The human microbiome and its ecological perspectives

Introduction

Human beings, like other animals, harbour a wide array of microorganisms either on or in their bodies. The normal microbial flora is more or less constant for each species. It can be broadly divided into residents and transients. The former constitute a constant population which cannot be completely removed permanently. The latter group varies from time to time and is not permanent. The residents prevent permanent colonization of the body by other organisms.

The adult human body contains 1014 cells, of which only 10% compose the body proper and 90% are members of the microflora. The predominating types of species in humans differ according to the body niche (oral cavity, skin, vagina, stomach, ileum, colon or urinary tract), as shown in Figure No. 1

Normal flora found in the oral cavity varies from area to area (tooth enamel, tongue, gingival surface, saliva) and also with the state of the health. The oral cavity contains a wide mixture of microbes, which are mainly anaerobic bacteria. Lactobacilli and alpha hemolytic Streptococcus species are frequently isolated on tonsils of healthy children. Gagliardiet al. sampled normal flora in the healthy esophagus and the predominant flora was found to be Streptococcus viridans. Fewer bacteria exist in the stomach (usually below 103/g due to acidic lumen). Helicobacter pylori have not only been found in patients with peptic ulcers and gastric neoplasia but are also found in 60% of healthy hosts. The concentration of microbes increase as we progress down the intestinal tract: from small intestine (104/ml contents) to ileocecal region (106 –107/ml) to colon (1011 –1012/g). The intestinal microflora consists of 1011 organisms/gram of feces with over 500 different species, ranging in concentrations from 102 –1011/ml of luminal contents. Although the variety of organisms is complex, generally there are more anaerobic microbes than aerobes.

Flora in the healthy vagina is typically a mixture of aerobic Lactobacillus species, including L. jensenii, L. acidophilus or L. rhamnosus. Two strains of lactobacilli (L. crispatus and L. jensenii) protect vaginal surfaces by producing H2O2. This inhibits the colonization of pathogenic anaerobes and mycoplasmas associated with bacterial vaginosis. Neisseria

Abstract

The normal microbial flora plays an important role in body economy. The human body, which contains about 1013 cells, routinely harbors about 1014 bacteria. This bacterial population constitutes the normal microbial flora. The normal flora influences the anatomy, physiology, susceptibility to pathogens, and morbidity of the host. Vaginal delivery inevitably results in exposure of the newborn to the normal maternal genital tract microflora. Although the newborn’s oral cavity is usually free microorganisms colonization begins within a few hours after birth with microorganisms from the mother, nurses and sometimes from the environment. The process begins with the colonization of the habitat by pioneer microbial populations. Pioneer microorganisms fill the niche of this new environment and modify the habitat, and as a result, new populations may develop.

This review covers what is meant by ‘normal flora’, how the microecology differs by the niche in the body, type of diet, age and health status. The knowledge of normal flora of the body is essential to an understanding of the interaction of human beings and their pathogen laden environment.

Key Words

‘Normal flora’, Microbial Colonization, oral cavity
gonorrhoaeae other sexually transmitted diseases. Vaginal flora has been shown to change over the menstrual cycle, sexual activity, hygiene habits and use of intravaginal microbicides. The knowledge of normal flora of the body is essential to an understanding of the interaction of human beings and their pathogen laden environment. The normal microbial flora plays an important role in body economy. They can 1) become pathogenic when host defences falter, 2) prevent or interfere with colonization/invasion of the body by pathogens, 3) raise the overall immune status of the host against pathogens having related or shared antigens and 4) cause confusion in diagnosis due their ubiquitous presence in the body and their resemblance to some of the pathogens. The beneficial and harmful effects of commensals are summarized below in Figure No.2.

Development Of Normal Microbial Flora
The process of the development of normal flora starts at birth. It is thought that colonization begins during parturition when the neonate's intestine is seeded with mostly Gram-positive facultative anaerobes from the vaginal microorganisms during delivery. Karvonen et al. documented that the vaginal organisms collected from mothers after delivery was the same as organisms found in the stools of neonates. Neonates born by caesarian section usually acquire their first microbes from the environment of the hospital nursery. Neonates are quickly colonized by facultative anaerobes (E. coli and Streptococcus), reaching concentrations of 10^10/g feces within 1–2 days. Previous studies reported that anaerobic organisms do not become established until the second month of life. This observation is explained by the growth of newly seeded facultative microbes which produce an anaerobic environment that is suitable for anaerobes.

Normal Microbial Flora In Infants
The foetus in the womb is normally sterile. Acquisition of the resident microflora of any surface depends on the successive transmission of microorganisms to the site of potential colonization. In the mouth, this is by passive transfer from the mother, from organisms present in milk, water (and eventually food), and the general environment, although saliva is probably the main vehicle for transmission. The mouth of an infant is not sterile at birth. It contains the same types of organisms in about the same relative numbers as those present in vagina of mother, i.e., mixture of Micrococci, Streptococci, Clostridiumbacilli and Doderlien's bacilli. These organisms diminish in number in first 2-5 days after birth and are replaced by the types of bacteria present in the mouth of the mother and nurse. 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. Also oral Actinomycetes and various anaerobic species establish during the first year of life. Actinomyces odontolyticus is the primary colonizer within the genus Actinomyces. Veillonellae species and the Prevotellamelanogenica group are the first obligate anaerobes. Enuclatum, nonpigmented Prevotella species and Porphyromonas species are common anaerobic members among early bacterial communities of the mouth, even the edentulous one. After the first year of life, the versatility among the oral microflora increases remarkably; the prevalence of early colonizing species further increases and various new species establish in the mouth. This may be caused by the emergence of teeth and in addition, by more matured biofilms. Before the age of 4 years, children already harbour a multiform oral microflora. Within 12 hours after birth, alpha hemolytic Streptococci are found in upper respiratory tract. They become the dominant organisms of the oropharynx and remains so for life. The type of diet largely influences the types of organisms in pre-weaning infants. Several studies have reported that infants who are breast-fed have higher concentrations of bifidobacteria as compared to formula-fed infants. Breast milk contains low protein content and high levels of oligosaccharides and glycoproteins, which are considered to be growth factors for bifidobacteria. Formula-fed infants have a more complex microbiota consisting of Bifidobacterium, Bacteroides, Clostridium and Streptococcus species. However, other researchers have not found a significant difference in breast-fed and formula-fed infants.

Oral Microbial Ecology In Adults
The indigenous microflora plays an important role in health and diseases of humans and animals. It contributes to the

| Text Table No.1: Predominant bacterial species in infants and children. |
|-------------------------|---------------------|---------------------|
| Age period              | Aerobic facultative anaerobic species | Obligatory anaerobic Species |
| 0 – 6 months            | Streptococcus mitis S. salivarius Actinomyces odontolyticus Stomatococcus species Staphylococci Enteric/environmental gram negative rods Neisseria species Haemophilus species | Veillonella species Prevotellamelanogenica group Porphyromonas species Fusobacterium nucleatum Non pigmented Prevotella species |
| 6 – 12 months           | S. oralis S. sanguis Corrodin rods | |
| 1 – 3 years             | Capnocytophaga species A. naeslundii Mutans streptococci | P. nigrescens P. pallens Leptotrichia species Other fusobacteria Selenomonas species Peptostreptococcus species |
development of the immune system and provides resistance to colonization by allochthonous or pathogenic microorganisms. **Ecology** is the science that studies interrelationships between organisms and their living (biotic) and nonliving (abiotic) environment. An ecosystem consists of the microbial community living in a defined habitat and the abiotic surroundings consisting of physical and chemical elements. In its simplest expression, the oral ecosystem is thus composed of the oral microorganisms and their surroundings, the oral cavity. Within an ecosystem, the development of a community usually involves a succession of populations. The process begins with the colonization of the habitat by pioneer microbial populations. Pioneer microorganisms fill the niche of this new environment and modify the habitat, and as a result, new populations may develop. As the process continues, the diversity and the complexity of the microbial community increases. Succession ends when no additional niche is available for new populations. At this stage, a relatively stable assemblage of bacterial populations is achieved. It is called a climax community. The ecology of the mouth is unique. The mouth consists of teeth, saliva and crevicular fluid, not present elsewhere in the body. It contains both distinct mucosal (lips, cheek, tongue, palate) and uniquely non-shedding surfaces (teeth) for microbial colonisation. Even the tooth has several ecological locales. Dental plaque (dental biofilm) is a diverse microbial community with channels and voids. It has several habitats with distinct microflora. The mouth provides a home for such a diversity of species that it has been called the tropical rainforest of the body.

All together 771 bacterial species have been detected in the oral cavity. The composition of this microflora varies at distinct habitats but is relatively consistent over time at each individual site among individuals. The mouth is similar to other environmentally-exposed sites in the body in having a diversity of species that it has been called the tropical rainforest of the body. The human mouth is lined by stratified squamous epithelium. This is modified in areas according to function (e.g. the tongue) and interrupted by 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 mucosa.

The major oral habitats present in the oral cavity are:

- buccal mucosa
- dorsum of tongue
- tooth surfaces (both supragingival and subgingival)
- crevicular epithelium
- prosthodontic and orthodontic appliances, if present.

### Buccal mucosa and dorsum of tongue

Special features and niches of the oral mucosa contribute to the diversity of the flora; for instance, the cheek mucosa is relatively sparsely colonized, whereas the papillary surface of the tongue is highly colonized because of the safe refuge provided by the papillae. The oral mucosa of the gingiva, palate, cheeks, and floor of the mouth are colonized with few microorganisms (0 to 25 CFU/epithelial cell). Streptococci constitute the highest proportion of the microbiota in these sites with a predominance of *S. oralis* and *S. sanguis*. The genera Neisseria, Haemophilus, and Veillonella have also been isolated. On the tongue, a higher bacterial density (100 CFU/epithelial cells) and diversity is found. In all studies, Streptococcus, Veillonella, *S. salivarius* and *S. mitis* and Veillonella species were the predominant members of the microbiota. Other major groups isolated include Peptostreptococcus, gram-positive rods (mainly Actinomyces species), Bacteroides species and other gram-negative rods. Black-pigmented obligate anaerobic rods and spirochetes which are closely associated with periodontal diseases have been recovered in small numbers. The papillary surface of the tongue has a low redox potential (Eh) thus promoting the growth of anaerobic flora. It also serves as a reservoir for some of the gram-negative anaerobes implicated in periodontal disease.

The mucosal surfaces (such as the lips, cheek, palate, and tongue) and teeth support the growth of a distinctive microbial community due their distinct biological features.

### Distinct microbial habitats within the healthy mouth

- **Habitat**
- **Predominant microbial group**
- **Comments**

| Lips, palate, cheek | Streptococci, Neisseria, Veillonella | desquamation restricts biomass; surfaces have distinct cell types; *Candida* act as opportunistic pathogens; Staphylococci may be present. |
| Tongue | Streptococci, Actinomyces, Veillonella, obligate anaerobes - Simonsiella | highly papillated surface - reservoir for anaerobes |
| Teeth | Streptococci Actinomyces, Veillonella, Eubacterium, obligate anaerobes - Spirochaetes, Haemophilus | non-shedding surfaces - promote biofilm formation (dental plaque). Distinct surfaces for colonisation (fissures, approximal, gingival crevice) which support a characteristic flora due to their intrinsic biological properties. Teeth harbour the most diverse oral microbial communities |

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### Table No.2: Predominant bacterial species in infants and children

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[Refer Text Table No.2]
**Teeth**

The surfaces of the teeth are the only non-shedding area of the body that harbours a microbial population. Large masses of bacteria and their products accumulate on tooth surfaces to produce dental plaque, present in both health and disease. Plaque is a classic example of a natural biofilm and is the major agent initiating caries and periodontal disease. A range of habitats are associated with the tooth surface [Refer Figure No. 3]. The nature of the bacterial community varies depending on the tooth concerned and the degree of exposure to the environment. Smooth surfaces are colonized by a smaller number of species than pits and fissures. Differences between the supragingival and subgingival environments are more readily apparent. The supragingival environment is bathed in saliva whereas subgingival in crevicular fluid. The subgingival zone is shaped like blind sack with little fluid flow. The chances of mechanical disruption of organisms are low. The supragingival areas are constantly being washed by saliva and exogenous fluids. They are subjected to mechanical disruption during chewing. These differences are reflected by difference in organism present.

Supragingival plaque typically demonstrates a stratified organization of the bacterial morphotypes. Gram-positive cocci and short rods predominate at the tooth surface. The gram-negative rods and filaments as well as spirochetes predominate in the outer surface of the mature plaque mass. Highly specific cell-to-cell interactions are also evident from the "corncob" structures often observed [Refer Figure No. 4]. Corncob formations have been observed between rod-shaped bacterial cells (e.g., *Bacterionemamatruchotior F. nucleatum*) that form the inner core of the structure and coccal cells (e.g., *Streptococci* or *P. gingivalis*) that attach along the surface of the rod shaped cell.

Morphologic and microbiologic studies of subgingival plaque reveal distinctions between the tooth-associated and tissue-associated regions of subgingival plaque [Refer Figure No. 5]. Host inflammatory cells and mediators are likely to have considerable influence on the establishment and growth of bacteria in this region. The tooth-associated (attached) plaque is characterized by gram-positive rods and cocci, including *Streptococcus mitis, S. sanguis, A. viscosus, Actinomyces naeslundi* and *Eubacterium* spp. The apical border of the plaque mass is separated from the junctional epithelium by a layer of host leukocytes, and the bacteria of this apical tooth-associated region show an increased concentration of gram-negative rods.

![Figure No. 3: Habitats associated with tooth surface](image)

**Crevicular epithelium and gingival crevice**

Although this habitat is only a minor region of the oral environment, bacteria that colonize the crevicular area play a critical role in the initiation and development of gingival and periodontal disease. The portion of plaque adjacent to the tissue surfaces (tissue-associated) is more loosely organized than the very dense tooth-associated region. It contains primarily gram-negative rods and cocci, as well as large numbers of filaments, flagellated rods, and spirochetes. Host tissue cells (e.g., white blood cells and epithelial cells) also may be found in this region. Studies of tissue-associated...
plaque indicate a predominance of species such as *S. oralis*, *S. intermedius*, *P. micros*, *P. gingivalis*, *P. intermedia*, *Bacteroides forsythus*, and *F. nucleatum*. Bacteria found in tissue-associated plaque (e.g., *P. gingivalis*) also have been found in host tissues. Thus the physical proximity of these bacteria to the host tissues in the plaque mass may be important in the process of tissue invasion.

**Prosthodontic and orthodontic appliances**

If present and not kept scrupulously clean, dental appliances may act as inanimate reservoirs of bacteria and yeasts. Yeasts on the fitting surface of full dentures can initiate Candida-associated denture stomatitis due to poor denture hygiene.

**References**