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Normal Oral Microflora- A Review

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Normal flora is a population of microorganisms that inhabit the skin and mucous membranes of healthy normal persons. The oral cavity normally supports a dense microbial population. Healthy clean mouth contains a considerable amount of organic matter, which provides nutrients for bacteria. The oral cavity is comprised of many surfaces, each coated with a plethora of bacteria, the proverbial bacterial biofilm. Some of these bacteria have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans. The mouth is a heterogeneous environment for microbial colonisation. Different habitats namely, shedding (lips, cheek, palate, and tongue) & hard non-shedding (teeth) surfaces exists and because of their biological features, support the growth of a distinctive microbial community.

Key words: Symbiosis, Aerobic Organism, Anaerobe, Strict anaerobe, Facultative anaerobe.

The term "normal microbial flora" denotes the population of microorganisms that inhabit the skin and mucous membranes of healthy normal persons. It is doubtful whether a normal viral flora exists in humans. The human body is made up 10^{14} cells, of which only 10% are mammalian. The oral cavity is an localized ecosystem. Ecosystem can be defined as a complex of organisms in a specified environment and the non-microbial surroundings with which the organisms are associated. The local environment of the oral cavity is rather small, with surface area around 215cm^2 but its ecological characteristics are rather unique & complex.

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Microbiota found in the oral cavity

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 part of the normal flora.[1] The oral cavity is the habitat of around 300 bacterial species (Loesche, 1996). Microflora found in the mouth is extremely variable. The foetus in the womb is normally sterile. Successive transmission of microorganisms takes place by passive transfer from the mother ,through the organisms present in milk, water (and eventually food), and the general environment, although saliva is probably the main vehicle for transmission.[2,3,4,5]

Microorganisms such as lactobacilli and candida may also be acquired transiently from the birth canal. The mouth is highly selective for microorganisms even during the first few days of life.[6]

The first microorganisms to colonize are termed pioneer species, and collectively they make up the pioneer microbial community. In the mouth, the predominant pioneer organisms are streptococci and in particular, *S. salivarius, S. mitis* and *S. oralis*.[7,8]

The oral cavity of the newborn contains only epithelial surfaces for colonization. The pioneer populations consist of mainly aerobic and facultative anaerobic species. The diversity of the pioneer oral community increases during the first few months of life, and several species of Gram-negative anaerobes appear. In a study of edentulous infants with a mean age of 3 months, *Prevotella melaninogenica* was the most frequently isolated anaerobe, being recovered from 76% of infants. Other commonly isolated bacteria were *Fusobacterium nucleatum, Veillonella* spp and non-pigmented *Prevotella* spp. In contrast, *Capnocytophaga* spp., *P. loescheii* and *P. intermedia* were recovered from 4–23% of infants, while *E. corrodens* and *Wolinella succinogenes* were only found in a single mouth[9]. During the eruption of the primary dentition. Gram-negative anaerobic bacteria were isolated more commonly, and a greater diversity of species were recovered from around the gingival margin of the newly erupted teeth Also, *Streptococcus mutans* and *S. sanguis* appear in the mouth following tooth eruption.[11,12] These findings confirm that a change in the environment, such as the eruption of teeth, has a significant ecological impact on the resident microflora. [13,14]

Composition of oral microflora from multiple oral sites

The bacterial diversity in the oral cavity is due to the various anatomical structures that support different ecological environments. Bacteria found in the mouth include Firmicutes, bacilli (Streptococci and Lactobacilli), Actinobacteria (especially Actinomyces), Spirochaetes, Proteobacteria and various other. Streptococci form a major component of the oral flora. *Haemophillus* spp are found in saliva[15] *Candida albicans* adhere to the buccal mucosa.[16] Gram negative anaerobic bacteria populate periodontal samples. This includes *Actinobacilli actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia*. Endodontal samples have been found to contain Gram negative species, *Porphyromonas endodontalis, P. gingivalis*, and many *Prevotella* species. In dental caries the species found are predominantly Gram positive facultative anaerobic bacteria. Among these, *Streptococcus mutans* is the most studied along with other streptococci found in the mouth. Corby, *et al*. (2005) performed a study to identify bacteria associated with dental caries in children. Their study found that there was an overabundance of *Actinomyces* spp, *Strepococccus mutans* and *Lactobacillus* spp in the caries active group and exhibited an inverse relationship to beneficial bacterial species such as *Streptococcus parasanguinis, Abiotrophia defective, Streptococcus mitis, Streptococcus oralis* and *Streptococcus sanguis*.[17]

The characteristic properties of the oral sites depend on the level of substances coming from the host. The subgingival areas have a rich blood supply and as a result they have a higher degree of nutrients and host defence molecules.

Oral flora changes with age

Stages in life	Major components & changes in oral flora
Newborn	Oral cavity sterile. Soon colonised by facultative andaerobic organisms; esp S. salivarius
6 months	Flora becomes more complex & includes anaerobic organisms Eg. Veillonella sp. & Fusobacteria
Tooth eruption	Increase in complexity. S sanguis, S mutans and Aviscosus appear. New habitats include hard surfacesand gingival crevice.
Child to adult	Various anaerobes frequently found inc. Membersof the Bacteroidaceae. Spirochaetes isolated morefrequently
Loss of teeth	Disappearance of S mutan, S sanguis, spirochaetesand many anaerobes
Denture	Reappearance of bacteria able to grow on hardsurfaces

The supragingival tooth surfaces are constantly exposed to saliva, which acts as a major source to molecules for bacteria. The soft tissue composed of mucosal surfaces have elevated renewal rate. This makes for a unique environment for bacteria to strive as the shedding of the outer epithelial cells affects the growth of bacteria and their ability to colonise.

Bacterial composition not only differs in each ecosystem found in the oral cavity, but also amongst individuals. Factors affecting the host's oral environments are the host's diet, oral hygiene, whole body health status, presence or absence of teeth, medication and genetic composition.

The oral cavity is mainly composed of soft tissues. An infant's oral cavity is a moist environment due to the secretions from the salivary glands and the lack of hard tissue. As a result *Streptococcus salivarius* is the principal bacterial species in an infant's oral cavity.[18] Many *Staphylococci* spp. are found in the oral cavity, but *Staphylococcus aureus* is often found in the oral cavity of young children but not adults. [15] *Actinomyces gerencseriae, Bifidobacteria, S. mutans, Veillonella, S. salivarius, S. constellatus, S. parasanguinis* and *Lactobacillus fermentum* are found to be associated with caries in childhood. *Actinobacilli* spp. is notably found in the gingival pockets in juvenile gingivitis. [19] During puberty bacteroides and spirochetes colonize.[15]

Important Oral Microorganism

Gram Positive organisms

1. Bulk of oral bacteria
2. Rods (bacilli), cocci or irregular shape (pleomorphic)
3. Oxygen tolerance varies from aerobes to strict anaerobes
4. Most are fermentative
5. Cell wall has thick peptidoglycan layer

Three important genera

· *Actinomyces*, facultative anaerobe

· *Lactobacillus*, produce lactic acid, facultative anaerobe, role in dentine caries rather than enamel caries

· *Streptococcus* facultative anaerobic cocci, produce lactic acid some implicated in caries.

Streptococci

· Isolated from all sights of the mouth

· Large proportion of resident microflora

· Majority α-haemolytic

Streptococcus mutans

· Associated with caries

· Associated with bacterial endocarditis

Streptococcus salivarius

· Colonise mucosal surfaces especially the tongue

Streptococcus angiosus

· Isolated dental plaque & mucosal surfaces

· Seen in maxillofacial infections, brain, liver etc

Streptococcus mitis

· Opportunistic pathogens e.g. endocarditis

Gram Negative organisms

1. Many Gram-negative bacteria found in the mouth, especially in established/subgingival plaque.
2. Cocci, rods, filamantous rods, spindle shaped or spiral shaped.
3. Range of oxygen tolerance but most important strict or facultative anaerobes.
4. Some fermentative, produce acids which other organisms use acids as an energy source, others

Distribution of Streptococci in the oral cavity

Species	Cheek	Tongue	Saliva	Tooth
S.mutans	-	-	+/-	+
S. mitior	+++	+++	+++	+++
S. salivarius	-	++	++	-

Important Streptococci in the oral cavity and their properties

Important Oral Species	Growth on hard surfaces	Production of		Cariogenic	Endocarditis isolates
		Insoluble Extracellular Polysaccharide	Acid		
S mutans	+	+	+	+++	+
S sanguis	+	+	+	++	++
S mitior	+/-	+/-	+	+/-	+++
S milleri	+	-	+	+	+
S salivarius	-	-	+	-	-

produce enzymes which break down tissue.

5. Cell wall different to Gram positive with a thin peptidoglycan layer, has B-lactamase which breaks down penicillin, also has LPS/endotoxin

- Porphyromonas: *P. gingivalis* major periodontal pathogen
- Prevotella: *P. intermedia* a periodontal pathogen
- Fusobacterium: *F. nucleatum* periodontal pathogen
- Actinobacillus: *A. actinomycetemcomitans* associated with aggressive periodontitis
- Treponema: In acute periodontal conditions i.e ANUG
- Neisseria
- Veillonella

Characterizing Interactions Between Oral Microorganisms

Until recently, planktonic bacteria have not been studied in settings with more than one species at a time. It was previously known that the commensal Streptococcus gordonii assists in minimizing dental plaque due to its production of hydrogen peroxide, which can kill many oral bacteria. More recently experiments with S. gordonii and Actinomyces naeslundii, two bacterial species that are highly represented in early oral biofilms, showed that A. naeslundii allowed S. gordonii to grow in the absence of arginine and removed hydrogen peroxide from coaggregate cultures, decreasing protein oxidation in S. gordonii. Conversely, hydrogen peroxide produced by S. gordonii inhibited growth of A. Naeslundii. [20]These observations illustrate the complexity of bacterial interactions in multispecies communities that occur widely in nature.

Fusobacterium nucleatum can aggregate with a large range of bacterial species and can bind to host tissues and immunoglobulin A, allowing *F. nucleatum* to invade epithelial cells and participate in biofilm formation. [21]

F. nucleatum can also associate with Streptococcus cristatus, and transports noninvasive S. cristatus into oral epithelial cells.[22] Conversely, *S. cristatus* attenuates *F. nucleatum*–induced cytokine expression in oral epithelial cells .[23] The relationship between the two bacterial species can be antagonistic or synergistic, depending perhaps on the composition of the remaining species in the biofilm or other environmental conditions.

Other relationships are purely antagonistic. *Streptococcus mutans*, leading cause of dental caries, uses Quorum Sensing and releases bacteriocin when introduced to other bacteria [24] while *Streptococcus*, *Actinomyces*, and *Lactobacillus* generate an acidic pH, thus inhibiting growth of a variety of bacterial species.[25]

Factors affecting the growth of microorganisms in the healthy oral cavity[6]

The number and types of bacteria present in the mouth at any time are the result of interaction of several factors:

1. Oxidation Reduction Potential (Eh value)
2. pH
3. Diet
4. Diurnal Variation
5. Oral Hygiene
6. Intake of Antibiotics
7. Presence of Dental Caries and Other Periodontal

Distinct microbial habitats within the healthy mouth [6]

Habitat	Predominant microbial groups	Comments
Lips, palate, cheek	Streptococci, Neisseria, Veillonella	Desquamation restricts biomass;Some surfaces have specialised hostcell types
Tongue	Streptococci, Actinomyces,Veillonella, Obligate anaerobes, Simonsiella	Highly papillated surface- acts as a reservoir for obligate anaerobes
Teeth	Streptococci, ActinomycesVeillonella, Eubacterium.Obligate anaerobes, spirochaetes, haemophili	Non-shedding surface enabling large masses of microbes to accumulate (dental plaque biofilm). Teeth have distinct surfaces for microbial colonisation (e.g. Smooth surfaces, pits & fissures etc) will support distinct micro flora due totheir biological properties. Teeth harbour the most diverse oral microbial communities.
Gingival crevice /Pocket	Health (aerobic), disease (anaerobic)	

Diseases
8. Nutrients

Oxidation reduction potential (Eh value)

The Eh value determines the types of bacteria whether aerobic or anaerobic. Before teeth eruption and after teeth loss, there is high Eh value thus allowing the growth of aerobic organisms. With teeth eruption, areas of low Eh values develop supporting the growth of anaerobic bacteria especially in deep layers of dental plaque and in gingival crevice. If the range is +200 to < -200mV, gradients do exist in biofilms such as plaque; lowest value in gingival crevice.

pH

It depends on Diet, saliva and presence of certain bacteria, thus affecting number and types of bacteria present. In general, lactobacilli and yeasts can tolerate acidic PH below 5.5 and are called aciduric. plaque pH falls during dietary sugar metabolism. Sub-gingival plaque pH rises during inflammation.

DIET

Increased carbohydrates in the diet, increases the number of Streptococci.

Diurnal variation

Oral bacterial flora usually increases at night and before meals. Flow of saliva is more in the daytime. The saliva tends to remove the bacteria mechanically and decrease its number. In case of decreased secretion of saliva there is significant increase in total bacterial population.

Oral hygiene

Poor oral hygiene leads to accumulation of food debris and formation of plaque. This causes increase in the number of bacteria, specially anaerobes. Good oral hygiene with removal of pellicle, before it forms plaque not only reduces the number of bacteria but also leads to a predominant aerobic population.

Intake of antibiotics

This causes temporary change in the relative number of species in the oral cavity, as the sensitive bacteria die with an increase in the number of the resistant species. Usually, the original condition is restored after stopping the antibiotic intake.

Presence of dental caries and other periodontal diseases

This is associated with changes in the microbial flora. Restoration of carious teeth and treatment of periodontal disease help to restore the composition of microbial flora.

Nutrients

Endogenous are peptides, proteins and glycoproteins in saliva and gingival crevicular fluid. Exogenous dietary sugars facilitate selection of acidogenic and acid-tolerating species in plaque; plaque pH falls and demineralises enamel.

CONCLUSION

The mouth is comprised of several sub niches that each function according to their environmental conditions and location. The throat and tongue epithelial cells are constantly shedding, creating a complex environment that bacteria must adapt to in order to form, let alone maintain a community. The mucosa covered surfaces are prime targets for bacteria to adhere, so bacteria are found on the throat, tongue, teeth and gums no matter how healthy someone tries to be. The mouth is subjected to environmental changes, and any disturbance to the conditions of the mouth lead to changes in the microflora. When conditions are disrupted, and the commensal bacteria are outnumbered by pathogenic bacteria, diseases occur.

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