutans* (incidence) (63.63%) than in the habitual individuals without caries, and also in non-habitual individuals without caries (75%) as compared to with caries of habitual. With this background, it is of interest that the bacteria with the greatest potential to induce dental caries are the species formerly included in *Streptococcus mutans*[19]. As the bacteria appears to be the most common species in humans. A key property of these bacteria, making them cariogenic, is their ability to rapid acid production.

CONCLUSION

Our study emphasizes, that even in without caries subjects, prevalence of Streptococcus mutans has a decisive effect on development of caries. If exposed to a cariogenic diet, are likely to develop a high number of carious lesions. With this background, it is of outmost importance to develop preventive programs for the population. Justifying the present study, it shows that, *Streptococcus mutans* are strongly associated with human dental caries, being the main organism responsible for the initiation of the disease. As being dominating species, to be widespread in populations among with and without caries subjects. Indicating that these bacteria are associated with caries promoting life style.

Regular Paper

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