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Bacterial flora in and around teeth and gums of healthy and infected individuals

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ABSTRACT

The samples of infected and healthy individuals were taken through sterilized swabs. The samples were collected from gums, tongue, soft palate, cheeks and throat. In most of the infectious samples collected during our study, the common diagnosis was of dental caries, dental plaque and dentoalveolar abscess that are caused when normal oral flora increase in number, and, irritation exceeds the host defense threshold. The bacterial flora isolated from healthy individuals were *Streptococcus mutans, S. salivarius, S. mitis, and Staphylococcus epidermidis,* and that from infected individuals were *Streptococcus mutans, S. mitis, S. sanguis, Staphylococcus epidermidis, Haemophilus haemolyticus* and *Neisseria subflava.* In both the cases, *Streptococcus mutans* was found in maximum number.

Key words: Bacterial flora, healthy and infected individuals, teeth and gums.

INTRODUCTION

Oral cavity may be considered as an ideal microbial incubator. More than 500 bacterial taxa are found in the oral cavity, of which approximately 22 predominant ones have been identified. Most members of the normal microbiota are non-pathogenic, some, however, may assume a pathogenic role when resistance of the host is lowered and also due to alterations in diet, medication, smoking, oral hygiene measures, etc. (Arora , 2003). Thus the commensal oral flora plays a significant role in dental infections.

Acquisition of normal oral flora

New born's have sterile mouth. After 8 hours of birth *Streptococcus salivarius* establish itself. Other species of *Streptococci, Staphylococci, Neisseria, Veillonellae* establish by the end of first year. Eruption of teeth provides hard surface. These are colonized by *Streptococcus mutans, S. sanguis* and *Actinomyces viscosus*. With adolescence *Spirochetes* and *Bacteroids* appear in the oral cavity. The two important regulators of oral cavity are - Saliva and Host diet.

Saliva is one of the most important regulators of the oral microbiota. Not does it vary with age and disease state, but the flow rate and composition demonstrate diurnal variations, which in turn affects the numbers and composition of the flora.

Microorganisms require an adequate nutrient supply for their growth and survival. A significant number of oral microorganisms use dietary carbohydrates as a major energy source.

Infectious bacteria of teeth and gums

Of all the bacteria present in oral cavity, *Streptococci* constitute the single largest group. *Viridans Streptococci*, *Streptococcus mutans, S.mitis, S.sanguis, S.milleri, S.salivarius.Enterococci (Enterococcus faecalis) Staphylococcus (S.aureus, S.epidermidis, S. asaccharolyticus) Lactobacilli (L. acidophilus, L. salivarius, L. casei, L. plantarum, L. fermentum, L. cellobiosus, L. brevis and L. buchneri), Diptheroids (Corynebacterium, Propionobacterium), Neisseria (N.sicca, N. subflava), Haemophilus (H.*

aphrophilus, H. parainfluenzae, H. paraprophilus, H. haemolyticus and H. segnis), Actinobacillus actinomyce-temcomitans, Helicobacter pylori.

MATERIAL AND METHODS

1. Day I - Samples are collected and inoculated on suitable agar plates and then incubated at 37°C for 24 hours.
2. Day II - Colony characters were observed. Smear preparation and Gram staining was done. Slides were observed under microscope in 100 X oil immersion. Colony was transferred to nutrient broth and kept overnight.
3. Day III - Second inoculation was done and the whole process was repeated as Day II.
4. Day IV - Biochemical tests were performed on the separated colonies transferred in the nutrient broth on the previous day. The tests performed were catalase, coagulase, phosphatase, sugar test, esculin hydrolysis, inulin, urease, oxidase, H_2S and indole test.
5. The results were obtained on the basis of microscopic observation and biochemical tests that helped in identifying the particular species of the bacteria.

Identification was done as per the method given by Collee *et al*., (1996) and Koneman *et al*.,(1997).

RESULTS AND DISCUSSION

This research work was undertaken to study and compare the bacterial flora in and around the teeth and gums of healthy and infected individuals. Total 100 healthy and 100 infected individuals were studied. A multitude of organisms are found in the oral cavity, but the bacterial flora isolated during this study were *Streptococcus mutans, S. salivarius, S. mitis, S. sanguis, Staphylococcus epidermidis, Haemophilus haemolyticus* and *Neisseria subflava*.

The bacterial flora and their frequency isolated from 100 healthy individuals were *Streptococcus mutans* (40%), *S. salivarius* (20%), *S. sanguis* (15%), *S. mitis* (10%) and *Staphylococcus epidermidis* (25%). All these microbes are reported as normal flora of oral cavity (Bhatia and Ichhpujani, 2003).

The bacterial flora and their frequency isolated from 100 infected individuals were *Streptococcus mutans* (50%), *S. sanguis* (20%), *S. mitis* (15%), *Staphylococcus epidermidis* (5%), *Haemophilus haemolyticus* (5%) and *Neisseria subflava* (5%).

It is to be noted that *Streptococcus mutans* has also been reported as opportunistic bacteria by Loesche *et al*., (1975) Featherstone, (2003) and Saini *et al*., (2003).

S. sanguis is also a reported pathogenic bacteria causing dental plaque by Kraus *et al*., (1953) and Hamada *et al*., (1980). *S. mitis* and *Staphylococcus epidermidis* were also isolated as opportunistic bacteria by Besic, (1943) , Torok and Day, (2005).

Though *Neisseria* make up a small fraction of the total cultivable microflora (Hemmens *et al*., 1946) but it may cause dental plaque. We also isolated it but only in one case.

Haemophilus haemolyticus is reported as pathogenic bacteria causing dentoalveolar abscess (Ljiljana and Brankovic; 2003).

As has been pointed out earlier, there is presence of a variety of microorganisms in the oral cavity of both males and females and despite their presence the subjects may not show any disease symptoms and therefore can be considered free from oral cavity or dental infections. In the present investigation, out of 100 samples collected from such healthy individuals, 16% were found to be inhabited by *Streptococcus mutans and Staphylococcus epidermidis* followed by 15% with *Streptococcus mutans and S. salivarius*. Twelve percent individuals were found to have *S. sanguis* and *S. salivarius*. Similar was the case with *S. sanguis*, *Staphylococcus epidermidis* and *Streptococcus mitis* and *S.mutans*, the combination of which accounted for 12% each. In 10% individuals *S. sanguis* and *S. mitis* were isolated whereas *S. mitis* and *S.mutans* were found to be present in 8% individuals which were all females.

Table 1: Percentage of bacterial flora isolated in teeth and gums of healthy individuals (No. of subjects = 100)

Sex	Age group	Species of bacteria isolated	Percentage
Female	(30-35) yrs	*Streptococcus mutans* *Staphylococcus epidermidis*	04
Female	(23-24) yrs	*Streptococcus salivarius* *S.mutans* *Staphylococcus epidermidis*	11
Female	(25-41) yrs	*Streptococcus sanguis* *S.salivarius*	12
Male	(35-43) yrs	*S.mutans* *S.salivarius*	15
Male	(36-45) yrs	*S.sanguis* *Staphylococcus epidermidis*	12
Female	(25-48) yrs	*Streptococcus mitis* *S.mutans*	12
Female	(23-47) yrs	*S.sanguis* *S.mutans*	10
Male	(29-51) yrs	*S.mutans* *Staphylococcus epidermidis*	16
Female	(23-26) yrs	*S.mitis* *S.mutans*	08

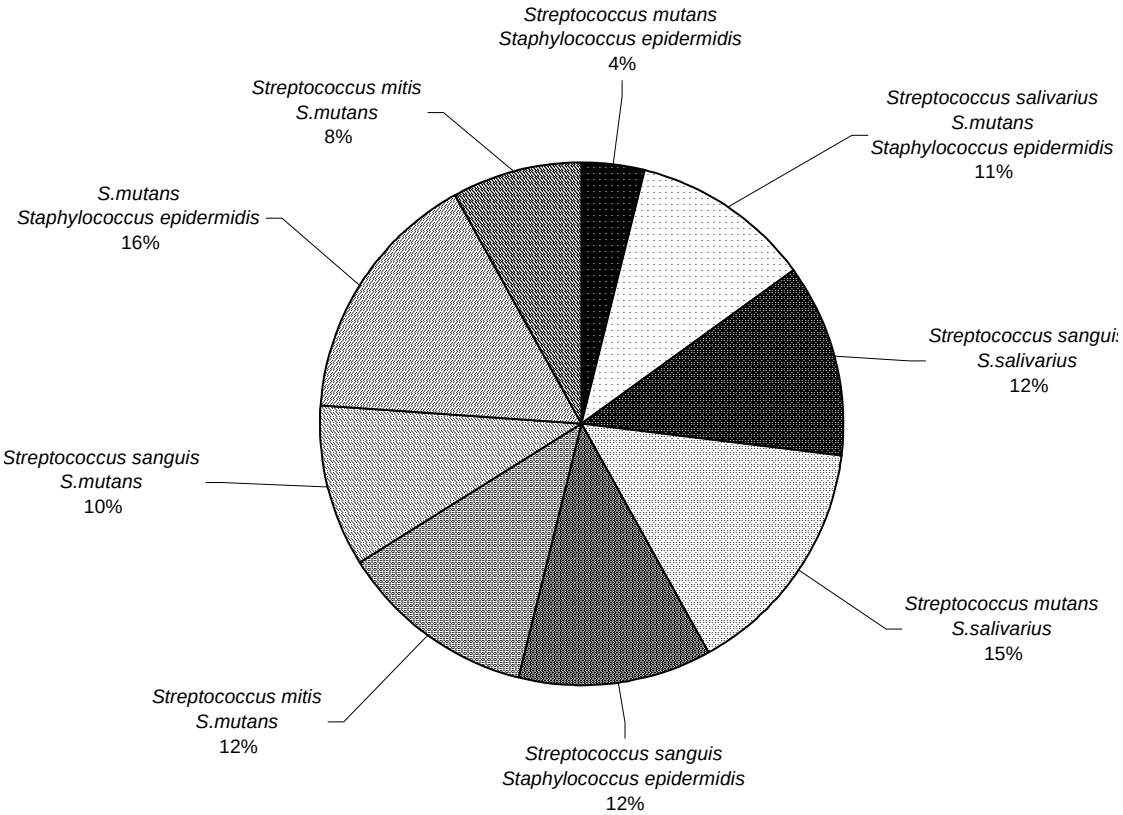

Fig. 1: Percentage of bacterial flora isolated in teeth and gums of healthy individuals

In 11% of cases there was presence of *S. salivarius*, *S.mutans* and *Staphylococcus epidermidis*. Incidentally they were all females in the age group of 23-24 (Table 1, fig.1).

In 50% of dental infection cases the causal organism for dental caries was *Streptococcus mutans*. The disease was equally prevalent among both male and female subjects in the age group 21-50 years (Table 2, Fig.2).

Among the individuals suffering from

Table 2: Percentage of bacterial flora responsible for various infections of teeth and gums of infected individuals

Age Group	Sex	Disease	Causal Organism	Percentage
(21-50) years	Both Males and Females	Dental caries	*Streptococcus mutans*	50
(41-51) yrs	Males only	Dental plaque	*S.sanguis*	20
(22-29) yrs	Female	Dentoalveolar abscess	*Haemophilus haemolyticus*	05
(32-45) yrs	Male and Female	Dental plaque	*Streptococcus mitis*	15
(38-45) yrs	Male	Dental plaque	*Staphylococcus epidermidis*	05
(45-51) yrs	Male	Dental plaque	*Neisseria subflava*	05

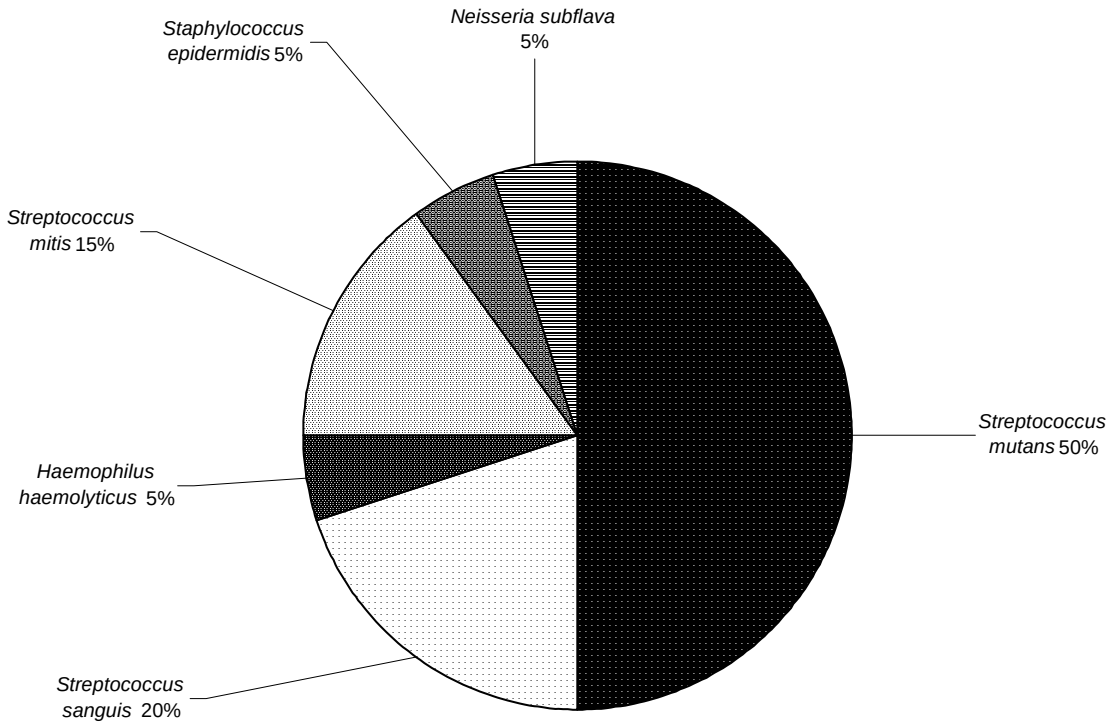

Fig. 2: Percentage of bacterial flora responsible for various infections of teeth and gums of infected individuals

dental plaques, 20% were found to be infected by *S. sanguis*, 15% by *S. mitis* and 5% each by *Staphylococcus epidermidis* and *Neisseria subflava*. Incidentally *Streptococcus sanguis* was isolated from males only in the age 41-51 years. Similar was the case with *Staphylococcus epidermidis* and *Neisseria subflava* which were also isolated from males only in the age group 38-45 years and 45-51 years, respectively. Five percent of the females in the age group 22-29 years were found to be infected by *Haemophilus haemolyticus*, responsible for dentoalveolar abscess.

When isolated from the mouth, throat or elsewhere the non-hemolytic *Streptococci* are generally regarded as harmless commensals. However when found in pure culture in blood, cerebrospinal fluid, an abscess or other closed lesion, they are likely to have a pathogenic role, their antibiotic sensitivities should then be tested and reported (Ross, 1996). Biochemical reactions of the principle non-hemolytic *Streptococci* were performed as per the method of Parker and Ball (1976). *S.mutans* was found to produce acid from mannitol, raffinose and sorbitol whereas *S. salivarius* sometimes in some cases tested positive and sometime negative for these biochemical tests. Probably because of different nature of strains isolated from persons of different sex and age group. So was the case with *S. sanguis* for raffinose and it tested negative for both mannitol and sorbitol. Ammonia was found to be produced from arginine by *S. sanguis* and both *S.mutans* and *S. salivarius* tested negative for this test. Some strains of *Staphylococcus epidermidis* produce slime, a complex glycoprotein, important ability of these strains to colonize foreign bodies such as vascular catheters or indwelling prosthesis. Slime producing colonies become encased in an extracellular glycocalyx, rendering them insusceptible to antibiotics (Bayston and Rodgers, 1990.). In the present investigation all the samples of *S.epidermidis* were found to form slimy substances.

The present investigation supports the view that oral cavity harbours a variety of microbial species especially the bacterial ones. When they behave opportunistically a person suffers from a host of dental disorders such as dental caries, dental plaques, dentoalveolar abscess, etc. The most common among these infectious organisms is *Streptococcus mutans* which is responsible for majority of dental caries cases. Dental plaques are usually caused by *S.sanguis* and dentoalveolar abscess is mostly caused by *Haemophilus haemolyticus*, a disease which is not frequently reported. The other causal organisms apart from *Streptococcus sanguis* for dental plaque are *S.mitis* and *Neisseria subflava* as well as *Staphylococcus epidermidis*. In the present study *Streptococcus mitis* stands second to *S.sanguis* in causing dental plaques. In most of the cases the above species of bacteria are either present as normal oral microbiota or in the form of transient microbiota. When this transient microbiota becomes hostile the person harbouring them may suffer from various dental disorders depending on the specific microbial species involved. Apart from the species listed above other bacterial genera such as *Lactobacillus*, *Actinomyces*, *Bacteroidis*, *Fusobacterium* and *Corynebacterium* as well as *Candida*, a fungus, are also encountered. These occur in the oral cavity because of very congenial atmosphere such as abundant moisture, warmth and constant presence of food that makes the mouth an ideal environment that supports very large and diverse microbial populations on the tongue, cheeks, teeth and gums.

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