Original Article

Diversity of Oral Microflora in children of age group 6-12years in Pedodontics Department of RCDSR

Reena Kulshrestha[1], Srinivas TS[2], Jayant Biswas[3]

[1]Assistant professor, Department of Microbiology, [2]Reader, Department of Periodontics, Rungta Dental College, Kohka-Kurud, Bhilai, C.G [3]President, National Cave Research and Protection Organization, India

Abstract

A comparative study of oral microflora in children of age group 6-12 years in Pedodontics department of RCDSR was carried. It resulted in microflora common in all types, gram positive facultative anaerobic rods and cocci. In normal children gram positive facultative anaerobic and fermenting cocci were predominant where as in children with caries growth of microbiota that were gram negative and positive, capnophilic, motile and anaerobic rods and cocci belonging to members of genera *Streptococcus* and *Actinomyces was seen*. But in patients with caries above age of 9 yrs there was subsequent increase in gram negative, obligate anaerobic, proteolytic, motile bacterial species. Numerous oral changes were seen in caries patients including alterations in the flora of oral cavity, greater predominance of *Hemolytic Streptococci*, *Lactobacillus* and *Staphylococcus*. Total bacterial loads were more in caries patients aged above 9-12 yrs than the microflora in normal children. Caries increases the risk and severity of periodontal diseases. Proportion of different periodontal pathogens was more in patients with caries.

Key Words: Oral, microflora, caries, periodontitis, micro-organisms

Author for correspondence: Dr. Reena Kulshrestha Email ID: reena_2k5@rediffmail.com

Introduction: The mouth is one of the key interface between the body and the external environment and can act as a site of entry for some microbial pathogens especially from the air or via ingestion from the diet, therefore it is equipped with a comprehensive array of defence strategic that includes elements of both the innate and adaptive immune system. The ability of the host is to recognize and respond to invading pathogens while simultaneously tolerating a diverse resident microflora. [1,2,3]

The human body is made up of over 10 cells which around 10% are mammalian. The remainder are the micro-organisms that comprise the resident microflora of the host. This resident micro-organism does not have merely a passive relationship with its host but contributes directly and indirectly to the normal development of the Physiology, Nutrition & Defence system of the organism. [4,5]

The mouth is the gateway of the body to the external world and represents one of the most biologically complex and significant sites in the body. [1,6] Recent studies have re-affirmed an earlier concept that oral health is inextricably linked to general health, and vice-versa maintaining a healthy mouth therefore is of vital importance for a person's self- esteem and general well-being. The oral cavity is the most complex and the most accessible microbial ecosystem of the human body. The teeth gingiva (gums), tongue, throat and buccal mucosa (cheeks) all provide different surface for microbial colonization. [5,6] The intermittent provision of sugar and Amino acid from

ingested food provide nutrients for microbial growth [3,7,19].

The human oral cavity is home to about 700 identified species of bacteria. It is home to fungus mainly of genera *Candida*, several species of protozoa which graze on bacteria for food and various intercellular viruses. [8,9]

Bacteria that first colonize salivary pellicle present on the tooth surface are mainly *Streptococcus (Str. oralis, Str. mitis, Str. sanguis, Str. parasanguinis, and Str. gordinii).* In addition , *Actinomyces, Veillonella, Gemelia, Abiotrophia* and *Granuliscanella* are usually detected.[9-13] WD Miller, an American Microbiologist was able to observe oral bacteria within tissues in particular *Streptococcus* penetrating the tubules of dentin. The acquisition of the oral microflora continues to change with age following tooth eruption, the isolation frequency of micro-organisms especially black-pigmented anaerobes increases.[12,13]

Dental caries is the single most common chronic childhood disease. [13,18,21] Both caries and Periodontal disease are for the most part acquired and preventable disturbances of the teeth and jaws. [15,16,18] Studies by Orland and by Fitzgerald, Jordan and Achard demonstrated that Dental caries will not occur in the absence of micro-organisms. Animals maintained in a germ free environment did not developed caries even when fed a high carbohydrate diet. [19,20]

A number of micro-organisms can produce enough acid to decalcify tooth structure, particularly aciduric *Streptococci, Lactobaccilii, Diptheroids, Yeast, Staphylococcus* & certain strains of *sarcinae.* Loesche concluded in his work that evidence suggests that *Str. mutans, Str. sorbius & lactobaccillliare human odontopathogens.* [23]

Wan et al reported *Str. mutans* colonization in infants as young as 3months - 2years of age. [13,14,,24,25,26] Investigations by Davey and Rogers and by Berkiwitz and Jones have confirmed that *Str. mutans* is transmitted orally from mother to infants. [27,28] Brown, Junner and liew have demonstrated a relationship between the numbers of *Str.mutans.* present in the mother and infants. Their findings also showed that children who carried *Str.mutans* at 3years of age had caries. [13,14,29]

Material and Methods**:** The study was carried out following the proper guidelines of the ethical committee of the Institute. Total 50 patients of age group 6-12 yrs were analyzed for their oral microflora.

COLLECTION OF SAMPLE:

The samples were taken by swabbing the oral cavity by rotating the sterile swab and where it had limitations, dental probes and scalers were used. Each sample was collected in 5ml of Thioglycollate broth media, vortexed with small, sterile glass bead. [8,11,12,31,33,34]

CULTIVATION:

The samples that were collected were incubated at 37^{o}C for 48 hrs. Once dispersed samples were taken and Gram staining was done, also they were spread on to a number of freshly prepared agar plates and incubated to allow cells to form microbial colony.

MEDIA USED

1. Nutrient agar (Basal Media) – was used as basal media for all aerobes, also to check pigment production
2. Blood Agar (Enriched Media) - to check hemolytic property of the bacteria like Streptococcus, Staphylococcus, Enteroccus, Pseudomonas, Actinomycetes, Fusobacteria, Eubacterium and Porphyromonas
3. Vancomycin + Blood agar- For Strict anaerobes – Bacteroides, Fusobacterium, Porphyromonas

4. Robertson Cooked Meat Media (Anaerobic media)-to check proteolytic or saccharolytic activity
5. Sabourauds Medi a- for candida albicans
6. Tryticase Soy Agar- For anaerobes – Bacteroides, Fusobacterium, Porphyromonas, Enterococcus, Eubacterium, Pseudomonas
7. Anaerobic Agar Media – basal media for all anaerobes
8. Thioglycollate – for sample collection & transportation
9. MacConkey Agar – for Streptococcus, Staphylococcus, Enterobacters, Pseudomonas

The above agar plates were inoculated by streak method and aerobes were subjected for incubation at 37^{o}C for 24 hrs, the anaerobes were kept in the McIntosh Jar and incubated at 37^{o}C for 48 hrs. After incubation period of 24-48 hrs the colonies were identified by colony morphology, Gram Staining & Biochemical reactions. [12,30,31,32,33,34] The various microflora were identified by the hemolytic zones & pigmentation on blood agar, pigment production on Nutrient Agar and Biochemical reactions such as IMViC Test, Fermentation test and other specific test (oxidase test, gelatin liquefaction, catalase test) were performed for identification of microbes are presented in Table No.1

For the organisms where fermentation and IMViC test was limitations, other specific tests such as Bile test, Esculin test, also germ tube test for identification of candida was performed.

ENUMERATION & IDENTIFICATION:

The samples were streaked with inoculating loop to produce isolated colonies. The colonies were counted and their concentration in the original sample was expressed as colony forming unit (CFU) by using colony counter (Hi-Media- LA660). Representative was sub-cultured to check for purity and for subsequent identification. This method also involves identifying 30-50 random colonies. [8,11,12,,31,32,34]

Result & Discussion: Fewer coccal cells, more motile rods were found in diseased sites, but there was rise in Gram Negative Bacteria in children with caries. [6] Cultivation of micro-organism from sites of caries reveals high percentage of anaerobic bacteria and Gram negative bacterial species. [8, 9] Numerous oral changes were seen in caries patients such as predominance of *Candida albicans, Hemolytic Streptococci*, *Staphylococci*, *Porphyromonas gingivalis*, *Actinobacillus actinomycetecomitans*. The number of bacteria determined by microscopic counts was twice as high in caries patients as in healthy sites. Microflora common in all types were gram positive facultative rods and cocci. Early studies with appropriate microscopy clearly demonstrated that the number & proportion of different subgingival bacterial groups varied in periodontal health compared with the disease state. [15,16]

The intermittent provison of sugar and Aminoacid from ingested food provide nutrients for microbial growth.[3,19] The presence of nutrients, epithelial debris, and secretions makes the mouth a favourable habitat for a great variety of bacteria. The oral bacteria exert microbial antagonism against non indigenous species by production of inhibitory substances such as fatty acids, peroxide and bacteriocins. Oral health is inextricably linked to general health, and vice-versa maintaining a healthy mouth therefore is of vital importance for a person's self- esteem and general well-being.

Table 1: Biochemical reactions as shown by various oral micro-biota

Oral Microflora	Hemolytic (blood agar)	Indole	MR	VP	Catalase	Nit redn	Gelatin	Coagulase	Oxi	G	L	S	M i	M ii
Streptococcus	-	-	+	+	-	+	+	-	-	-	+	-	+	+
Staphylococcus	Beta	-	+	+	+	+	+	+			-		-	+
Enterococcus	Cream	*	*	*	-	*	*	*	*	*	*	*	+	*
Enterobacters	-	+	-	+	+	-	-	*	-	+	+	+	+	+
Pseudomonas	+	-	-	-	+	+	+	*	+	-	-	-	+	-
Lactobacillus	-	*	*	*	-	*	*	*	*	*	*	*	*	*
Actinomycetes	-	*	*	*	*	+	*	*	*	+	-	+	+	+
Bacteroides	Grey	+	*	*	+	-	*	*	*	*	*	*	*	*
Fusobacterium	-	+	*	*	-	-	*	*	*	*	*	*	*	*
Eubacterium	Grey	+	*	-	*	-	*	*	*	*	*	*	*	*
Porphyromonas	Black pigment	+	*	*	*	*	*	*	*	-	-	-	-	-
Candida	----	*	*	*	*	*	*	*	*	+	*	+	*	*

MR-Methyl Red, VP- Voges Prausker, Nitr- Nitrate, Oxi- oxidase, G-Glucose, L-Lactose, S-Sucrose, M I – Mannose, M ii – Mannitol, *-- no test.

Table 2: Types of Oral Microflora in different groups of patients

Oral Microflora	6-8 years	9-12 years
Streptococcus*	++	+++
Staphylococcus	++	+++
Enterococcus	+	++
Enterobacteriaceae	+	++
Pseudomonas	+	++
Lactobacillus*	++	+++
Actinomycetes*	++	+++
Bacteroides	+	+++
Fusobacterium	+	++
Eubacterium	+	+++
Candida*	++	+++
Gram Positive Bacilli*	++	+++
Porphyromonas*	+	++

++++ = nearly 100 % ++ = nearly 50% + = common (about 25 %) +/- = rare (less than 5%) * = potential pathogen

Table 3: Gram reaction shown by various microflora

Oral Microflora	Gram Reaction	Morphology	Arrangement
Streptococcus	Gram +ve	Coccus	Small chains
Staphylococcus	Gram +ve	Coccus	Clusters
Enterococcus	Gram +ve	Coccus	Single/ short chain
Enterobacteriaceae	Gram -ve	Bacillus	Single
Pseudomonas	Gram -ve	Bacillus	Single
Lactobacillus	Gram +ve	Bacillus	Single
Actinomycetes	Gram +ve	Bacillus (Straight or curved with rounded ends)	Straight or curved with rounded ends
Bacteroides	Gram -ve	Bacillus	Single

References:

1. Ruby J, Goidner M. Nature of Symbiosis in oral disease. J Dent Res. 2007; 86:8-11.
2. Wilson M, Microbial inhabitants of humans: Their ecology and role in health and disease. Cambridge University press. Cambridge; 2005
3. Niew Amerongen AV, Veermani BC. Saliva- the defender of the oral cavty. Oral Disease. 2002; 8: 12-22
4. Aas IA, Paster RJ, Stoker IN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the Oral cavity. J Clin Microbiol. 2005; 43:5721-5732
5. Lamont RI, Jenkinson HF. Adhesion as an ecological determinant in the oral caity. In Kuramitsn HK, Ellen RP(eds) Oral Bacterial ecology. The molecular basis. Horizon Scientific Press. Wymondham. 2000; 131-168
6. Lucas VS, Gupta R, Ololade O, Gelbier M, Roberts GJ. Dental health indices and caries associated microflora in children with unilateral cleft lip and palate. *Cleft Palate Craniofac J.* 2000;37:447–452
7. Hobdel M, Peterson PE, Clarkson J. Global goals for oral health 2000 Int dent J. 2003; 53: 285-288
8. William DW, Lewis MA, Isolation and identification of *Candida* from the oral Cavity. Oral Disease. 2000; 6:3-11
9. Li I, Redding S, Dongari- Bagtzoglou A, Candida galbrata: an emerging oral opportunistic pathogen. J Dent Res. 2007; 86: 204-215
10. Percival RS.Changes in Oral microflora and host defences with advanced age In : Percival S, Hart A(eds) Microbiology and ageing Cinical manifestations Springer, NewYork. 2009; 131-152
11. Dowsett SA, Kowolik MJ, Oral *Helicobacter pylori* can we stomach it? Crit Rev Oral Biol Med. 2003; 14:226-233
12. Downes I, Munson MA, Spratt DA, Characterisation of Eubacterium like strains isolates from Oral Infections. J Med Microbiol. 2001; 50: 947-951
13. Tanner AC, Milgrom PM, Kent R.,The microbiota of young children from tooth and tongue samples. J Dent Res. 2002; 81:53-57
14. Kanonen E. Development of oral Bacterial flora in young children. *Ann Med.* 2000; 32:107-112
15. Marsh PD. Are dental diseases examples of ecological catastrophes? Micobiol. 2003; 149:279-294
16. Sinnian G, Shimizu T. Sugar C. Periodonpathic bacteria in young healthy subjects of different ethnic backgrounds In Los Angeles. J Periodontal. 2002; 73:283-288.
17. Socransky SS, Haffinjee AD. Peridontal Microbial Ecology. Periodontology 2000; 38: 185-187.
18. Johnsen DC. Dental caries patterns in preschool children. *Dent Clin North Am* 1984; 28:3–20.
19. Orland FJ. Bacteriology of Dental Caries: Formal Discussion. J Dent. 1964; 43: 1045-1047.
20. Fitzgerala RJ, Jordan HV, Archard HL. Dental caries in gnobiotic rats infected with a variety of Lactobacillus *acidophilus.* Arch Oral Biol. 1966; 11: 473-476,
21. Van Winkelhoff AI, Boutaga K, Transmission of Periodontal bacteria and models of infections. J Clin Periodontal. 2005; 32(suppl) 6: 16-27
22. Lingstrom P, Vanhoute J, Kashket S, Food Scatches and Dental Caries. Crit Rev oral Biol Med. 2000; 11:366-380.
23. Loesche WJ. Role of *Str. mutans* in human dental decay. Microbiol Rev. 1986; 50:353-380.
24. Wan AK. Association of *Str. mutans* infection & oral developmental nodules

in pre-dentate infants. J Dent Res. 2001; 80: 1945-48.

25. Wan AK, Seow WK, Purdie DM, Bird PS, Walsh LJ, Tudehope DI. Oral colonization of Streptococcus mutans in six-month-old predentate infants. *JDent Res.* 2001;80:2060–2065.

26. Wan AK, Seow WK, Purdie DM, Bird PS, Walsh LJ, Tudehope DI. A longitudinal study of Streptococcus mutans colonization in infants after tooth eruption. *J Dent Res.* 2003a;82:504–508.

27. Davey AL, Rogers AH. Multiple types of the bacterium *Str. mutans* in the human mouth and their Intrafamily transmission. Arch oral Biol. 1984; 29:453-460.

28. Berkowitz RJ, Jordan HV, White G. The early establishment of Streptococcus mutans in the mouths of infants. *Arch Oral Biol*1975;20:171–174.

29. Brown JP, Turner C, Liew V, A study of *Str. mutans* levels in both infants with bottle caries and their mothers. Aust Dent J. 1985; 30: 96-98.

30. Bridson EY , Brecker A. Design & formulation of microbial culture media. Methods in microbiology. Vol.3A, PP 229-295, Ed Norris & Ribbons. Academic Press; 1970.

31. Meynell GG, Meynell E. Book on Theory & practice in experimental bacteriology. Cambridge University Press; 1965.

32. Skyes G. Book on Constituents of bacteriological culture media. Cambridge University press; 1956.

33. Skinner FA. Shaptom DA, Board RG. Isolation of anaerobes. The society for applied bacteriology. Academic Press. Technical series 5; 1971.

34. Mukherjee, K.L. Medical Laboratory: A Procedure Manual For Routine Diagnostic Tests, New Delhi, 110002 Tata- McGraw- Hill Publishing Company Limited; 2006
.

Acknowledgement:

I am thankful to Rungta College of Dental Sciences and Research, for providing the Laboratory facility. I am also thankful to Dr. Srinivas T.S. for his valuable support.