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Establishment of Oral Bacterial Flora – An Overview

Abstract **: Until the time of birth the human infant is usually "germfree." The newborn then becomes suddenly exposed to millions of microorganisms, only a small portion of which will become a part of the newborn's normal flora. The oral microbiota is intimately related to oral health. It is generally accepted that a shift in microbial composition is an important step in the progression of oral disease. Most of the previous articles have focused on identification of several species of microbes specially bacteria, which may promote oral diseases such as dental caries, periodontitis etc.However the present article depicts an overview of establishment, maturation and progression of commensal oral bacterial flora in human being.**

Keywords: Oral commensals, microflora, bacteria, establishment, maturation

The human body is made up of over 10^{10} cells of which around 10% are mammalian. The remainder are the micro-organisms that comprise the resident microflora of the host. These resident micro-organisms does not have a passive relationship with its host but contributes directly and indirectly to the normal development of the Physiology, Nutrition and Defence system of the organism.[1]

Recent studies have re-affirmed an earlier concept that oral health is inextricably linked to general health, and vice-versa, maintaining a healthy mouth of vital importance for a person's self- esteem and general well-being.[2] The oral cavity is the gateway of the body to the external world and represents one of the most biologically complex and significant sites in the body. It is generally lined by stratified squamous epithelium and is modified in areas according to functions (e.g the tongue). The oral cavity consists of other structures such as teeth and salivary ducts .The gingival tissues form a cuff around each tooth and there is a continuous exudate of crevicular fluid from the gingival crevice. A thin layer of saliva bathes the surface of the oral mucous membrane. The oral cavity being an extension of an external body site, has a natural microflora. The commensal oral flora comprises a diverse array of organisms and includes eubacteria, fungi, mycoplasmas, protozoa and possibly a viral flora which may persist for time to time. Bacteria are by far the predominant group of organisms and there are probably some 350 different cultivable species and a further proportion of unculturable bacterial flora which are currently being identified using molecular technique[3]. Some of these bacteria have been implicated in oral diseases such as dental caries and periodontitis, which are among the most common bacterial infections in humans. The prevalence of dental caries has been reported from 70 % - 80 %[4]. It has been estimated that at least 35% of dentate U.S. adults aged 30 to 90 years have periodontitis[5]. In addition, specific oral bacterial species have been implicated in several systemic diseases, such as bacterial endocarditis[6], aspiration pneumonia[7], osteomyelitis in children[8], preterm low birth weight [6]and cardiovascular disease[9]. In order to understand the aetiology of many oral and dental diseases a thorough knowledge of the oral micro-organisms specially oral bacteria, which comprise the resident oral flora is essential. A better understanding of the acquisition of commensal oral microbiota is essential in developing new frontier for prevention of oral diseases. So the present article is an overview of acquisition of commensal oral bacterial flora in different developmental stages.

Over View : The earliest form of life in our planet earth were micro-organisms. Antonie van Leeuwenhoek first time saw micro-organisms (bacteria) using high resolution single lens microscope, while using material scraped from his own teeth as specimen. The skin or external surface and most of the mucosal surfaces of the human body are colonized by normal human microbiota, often referred as the commensal flora which exist in stable equilibrium with the host. Some of them may become opportunist pathogen if the opportunity presents.[3]

Oral cavity, which is a part of G.I.tract is the habitat of extremely variable, commensal bacterial flora of around 1000 species[10]. The diversity in the oral cavity is due to the various anatomical structures that support different ecological environments. The composition of microflora differs in each ecosystem found in the oral cavity as well as amongst individuals. Factors affecting the host's oral environments are the host's diet, oral hygiene, general health status, presence or absence of teeth, medication and genetic composition[11]. Commensal bacterial flora colonizing the mouth can also vary depending on age.

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Discussion : The GI tract including the oral cavity of the fetus is sterile, except in case of chorioamnionitis[12]. Rapid contamination of external and internal surfaces occurs when the fetus is exposed to the flora of the birth canal during vaginal birth and to the external environment immediately after birth with G.I. tract becoming colonized within 24 hrs. of birth.[13] The rate and extent of neo-natal GI tract colonization depends on various perinatal and neonatal factors, such as gestational age,mode of delivery, place of hospitalization, type & mode of feeding and anti bacterial treatment. However , colonization of G.I. tract after birth could be delayed because of prematurity, cesarean delivery or total par - enteral nutrition.[13,14]

The oral cavity which is a very important part of G.I. tract is a highly selective environment for bacteria and only a few species are able to colonize the mouth of the new born. From the first feeding, microorganisms are transferred from the surrounding environment such as maternal saliva or the skin flora of the mother and nursing staff. By 24 hours after birth the first (pioneer) species have become established. *Streptococci*, particularly *S.salivarius*, which bind to epithelial cells are usually the first to colonize. The early colonizer develop into a pioneer microbial community and begin to modify their environment by producing extracellular products, which enhance conditions for growth of other species. For example, *S.salivarius* produces extracellular polymers from sucrose to which other bacteria, for example *Actinomyces*spp., can attach. This process of microbial succession and increasing diversity will result in eventual formation of a climax community.

The oral cavity of the neonate lacks teeth and only mucosal surfaces are available during the first months of life, organisms with ligands for the tooth are absent**[2,15].** Epithelial binding sites for group A *Streptococci* and their lipoteichoic acid in the oral cavity of newborn infants are absent or minimal at birth. It reaches adult levels between 48 and 72 hours after birth . The oral colonization patterns differ among individual atinfancy; variable bacterial load in saliva and other close contacts and the frequency of this bacterial exposure may partly account for individual differences . Systemic and oral antibiotic could affect the oral flora of infants and adults**[16].**

By one year of age, when teeth have erupted, the predominant species isolated as *Streptococcus spp., Neisseria spp., Veillonella spp., and Staphylococcus spp.* Less frequently isolated species include *Lactobacillus, Actinomyces , Prevotella and Fusobacterium***.** Tooth surface and gingival tissue provide new habitats for colonization, with resultant formation of dental plaque. Other shifts in the microbial floral take place during the lifetime of an individual; for example only 18-40% of 5 year olds have spirochaetes and black pigmenting anaerobes, compared with 90% of 13-16 year old. The flora of adults remains relatively stable but denture wearers have an increased carriage rate of *Candida albicans*. From approximately 70 years of age there is an increased proportion of *Lactobacillus* and *Staphylococcus* species in saliva of non-denture wearers, whilst after 80 years of age the number of yeasts increases.

By using a longitudinal study design and culture techniques, Sarkonen et al examined the age-related occurrence of *Actinomyces* species in saliva from 39 healthy children at 2, 6, 12, 18, and 24 months of age. Altogether 428 *Actinomyces* isolates were available for this study.Identification was based on biochemical tests and gas chromatographic demonstration of metabolic end-products, and when needed, cellular fatty acid profiles were determined. Based on the present results, they suggested that *A. odontolyticus* is the main primary *Actinomyces*species on oral mucosal surfaces in infants up to 2 years of age. *A. naeslundii* was the second most common *Actinomyces sp.* but was not detected before the age of 1 year.[17]

Crielaard et al studied the oral microbial composition of children aged 3 - 18 years in natural transition from their deciduous to a permanent dentition and related the microbial profiles to their oral health status. The microbial composition of saliva was assessed by barcoded pyrosequencing of the V5-V6 hypervariable regions of the 16 S rRNA, as well as by using phylogenetic microarrays**[18].** Pyrosequencing reads (126174 reads, 1045 unique sequences) represented 8 phyla and 113 higher taxa in saliva samples. Four phyla - *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* - predominated in all groups. The deciduous dentition harboured a higher proportion of *Proteobacteria* (*Gammaproteobacteria*, *Moraxellaceae*) than *Bacteroidetes*, while in all other groups *Bacteroidetes* were at least as abundant as *Proteobacteria.Bacteroidetes* (mainly genus *Prevotella*), *Veillonellaceae* family, *Spirochaetes* increased with increasing age, reflecting maturation of the microbiome driven by biological changes with age. A caries-free oral status was significantly associated with the higher signal

of the probes targeting *Porphyromonascatoniae* and *Neisseria flavescens*..The study concluded that the potential role of *P. catoniae* and *N. flavescens* as oral health markers should be assessed in large scale clinical studies.The same study, through a combined approach of pyrosequencing, as well as taxonomic microarray analysis, showed that salivary microbiome of children is already complex by the age of 3, and it matures with increasing age. However, at the age of puberty it still differs from the adult microbiome.Although the analysis of the adult oral microbiome was performed on a different sequencing platform (GS20), and the forward primers differed from the ones used inthis study, this is unlikely to account for the major differences been observed. As in previous observations, the oral microbiome was shown to be relatively stable, despite the significant biological changes that occur during the eruption of teeth.Striking differences were observed, between the deciduous dentition andthe rest of the stages.[18]

TABLE I : Oral bacterial flora changes with age

Time During a Lifetime	Major Components & Changes in Oral Flora
Newborn	Oral cavity sterile. Soon colonised by facultative and aerobic organisms; esp *S. salivarius*
6 months	Flora becomes more complex & includes anaerobic orgs eg. *Veillonella sp.* & *Fusobacteria*
Tooth eruption	Increase in complexity. *S sanguis, S mutans* and *A Viscosus* appear. New habitats include hard surfaces and gingival crevice.
Child to adult	Various anaerobes frequently found inc. Members of the *Bacteroidaceae*. *Spirochaetesisolated* more frequently
Loss of teeth	Disappearance of *S mutan, S sanguis, spirochaetes* and many anaerobes
Dentures	Reappearance of bacteria able to grow on hardsurfaces

Kulshrestha et al. did a comparative study of oral microflora in children with or without cariesat an age group 6-12 years. In normal children gram positive facultative anaerobic and fermenting cocci were predominant where as in children with caries 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 dental caries above the age of 9 years there was subsequent increase in gram negative, obligate anaerobic, proteolytic, motile bacterial species. Numerous oral changes were seen in patients with dental caries including alterations in the flora of oral cavity, greater predominance of *Hemolytic Streptococci***,** *Lactobacillus*and *Staphylococcus*. Total bacterial loads were more in carious 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.[19]

A variety of changes take place from childhood to adulthood which may be of importance to the development of periodontal disease. If there are subgingival restorations or defective restorations, one would expect a particular site to be more likely to develop periodontal destruction. Likewise, puberty (hormonal changes) may increase the susceptibility to gingival inflammatory reactions. An age effect can be of importance as well if a particular individual is chronically infected by periodontopathic bacteria, which increases the probability of multiple challenges from the bacteria with subsequent development of periodontal destruction.[20]

Bacterial culture, particularly anaerobic culture, has been critical in the appreciation of the diversity of the subgingival microbiota. Use of non-selective and selective media and various atmospheric conditions, has aided in growth of multiple bacterial species from the oral cavity. Not all bacteria, however, present in oral samples have been cultured to date. The fact that certain microorganisms cannot be cultured and that culture has recognized limitations including sample transportation, time constraints, labor intensity, and high expense, has led to increasing use of molecular detection and identification methods. DNA based methods are currently being extensively used and renewed possibilities to study bacterial etiology of dental diseases have risen. DNA methods, such as PCR, DNA-DNA hybridization, 16S rDNA cloning and sequencing, and microarray analysis, allow simultaneous identification of numerous previously known bacteria and/or novel phyla and phylotypes .These approaches providing the possibility of identification of novel pathogens associated with dental diseases.

In the human oral cavity, 700 bacterial species and phylotypes have been recently identified by molecular methods (Aaset al., 2005; Aaset al., 2008; Becker et al., 2002; Chhouret al., 2005; Corby et al., 2005; Kazoret al., 2003; Kroeset al., 1999; Pasteret al., 2001; Prezaet al.,

2008). These molecular techniques open intriguing possibilities for identification of novel bacterial phylotypes. It has been estimated that half of the oral bacteria have not yet been cultured (Pasteret al., 2001). Although 50% successful cultivation of bacteria in the oral cavity by far exceeds the 1% cultured on Earth (Hugenholtzet al., 1998), cultivation-based approaches have provided an incomplete picture of the microbial diversity of dental diseases and oral health. The conclusions drawn about bacteria associated with dental diseases have been dependent on the available technology. Molecular approaches provide the possibility of expanding the knowledge of the diverse array of microorganisms present in the oral cavity. In order to be able to identify candidate pathogens and beneficial species a comprehensive approach of the oral microbiota involved in oral health and disease is needed.

Conclusion : Predominantly, most of the earlier studies have focused on the differences in microbial carriage between healthy and diseased oral cavities and have identified several species which may be causative and promote oral diseases such as dental caries, periodontitis and halitosis. Less is known about how co-habitants of a healthy oral cavity interact and how those interactions are mediated and controlled through ages. An understanding of the relation of commensal microbiota to oral health from infancy through adulthood is essential in preventing disease. The combination of both, open-ended and targeted molecular approaches can provide us with information that will increase our understanding of the interplay between the human host and its microbiome. ❐

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