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Review Article

Acquisition and maturation of oral microbiome throughout childhood: An update

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ABSTRACT

Traditional microbiology concepts are being renewed since the development of new microbiological technologies, such as, sequencing and large-scale genome analysis. Since the entry into the new millennium, a lot of new information has emerged regarding the oral microbiome. This revision presents an overview of this renewed knowledge on oral microbial community acquisition in the newborn and on the evolution of this microbiome to adulthood. Throughout childhood, the oral microbial load increases, but the microbial diversity decreases. The initial colonizers are related to the type of delivery, personal relationships, and living environment. These first colonizers seem to condition the subsequent colonization, which will lead to more complex and stable ecosystems in adulthood. These early oral microbial communities, therefore, play a major role in the development of the adult oral microbiota and may represent a source of both pathogenic and protective microorganisms in a very early stage of human life. The implications of this knowledge on the daily clinical practice of odontopediatrics are highlighted.

Key Words: Oral health, Oral microbiome, pediatrics

INTRODUCTION

The concepts of oral microbiology are in revolution since the entry into the new millennium. This profound change comes in the light of new technologies developed for microbiological analysis such as sequencing and large-scale genome analysis. Prior to this new era, it was thought that the number of microorganisms that colonize the oral cavity was around 700 species; today is thought that it may reach 19,000 phylotypes.[1] These recent studies have shown that most oral microorganisms are uncultivable; that the oral microbiome is much more diverse than previously thought; and that oral infections are of a polymicrobial nature.[2-5] The microorganisms residing in the oral cavity, and their inevitable inter-relationships, are essential components in changing the balance between health and disease. Thus, understanding what constitutes microbial communities in health, as opposed to disease, is a crucial goal in studying the microbiology of the human mouth, the portal of entry to both the gastrointestinal and respiratory tracts.[6,7] This revision presents an overview of this renewed knowledge on oral microbial community acquisition in the newborn and on the evolution of this microbiome to adulthood.

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Intrauterine life and microbial colonization

At present, the medical community assumes that, in normal conditions, intrauterine fetal development occurs in an aseptic environment. However, recent studies have reported intrauterine environment colonization, specifically the amniotic fluid, by oral microorganisms, in up to 70% of the pregnant women.[8] The cultivable microorganism most often found was *Fusobacterium nucleatum*, a species associated with periodontal disease.[9] This data further supports the notion that in pregnant women,

periodontal disease represents a risk factor for preterm birth and low birth weight babies.[10] During pregnancy, the bacteria found in the oral cavity may reach the amniotic fluid via transient bacteremia, especially in the presence of oral diseases such as gingivitis or periodontitis. Thus, oral screening and/or oral treatment should integrate the preconception care and oral health maintenance should be a concern throughout pregnancy.

The oral microbiome — from birth to adulthood

During and after birth, the newborn comes in contact with a wide variety of microorganisms. Given their state of immune tolerance,[11] the newborn may be colonized by this initial inoculation. However, only a subgroup of these microorganisms is able to permanently colonize the subject.[12] The set of initial colonizers seems to condition the subsequent colonization, which will lead to more complex and stable ecosystems in adulthood.[13] These early microbial communities, therefore, play a major role in the development of the microbiota of the adult body and may represent a source of both pathogenic and protective microorganisms in a very early stage of human life. In the following sections, the evolution of oral bacteria, Archaea, fungi, parasitic, and viral colonization from birth to adulthood, will be described.

Oral bacterial colonization

A significant number of the first bacteria colonizing the human body are of maternal origin. The type of delivery, eutocic or dystocic, may affect the type of microorganisms that the newborn is first exposed to. Immediately after birth (< five minutes), the bacterial communities present in different habitats of the newborn (oral, nasopharyngeal, skin, and intestines) are very similar to each other.[14] However, babies born by vaginal birth have similar bacterial communities to the mother's vaginal bacterial communities; predominantly *Lactobacillus*, *Prevotella,* and *Sneathia* spp., while babies born by Cesarean section (dystocic) have bacterial communities similar to those present in the mother's skin, predominantly *Staphylococcus*, *Corynebacterium,* and *Propionibacterium* spp.[14]

At birth and in the subsequent hours, the baby's mouth will be exposed to a large number of microorganisms by contact with the outside world through breathing, breastfeeding, and contact with parents and medical staff. In the postpartum period, it begins the process of permanent colonization of the oral cavity. When the newborn has only twenty-four hours of life, the establishment of the so-called pioneer microorganisms in the oral cavity has already begun. At this stage, the most frequent colonizers of the oral cavity are Gram-positive cocci, including *Streptococcus* and *Staphylococcus*.[15,16]

The pioneer microorganisms begin to promote the change of the environment through the production and excretion of products of their metabolism, which often potentiate the growth of other species. For example, *Streptococcus salivarius* is most often found in the oral cavity of the newborn, since it has the ability to adhere to epithelial cells. This species produces extracellular polymers from sucrose to which other bacteria such as *Actinomyces* spp., can adhere.[15] This process of microbial succession and increasing diversity will result in the eventual formation of a complex and more stable microbial community.

As the baby grows, the microbial communities also evolve. Around five months of age, infants already show a distinct oral microbiota from the mother, due to environmental exposure that occurs in the first months of life, particularly through the ingestion of food, contact with other adults and children, contact with domestic animals, hygiene habits, and so on.[17] This microbiota consists mostly of bacteria, including the six phyla: *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Fusobacteria,* and *Spirochaetes*. The most prevalent genera are *Streptococcus*, *Haemophilus*, *Neisseria,* and *Veillonella*.[17] Many of these microorganisms, such as *S. mitis* or *S. oralis*, produce immunoglobulin A (IgA) proteases that specifically degrade the secreted salivary IgA. It is speculated that this feature is an advantage for the survival of these species in an IgA-rich environment, which is secreted from the breast milk.[18] Interestingly, in this phase, although the infants show fewer oral microorganisms than their parents, they have a greater microbial diversity.[17]

With the eruption of the first teeth, a new ecological event takes place in the oral environment, with the emergence of new adhesion surfaces. It was thought that some cariogenic *Streptococcus* species, such as *S. mutans*, only began their colonization at this stage, due to the fact that their preferable adhesion surfaces are the teeth. This phase was named by Caufield *et al.* as the, 'window of infectivity'.[19] However, recent studies have demonstrated the presence of this species in edentulous children, suggesting that soft tissues may play the role of a reservoir for oral pathogenic

microorganisms.[17,20] This highlights the importance of oral hygiene practice in the baby, even before tooth eruption.

At three years of age, the salivary microbiome is already complex, but its maturation process continues until adulthood.[21] The children's oral microbiota varies throughout the development of teeth; deciduous, mixed or permanent dentition. The oral microbiota of children with primary dentition in relation to other groups has a higher prevalence of bacteria belonging to the class *Gammaproteobacteria*, particularly the families of *Pseudomonaceae* (genus *Pseudomonas*), *Moraxellaceae* (genera *Acinetobacter*, *Moraxella,* and *Enhydrobacter*), *Enterobacteriaceae* and *Pasteurellaceae* (genus *Aggregatibacter*).[21] As the dentition evolves from deciduous to permanent, the population of the bacteria belonging to the *Veillonellaceae* family (genus *Veillonella* and *Selenomonas*) and the genus *Prevotella* increases, while the bacteria of the *Carnobacteriaceae* family (genus *Granulicatella*) decreases.[21]

The emergence of teeth in the oral habitat leads to a major worldwide health problem, that is, dental caries. According to the Surgeon General's report on oral health in America, published in May 2000, dental caries is the most common chronic childhood disease.[22] The Global Oral Data Bank of the World Health Organization (WHO) reports that, at 12 years of age, 70 to 85% of the population has or had carious lesions.[23] Recent studies evaluating the oral microbial population in children aged three to twelve years, suggest that the entire population of the tooth-bound bacteria, and not just a small number of specific pathogenic bacteria, influences the development of caries.[3,20,24-27] Aas *et al.*,[24] showed that 10% of the children and young adults (aged between two and twenty-one years) with dental caries did not have detectable levels of *S. mutans*, and also suggested the involvement of other bacterial species in the development and progression of dental caries, such as, *Lactobacillus*, *Veillonella*, *Bifidobacterium*, *Propionibacterium*, acidogenic non-mutans *Streptococci* (*S. gordonii*, *S. oralis*, *S. Mitis,* and *S. anginosus*[28]), *Actinomyces*, and *Atopobium*, thus revealing the polymicrobial nature of this infectious disease. More specifically, in white-spot lesions, the proportion of *S. mutans* found in the plaque associated with the lesion was often higher than in clinically healthy sites, although still quite low, ranging between 0.001 and 10%.[29] The non-mutans, *Streptococci* and *Actinomyces,* represented the major groups of bacteria in the enamel lesions. In fact, it was seen that in the absence of *S. mutans* and *Lactobacillus*, the initial demineralization of the enamel could be induced by the early colonizers alone (*S. sanguinis, S. Mitis,* and *S. oralis*).[3,30,31] In cavitated lesions reaching the dentin, *S. mutans* constituted about 30% of the total microbiota, indicating that these species were associated with advanced stages of decay. However, *S. mutans* were less prevalent in the progress area of dental caries, where species of *Lactobacillus*, *Bifidobacterium*, and *Prevotella* prevailed.[3,24,30,32-35] Studies evaluating the microbiota associated with early childhood caries, in particular, found the genera of *Streptococcus*, *Veillonella*, *Actinomyces*, *Propionibacterium*, *Granulicatella*, *Leptotrichia*, *Thiomonas*, *Bifidobacterium,* and *Atopobium*, suggesting that there was not a single pathogen, but a pathogenic population that correlated with the development of early childhood caries. It is worth reinforcing that it is not the genotype of bacteria *per se*, but the phenotype adopted in a particular environment, that is, the acidogenic and aciduric potential of the microorganism, that may induce an environmental shift leading to dental caries.[24,36-38]

With regard to health, children's oral cavities have a higher proportion of bacteria from the phyla *Firmicutes* (genus *Streptococcus*, *Veillonella*, *Lactobacillus,* and *Granulicatella*) and *Actinobacteria* (*Rothia* and *Actinomyces* genera), and a smaller proportion of bacteria from the phyla *Bacteroidetes* (genus *Prevotella* and order *Bacteroidales*), *Fusobacteria* (genus *Fusobacterium*), *Spirochaetes,* and candidate division TM7, in comparison to adults.[21] Interestingly, as the child grows the proportion of periopathogenic bacteria increase. There is a change in the bacterial population from aerobic or facultative gram-positive cocci to anaerobic fastidious gram-negative bacteria.[39]

Puberty is a time of major hormonal changes, which is accompanied by nutritional enrichment of the oral environment. Commonly, this phenomenon leads to an increase in some groups of oral microorganisms, including gram-negative anaerobes and spirochetes.[40] This change in the oral microbiota may be associated with the increased incidence and severity of gingivitis during puberty.[41]

It is also important to note that the oral microbiome may play a role in the development of oral and systemic pathology. For example, the increased consumption of fermentable carbohydrates can induce a change, with the oral microbiota favoring the growth of aciduric and acidogenic species, allowing the development of dental caries, as previously described.[30] Also, an association

between oral microorganisms and cancer has been suggested relatively recently.[42-44] The major mechanism associated is hypothesized to be a chronic oral infection-based carcinogenesis, being a subjacent inflammation process and the key feature.[45-48] In accordance, poor oral health and dental care, tooth loss, and a history of periodontitis are considered risk factors for cancer development in the oral cavity or other body sites.[49-59] In addition, several oral microorganisms, including the commonly encountered oral *Streptococci* (and yeasts), possess metabolic pathways for the conversion of alcohol to carcinogenic acetaldehyde.[60-64] Similarly, smoking also causes an increase in salivary acetaldehyde concentrations, hence adding to the risk related to alcohol,[65] thus making the effects of smoking and alcohol consumption on cancer development synergistic.[66] Virus, are also recognized etiological agents of cancer; the Human Papilloma Virus (HPV) being of particular relevance in the oral cavity, as mentioned a little later in the text, in the chapter on oral virus colonization.[67]

The placement of intraoral biomaterials, such as dental prostheses or orthodontic appliances, may also induce alterations in the oral microbiome.[68-70] Nowadays, orthodontic treatment is a frequent procedure in children for correction of malocclusion and for the improvement of mastication, speech, and appearance, as well as for overall health, comfort, and self-esteem.[71] However, orthodontic treatment is being associated with a higher risk of caries development or exacerbation of any pre-existing periodontal disease.[72-80] Fixed and removable orthodontic appliances, namely brackets, bands, and space maintainers, may frequently cause enamel demineralization, gingival inflammation, and increase in periodontal pocket depth.[74,76,78,79,81-83] These can be explained by the increase in plaque accumulation due to a higher number of plaque-retentive sites and impaired mechanical plaque or food residue removal, as well as, by mechanical or chemical irritation due to exposed cement.[83-85] Furthermore, it has been observed that the surface physicochemical properties of the orthodontic devices, such as, surface roughness, hydrophobicity, and elemental composition can influence bacterial attachment, plaque retaining capacity, microbial diversity, microorganism interaction, as well as, the biofilm matrix.[73,86-88] As an example, two recent studies evidenced the virulence modulation of *Streptococcus mutans* and *Candida albicans* biofilms by the metal ions released from orthodontic appliances.[89,90]

Sukontapatipark *et al.*,[91] in a time-dependent scanning electron microscopy (SEM) study on dental plaque adjacent to orthodontic brackets showed that the early stage of plaque formation began in the first week after the appliances were placed. Although most studies available do not compare oral microbiota before and after orthodontic treatment, the high concentrations of cariogenic microorganisms in the plaque and saliva of children with orthodontic devices,[92] namely *Streptococcus mutans* and *S. sobrinus,*[93,94] is being associated with recurrent enamel decalcification and white spot lesion formation in patients treated with fixed orthodontic appliances.[95-98]

In 2006, Naranjo *et al.*,[78] reported an increase in the *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Tannerella forsythia*, and *Fusobacterium* species after bracket placement. In accordance with this, in a recent study by Andrucioli *et al.,*[93] using the checkerboard DNA–DNA hybridization technique, the bacterial species of the orange complex (namely *P. intermedia*, *P. melaninogenica*, *P. nigrescens*, *S. noxia*, *F. nucleatum sp nucleatum*, *F. nucleatum sp vincentii*, *F. nucleatum sp polymorphum*, *F. periodonticum*, *Campylobacter gracilis*, *C. rectus*, *C. Showae,* and *C. ochracea*) were the most prevalent on metallic brackets, representing 40% of the total bacterial counts, followed by *Veillonella parvula*, representing 22% of the total bacterial counts. These microorganisms may be associated with the enhanced gingival inflammation observed in these patients. Some studies reported that removable devices show less plaque formation in relation to fixed orthodontic appliances.[99,100]

Considering the wide array of bacterial species found on orthodontic appliances *in vivo*, further studies are needed to guide the establishment of preventive clinical protocols that can be effective in controlling microbial contamination and preventing the development of bacteremias and pathologies, such as dental caries and periodontal disease, during orthodontic treatment.[93] Furthermore, oral health education supported by supplementary materials (brochures, paintings, etc.) for both children and parents are strongly recommended.[99]

Furthermore, it is of interest to emphasize that systemic changes in the overall host's health status can also influence the composition of the oral microbiome and the host's oral health.[101]

Oral Archaea colonization

Archaea represent a small minority of the oral microbiome, which are restricted to a small number

of methanogenic species/phylotypes, namely, *Methanobrevibacter oralis*, *Methanobacterium curvum/congolense,* and *Methanosarcina mazei*.[102,103] Archaea can be detected in healthy individuals, but its prevalence seems to increase in subjects with periodontitis. However, studies with these microorganisms are very scarce.

Oral fungal colonization

The oral cavity of newborns may be colonized by yeasts, specifically *Candida*, on their first day of life; and during the first year, the rate of oral colonization by *Candida* may vary between 40 and 82%.[104-106] However, in older children the frequency of colonization decreases to values between 3 and 36%.[106] These variations in the frequency of oral *Candida* colonization in children may be due to the physiological factors related to age, namely immune maturation, as well as other factors such as environmental changes (hospital vs. home) and diet alterations (breastfeeding vs. formula feeding).[104,107-109] After infancy, the prevalence of oral *Candida* colonization gradually increases until old age, reaching up to 75% in healthy subjects.[106,107,110,111] Although *C. albicans* is the most frequently detected fungi in the oral cavity of healthy children, the species *C. parapsilosis* has also gained some importance.[106-108,112,113]

For a long time, yeast *Candida* was the only fungus recognized as part of the normal oral microbial population, despite its opportunistic character.[114] However, in 2010, a metagenomic study identified 74 genera of cultivable fungi and 11 uncultivable ones in the oral cavity of healthy adults. Although *Candida* was the most frequent genus isolated in 75% of the subjects, other fungi groups presented a relevant prevalence, such as, *Cladosporium* (65%), *Aureobasidium* (50%), *Saccharomycetales* (50%), *Aspergillus* (35%), *Fusarium* (30%), and *Cryptococcus* (20%). However, the role of this oral mycobiome and its identification in the children's oral cavity is yet to be explored.[110] More recently, using improved culture techniques, it was demonstrated that a group of healthy young adults show 100% growth of filamentous fungi in their saliva and 92.5% showed growth of yeast, especially belonging to the genus *Candida*.[115] In this study, the most prevalent filamentous fungi identified were *Penicillium* sp., *Aspergillus* sp., and *Cladosporium* sp. Interestingly, the individual profile of fungal colonization was maintained over a six-month period, which might question the assumption that the presence of these fungi in the oral cavity represented only a transient colonization.[115] However, the role of this oral 'mycobiome' in adults and their identification in the oral cavity of children remains unexploited.

Oral parasitic colonization

Compared to other groups of microorganisms, few parasites colonize the oral cavity, although several recent studies have revealed that the protozoa are more frequent than previously thought.[116,117] Notwithstanding, its prevalence may vary significantly with the worldwide geographic distribution, ranging from 4 to 53%.[116] Within oral parasites, the protozoan *Entamoeba gingivalis* and *Trichomonas tenax* are the most frequent and are normally non-pathogenic commensal microorganisms. Although their oral colonization is associated with poor oral hygiene and a low socioeconomic status, these protozoa can also be found in caries-free children and adolescents.[117-119] The protozoa's rate of colonization increases with age, being more frequent in children aged between 11 and 19 years than in younger children.[116] However, protozoa are much more prevalent in adults, particularly in those with periodontal disease.[116] It is interesting to note that both protozoa can occur simultaneously, but the rate of colonization of *E. gingivalis* appears to increase more rapidly with age in relation to that of *T. tenax*.[119]

Oral viral colonization

The complexity of the human virome and its relationship with the host's health is not yet completely understood. The recent studies of Pride *et al.,*[120] show that there is a persistent community of double-stranded DNA viruses in the saliva of healthy human subjects, almost exclusively identified as bacteriophages. This finding is not surprising, taking into account the massive oral bacterial community. The fact that the vast majority of human oral viruses are bacteriophages, which play a prominent role in lysogeny, suggests that these viruses may play an important role in regulation of the microbial diversity of the human oral cavity.[120] However, salivary virus may serve as reservoirs of pathogenic gene function in the human oral environment.[120]

Other viruses associated with human disease may also be found in the oral cavity; however, their presence is primarily viewed as a pathological state. The course of viral diseases in children differs from adults due to the incomplete maturation of the immune system.[121] In children, unlike adults, the severity of symptoms is related to the age at which the infection was acquired.

Several viral agents can infect oral cells, however, only a few cause clinical alterations. Some examples include: Herpes simplex virus-1 (HSV-1) and HSV-2, which cause herpetic gingivostomatitis, orofacial herpes, and aphthous stomatitis; the Coxsackie A virus, which causes herpangina and hand, foot, and mouth disease; the Morbilli virus that causes measles; the Rubulavirus that causes mumps; and the human papilloma virus that causes oral papilloma (warts).[121]

The microbiome on different oral habitats

The oral microbiome is one of the most complex microbiome of the human body.[122] Its complexity results from a variety of oral habitats that comprise the oral cavity. These different oral habitats vary in relation to oxygen tension, nutrient availability, temperature, and host immunological factor exposure, due to their anatomical and physiological characteristics.[20,123] Most oral microorganisms colonize all oral habitats, including the mucosa, the tongue, and the teeth, however, their proportion may differ depending on the colonization site. In comparison to the oral mucosa and saliva, the teeth and tongue present a higher microbial load.[20]

With respect to microorganism distribution, the genus *Streptococcus* is present in a high proportion in the soft tissue, saliva, tongue, and supragingival area. The species *S. mitis* and *S. oralis* are found in high proportions in soft tissues, and the species *S. salivarius* is found in greater proportions in the saliva, soft tissues, and tongue.[20] Species of the genus *Actinomyces* are detected more frequently in the supra- and subgingival samples. Gram-negative bacilli are found in the subgingival tooth surfaces and also in the tongue fissures. The species *Lactobacillus acidophilus* is found in low proportions in all oral habitats, except in the tongue, where their proportion may be higher. Other bacteria such as *Veillonella parvula* and *Neisseria mucosa*, common colonizers of the oral cavity, are relatively abundant in all oral habitats.[20] It should be noted, however, that these proportions may change in case of oral pathology.

Saliva collects the released microorganisms working as a transition fluid, whereas, the dorsum of the tongue acts as a reservoir for several microorganisms, which will later fill other niches in the sub- and supragingival tooth surfaces.[20] In children, the colonization of oral epithelial cells appears to decrease with age,[124] perhaps due to improved oral hygiene habits or to the maturation of the immune system, given that during childhood, the levels of secreted IgA increase progressively.[125] One of the most dramatic results of the interactions between certain oral bacteria and epithelial cells is the internalization of microorganisms within the host cell. This is an active process, driven by the bacteria, where the signal transduction pathways of epithelial cells, which are otherwise non-phagocytic, are subverted to induce the entry of bacteria.[6] Epithelial cells can be infected not only by isolated strains, but also by complex consortia of bacteria, as exemplified by the consortium constituted by *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis,* and *Tannerella forsythia*.[126] The intracellular colonization has several advantages for the microorganisms, including protection against action of the humoral immune system and the action of many antibiotics.

The discovery that other oral habitats rather than teeth have relevant microbial colonization emphasizes that when the dentist designs a preventive approach he should take into account the oral cavity as a whole.

Routes of transmission of oral microorganisms

Oral microorganisms may have different origins. Studies that have focused on the phenotypic and genotypic characteristics of oral microorganisms suggest that the mother's or the primary caregiver's oral microbiota represent one of the most important sources of infants' and young children's oral microbiota.[39,127,128] A good example of vertical transmission is the transmission of the mother's vaginal *C. albicans* to ~80% of their vaginally delivered newborns.[129] Also, recent studies have shown that breast milk has a specific microbiome that varies throughout lactation.[130-132] Bacterial communities of milk typically include oral bacteria such as those belonging to the genera *Veillonella*, *Prevotella,* and *Leptotrichia*, suggesting that breastfeeding may represent a significant source of oral microorganisms. Moreover, 30 to 60% of the parents of children colonized with *S. mutans* and *Aggregatibacter actinomycetemcomitans*, important oral pathogens, presented identical bacteria genotypes.[133-135] Although the research on the transmission of cariogenic and periopathogenic microorganisms is scarce and limited to a few agents, most experts agree that early transmission is a risk factor for disease.[136,137] Thus, the prevention of oral colonization by pathogenic microorganisms in children should start with the prevention / treatment of the caregiver's oral cavity.

However, it is known that the oral transmission of microorganisms can occur not only by vertical transmission, but also horizontally, between brothers and/or colleagues.[138,139] This is particularly important if we take into account the socioeconomic changes taking place in the last two to three decades in the Western culture. The children that are under the care of a nanny or in contact with other children in a day care center present additional vectors for oral microorganism acquisition. The genotyping of *Streptococcus mutans* in children aged between 12 to 30 months, attending a day care center, revealed that 29% of the children had two or more corresponding genotypes, strongly suggesting the occurrence of horizontal transmission within this population.[140] It is interesting to note that those children attending day care centers present a lower level of their mothers' *S. mutans* genotypes in comparison to children staying with their moms'.[141,142]

In addition to the microbial route of transmission, the host genetic factors may also influence the proportion of species in genetically related individuals. In twins, it was demonstrated that the oral microbiota is more alike than in non-related persons.[143] Despite these intrafamilial similarities, the oral microbiota of children is unique and differs significantly from their parents and siblings, from an early age.[21] These findings support the possibility of using the oral microbiota as a fingerprint.

CONCLUSION

Throughout childhood the oral microbiome changes, maturates, and evolves. Along with the growth of the child, the oral microbial load increases, but the microbial diversity decreases. The type of first colonizers is related to different factors, such as, type of delivery, personal relationships, living environment, and so on. However, the set of initial oral colonizers seems to condition the subsequent colonization, which leads to more complex and stable ecosystems in adulthood. Therefore, these early microbial communities play a major role in the development of the microbiota in the adult oral cavity and may represent a source of both pathogenic and protective microorganisms at a very early stage of human life. Thus, the paediatric oral health care and prevention should start as early as its conception. The discovery that other oral habitats rather than teeth have relevant microbial colonization emphasizes the fact that when the dentist designs a preventive approach, he should take into account the oral cavity as a whole. Given that the child's family members, caregivers, and colleagues may represent an important source of oral microorganisms, the prevention of oral colonization by pathogenic microorganisms in children should start with the prevention/treatment of the life-sharing individuals' oral cavity.

REFERENCES

1. Keijser BJ, Zaura E, Huse SM, van der Vossen JM, Schuren FH, Montijn RC, *et al.* Pyrosequencing analysis of the oral microflora of healthy adults. J Dent Res 2008;87:1016-20.
2. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-14.
3. Ling Z, Kong J, Jia P, Wei C, Wang Y, Pan Z, *et al.* Analysis of oral microbiota in children with dental caries by PCR-DGGE and barcoded pyrosequencing. Microb Ecol 2010;60:677-90.
4. Nasidze I, Li J, Quinque D, Tang K, Stoneking M. Global diversity in the human salivary microbiome. Genome Res 2009;19:636-43.
5. Zaura E, Keijser BJ, Huse SM, Crielaard W. Defining the healthy "core microbiome" of oral microbial communities. BMC Microbiol 2009;9:259.
6. Jenkinson HF, Lamont RJ. Oral microbial communities in sickness and in health. Trends Microbiol 2005;13:589-95.
7. Pennisi E. A mouthful of microbes. Science 2005;307:1899-901.
8. Bearfield C, Davenport ES, Sivapathasundaram V, Allaker RP. Possible association between amniotic fluid micro-organism infection and microflora in the mouth. BJOG 2002;109:527-33.
9. Hill GB. Preterm birth: Associations with genital and possibly oral microflora. Ann Periodontol 1998;3:222-32.
10. Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Maynor G, *et al.* Periodontal infection as a possible risk factor for preterm low birth weight. J Periodontol 1996;67(10 Suppl):1103-13.
11. Mold JE, Michaelsson J, Burt TD, Muench MO, Beckerman KP, Busch MP, *et al.* Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. Science 2008;322:1562-5.
12. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. PLoS Biol 2007;5:e177.
13. Gronlund MM, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: Permanent changes in intestinal flora after cesarean delivery. J Pediatr Gastroenterol Nutr 1999;28:19-25.
14. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 2010;107:11971-5.
15. Bagg J, MacFarlane T, Poxton L, Smith A. Essentials of Microbiology for Dental Students. New York: Oxford University Press; 2006.
16. Hegde S, Munshi AK. Influence of the maternal vaginal microbiota on the oral microbiota of the newborn. J Clin Pediatr Dent 1998;22:317-21.

17. Cephas KD, Kim J, Mathai RA, Barry KA, Dowd SE, Meline BS, *et al.* Comparative analysis of salivary bacterial microbiome diversity in edentulous infants and their mothers or primary care givers using pyrosequencing. PloS one 2011;6:e23503.
18. Cole MF, Evans M, Fitzsimmons S, Johnson J, Pearce C, Sheridan MJ, *et al.* Pioneer oral streptococci produce immunoglobulin A1 protease. Infect Immun 1994;62:2165-8.
19. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: Evidence for a discrete window of infectivity. J Dent Res 1993;72:37-45.
20. Gizani S, Papaioannou W, Haffajee AD, Kavvadia K, Quirynen M, Papagiannoulis L. Distribution of selected cariogenic bacteria in five different intra-oral habitats in young children. Int J Paediatr Dent 2009;19:193-200.
21. Crielaard W, Zaura E, Schuller AA, Huse SM, Montijn RC, Keijser BJ. Exploring the oral microbiota of children at various developmental stages of their dentition in the relation to their oral health. BMC Med Genomics 2011;4:22.
22. General S. Oral health in America: A report of the Surgeon General. J Calif Dent Assoc 2000;28:685-95.
23. Nithila A, Bourgeois D, Barmes DE, Murtomaa H. WHO Global Oral Data Bank, 1986-96: An overview of oral health surveys at 12 years of age. Bull World Health Organ 1998;76:237-44.
24. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, *et al.* Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol 2008;46:1407-17.
25. Mantzourani M, Gilbert SC, Sulong HN, Sheehy EC, Tank S, Fenlon M, *et al.* The isolation of bifidobacteria from occlusal carious lesions in children and adults. Caries Res 2009;43:308-13.
26. Kanasi E, Dewhirst FE, Chalmers NI, Kent R Jr, Moore A, Hughes CV, *et al.* Clonal analysis of the microbiota of severe early childhood caries. Caries Res 2010;44:485-97.
27. Tanner AC, Mathney JM, Kent RL, Chalmers NI, Hughes CV, Loo CY, *et al.* Cultivable anaerobic microbiota of severe early childhood caries. J Clin Microbiol 2011;49:1464-74.
28. van Houte J, Lopman J, Kent R. The predominant cultivable flora of sound and carious human root surfaces. J Dent Res 1994;73:1727-34.
29. Van Houte J, Sansone C, Joshipura K, Kent R. Mutans streptococci and non-mutans streptococci acidogenic at low pH, and in vitro acidogenic potential of dental plaque in two different areas of the human dentition. J Dent Res 1991;70:1503-7.
30. Takahashi N, Nyvad B. Caries ecology revisited: Microbial dynamics and the caries process. Caries Res 2008;42:409-18.
31. Boyar RM, Thylstrup A, Holmen L, Bowden GH. The microflora associated with the development of initial enamel decalcification below orthodontic bands *in vivo* in children living in a fluoridated-water area. J Dent Res 1989;68:1734-8.
32. Gross EL, Leys EJ, Gasparovich SR, Firestone ND, Schwartzbaum JA, Janies DA, *et al.* Bacterial 16S sequence analysis of severe caries in young permanent teeth. J Clin Microbiol 2010;48:4121-8.
33. Becker MR, Paster BJ, Leys EJ, Moeschberger ML, Kenyon SG, Galvin JL, *et al.* Molecular analysis of bacterial species associated with childhood caries. J Clin Microbiol 2002;40:1001-9.
34. Munson MA, Banerjee A, Watson TF, Wade WG. Molecular analysis of the microflora associated with dental caries. J Clin Microbiol 2004;42:3023-9.
35. Chhour KL, Nadkarni MA, Byun R, Martin FE, Jacques NA, Hunter N. Molecular analysis of microbial diversity in advanced caries. J Clin Microbiol 2005;43:843-9.
36. Li Y, Ge Y, Saxena D, Caufield PW. Genetic profiling of the oral microbiota associated with severe early-childhood caries. J Clin Microbiol 2007;45:81-7.
37. Ling Z, Kong J, Jia P, Wei C, Wang Y, Pan Z, *et al.* Analysis of oral microbiota in children with dental caries by PCR-DGGE and barcoded pyrosequencing. Microb Ecol 2010;60:677-90.
38. Kanasi E, Dewhirst FE, Chalmers NI, Kent R Jr, Moore A, Hughes CV, *et al.* Clonal analysis of the microbiota of severe early childhood caries. Caries Res 2010;44:485-97.
39. Tanner AC, Milgrom PM, Kent R Jr, Mokeem SA, Page RC, Liao SI, *et al.* Similarity of the oral microbiota of pre-school children with that of their caregivers in a population-based study. Oral Microbiol Immunol 2003;17:379-87.
40. Jenkinson H, Lamont R. Oral Microbial Ecology. In: Lamont R, Burne RA, Lantz MS, Leblanc DJ, editors. Oral microbiology and immunology. Washington DC: ASM Press; 2006. p. 89-105.
41. Mombelli A, Gusberti FA, van Oosten MA, Lang NP. Gingival health and gingivitis development during puberty. A 4-year longitudinal study. J Clin Periodontol 1989;16:451-6.
42. Hooper SJ, Wilson MJ, Crean SJ. Exploring the link between microorganisms and oral cancer: A systematic review of the literature. Head Neck 2009;31:1228-39.
43. Meurman JH. Oral microbiota and cancer. J Oral Microbiol 2010;2:5195.
44. Meurman JH, Uittamo J. Oral micro-organisms in the etiology of cancer. Acta Odontol Scand 2008;66:321-6.
45. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860-7.
46. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436-44.
47. Meurman JH. Infectious and dietary risk factors of oral cancer. Oral Oncol 2010;46:411-3.
48. Allavena P, Garlanda C, Borrello MG, Sica A, Mantovani A. Pathways connecting inflammation and cancer. Curr Opin Genet Dev 2008;18:3-10.
49. Abnet CC, Kamangar F, Islami F, Nasrollahzadeh D, Brennan P, Aghcheli K, *et al.* Tooth loss and lack of regular oral hygiene are associated with higher risk of esophageal squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 2008;17:3062-8.
50. Abnet CC, Qiao YL, Mark SD, Dong ZW, Taylor PR, Dawsey SM. Prospective study of tooth loss and incident esophageal and gastric cancers in China. Cancer Causes Control 2001;12:847-54.
51. Demirer T, Icli F, Uzunalimoglu O, Kucuk O. Diet and stomach cancer incidence. A case-control study in Turkey. Cancer 1990;65:2344-8.
52. Hujoel PP, Drangsholt M, Spiekerman C, Weiss NS. An exploration of the periodontitis-cancer association. Ann Epidemiol 2003;13:312-6.
53. Michaud DS, Joshipura K, Giovannucci E, Fuchs CS. A prospective study of periodontal disease and pancreatic cancer in US male health professionals. J Natl Cancer Inst 2007;99:171-5.
54. Michaud DS, Liu Y, Meyer M, Giovannucci E, Joshipura K. Periodontal disease, tooth loss, and cancer risk in male health professionals: A prospective cohort study. Lancet Oncol 2008;9:550-8.

55. Stolzenberg-Solomon RZ, Dodd KW, Blaser MJ, Virtamo J, Taylor PR, Albanes D. Tooth loss, pancreatic cancer, and Helicobacter pylori. Am J Clin Nutr 2003;78:176-81.
56. Tezal M, Sullivan MA, Hyland A, Marshall JR, Stoler D, Reid ME, *et al.* Chronic periodontitis and the incidence of head and neck squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 2009;18:2406-12.
57. Tezal M, Sullivan MA, Reid ME, Marshall JR, Hyland A, Loree T, *et al.* Chronic periodontitis and the risk of tongue cancer. Arch Otolaryngol Head Neck Surg 2007;133:450-4.
58. Watabe K, Nishi M, Miyake H, Hirata K. Lifestyle and gastric cancer: A case-control study. Oncol Rep 1998;5:1191-4.
59. Holmes L Jr, desVignes-Kendrick M, Slomka J, Mahabir S, Beeravolu S, Emani SR. Is dental care utilization associated with oral cavity cancer in a large sample of community-based United States residents? Community Dent Oral Epidemiol 2009;37:134-42.
60. Homann N, Tillonen J, Meurman JH, Rintamaki H, Lindqvist C, Rautio M, *et al.* Increased salivary acetaldehyde levels in heavy drinkers and smokers: A microbiological approach to oral cavity cancer. Carcinogenesis 2000;21:663-8.
61. Homann N, Tillonen J, Salaspuro M. Microbially produced acetaldehyde from ethanol may increase the risk of colon cancer via folate deficiency. Int J Cancer 2000;86:169-73.
62. Kurkivuori J, Salaspuro V, Kaihovaara P, Kari K, Rautemaa R, Gronroos L, *et al.* Acetaldehyde production from ethanol by oral streptococci. Oral Oncol 2007;43:181-6.
63. Nieminen MT, Uittamo J, Salaspuro M, Rautemaa R. Acetaldehyde production from ethanol and glucose by non-Candida albicans yeasts in vitro. Oral Oncol 2009;45:e245-8.
64. Uittamo J, Siikala E, Kaihovaara P, Salaspuro M, Rautemaa R. Chronic candidosis and oral cancer in APECED-patients: Production of carcinogenic acetaldehyde from glucose and ethanol by Candida albicans. Int J Cancer 2009;124:754-6.
65. Morse DE, Psoter WJ, Cleveland D, Cohen D, Mohit-Tabatabai M, Kosis DL, *et al.* Smoking and drinking in relation to oral cancer and oral epithelial dysplasia. Cancer Causes Control 2007;18:919-29.
66. Salaspuro V, Salaspuro M. Synergistic effect of alcohol drinking and smoking on in vivo acetaldehyde concentration in saliva. Int J Cancer 2004;111:480-3.
67. Giovannelli L, Campisi G, Lama A, Giambalvo O, Osborn J, Margiotta V, *et al.* Human papillomavirus DNA in oral mucosal lesions. J Infect Dis 2002;185:833-6.
68. Claro-Pereira D, Sampaio-Maia B, Ferreira C, Rodrigues A, Melo LF, Vasconcelos MR. In situ evaluation of a new silorane-based composite resin's bioadhesion properties. Dent Mater 2011;27:1238-45.
69. Sampaio-Maia B, Figueiral MH, Sousa-Rodrigues P, Fernandes MH, Scully C. The effect of denture adhesives on Candida albicans growth *in vitro*. Gerodontology 2012;29:e348-56.
70. Topaloglu-Ak A, Ertugrul F, Eden E, Ates M, Bulut H. Effect of orthodontic appliances on oral microbiota--6 month follow-up. J Clin Pediatr Dent 2011;35:433-6.
71. Lau P, Wong R. Risks and complications in orthodontic treatment. Hong Kong Dent J 2006;3:15-22.
72. Ai H, Lu HF, Liang HY, Wu J, Li RL, Liu GP, *et al.* Influences of bracket bonding on mutans streptococcus in plaque detected by real time fluorescence-quantitative polymerase chain reaction. Chin Med J (Engl) 2005;118:2005-10.
73. Anhoury P, Nathanson D, Hughes CV, Socransky S, Feres M, Chou LL. Microbial profile on metallic and ceramic bracket materials. Angle Orthod 2002;72:338-43.
74. Atack NE, Sandy JR, Addy M. Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. J Periodontol 1996;67:78-85.
75. Bjerklin K, Garskog B, Ronnerman A. Proximal caries increment in connection with orthodontic treatment with removable appliances. Br J Orthod 1983;10:21-4.
76. Boyd RL, Leggott PJ, Quinn RS, Eakle WS, Chambers D. Periodontal implications of orthodontic treatment in adults with reduced or normal periodontal tissues versus those of adolescents. Am J Orthod Dentofacial Orthop 1989;96:191-8.
77. Lo BA, Di Marco R, Milazzo I, Nicolosi D, Cali G, Rossetti B, *et al.* Microbiological and clinical periodontal effects of fixed orthodontic appliances in pediatric patients. New Microbiol 2008;31:299-302.
78. Naranjo A, Trivino M, Jaramillo A, Betancourth M, Botero J. Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. Am J Orthod Dentofacial Orthop 2006;130:275.e17-22.
79. Ogaard B. Prevalence of white spot lesions in 19-year-olds: A study on untreated and orthodontically treated persons 5 years after treatment. Am J Orthod Dentofacial Orthop 1989;96:423-7.
80. Sari E, Birinci I. Microbiological evaluation of 0.2% chlorhexidine gluconate mouth rinse in orthodontic patients. Angle Orthod 2007;77:881-4.
81. Behlfelt K, Ericsson L, Jacobson L, Linder-Aronson S. The occurrence of plaque and gingivitis and its relationship to tooth alignment within the dental arches. J Clin Periodontol 1981;8:329-37.
82. Silness J, Roynstrand T. Relationship between alignment conditions of teeth in anterior segments and dental health. J Clin Periodontol 1985;12:312-20.
83. Valderhaug J. Periodontal conditions and carious lesions following the insertion of fixed prostheses: A 10-year follow-up study. Int Dent J 1980;30:296-304.
84. Zachrisson S, Zachrisson BU. Gingival condition associated with orthodontic treatment. Angle Orthodt 1972;42:26-34.
85. Boyd RL. Longitudinal evaluation of a system for self-monitoring plaque control effectiveness in orthodontic patients. J Clin Periodontol 1983;10:380-8.
86. Eliades T, Eliades G, Brantley WA. Microbial attachment on orthodontic appliances: I. Wettability and early pellicle formation on bracket materials. Am J Orthod Dentofacial Orthop 1995;108:351-60.
87. Suljak JP, Reid G, Wood SM, McConnell RJ, van der Mei HC, Busscher HJ. Bacterial adhesion to dental amalgam and three resin composites. J Dent 1995;23:171-6.
88. Bos R, van der Mei HC, Busscher HJ. Physico-chemistry of initial microbial adhesive interactions — its mechanisms and methods for study. FEMS Microbiol Rev 1999;23:179-230.
89. Ronsani MM, Mores Rymovicz AU, Meira TM, Trindade Gregio AM, Guariza Filho O, Tanaka OM, *et al.* Virulence modulation of Candida albicans biofilms by metal ions commonly released from orthodontic devices. Microb Pathog 2011;51:421-5.

90. Rymovicz AU, Ronsani MM, Gregio AM, Guariza-Filho OG, Tanaka O, Rosa EA. Virulence modulation of Streptococcus mutans biofilms by metal ions released from orthodontic appliances. Angle Orthod 2013 [In Press].
91. Sukontapatipark W, el-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. Eur J Orthod 2001;23:475-84.
92. Menzaghi N, Saletta M, Garattini G, Brambilla E, Strohmenger L. Changes in the yeast oral flora in patients in orthodontic treatment. Prev Assist Dent 1991;17:26-30.
93. Andrucioli MC, Nelson-Filho P, Matsumoto MA, Saraiva MC, Feres M, de Figueiredo LC, *et al.* Molecular detection of in-vivo microbial contamination of metallic orthodontic brackets by checkerboard DNA-DNA hybridization. Am J Orthod Dentofacial Orthop 2012;141:24-9.
94. Scheie AA, Arneberg P, Krogstad O. Effect of orthodontic treatment on prevalence of Streptococcus mutans in plaque and saliva. Scand J Dent Res 1984;92:211-7.
95. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. Am J Orthod 1982;81:93-8.
96. Mizrahi E. Enamel demineralization following orthodontic treatment. Am J Orthod 1982;82:62-7.
97. Ogaard B, Rolla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1. Lesion development. Am J Orthod Dentofacial Orthop 1988;94:68-73.
98. Basdra EK, Huber H, Komposch G. Fluoride released from orthodontic bonding agents alters the enamel surface and inhibits enamel demineralization *in vitro*. Am J Orthod Dentofacial Orthop 1996;109:466-72.
99. Arikan F, Eronat N, Candan U, Boyacioglu H. Periodontal conditions associated with space maintainers following two different dental health education techniques. J Clin Pediatr Dent 2007;31:229-34.
100. Boyd RL, Baumrind S. Periodontal considerations in the use of bonds or bands on molars in adolescents and adults. Angle Orthod 1992;62:117-26.
101. Pereira-Lopes O, Sampaio-Maia B, Sampaio S, Vieira-Marques P, Monteiro-da-Silva F, Braga A, *et al.* Periodontal inflammation in renal transplant recipients receiving Everolimus or Tacrolimus — preliminary results. Oral Dis 2013;19:666-72.
102. Lepp PW, Brinig MM, Ouverney CC, Palm K, Armitage GC, Relman DA. Methanogenic Archaea and human periodontal disease. Proc Natl Acad Sci U S A 2004;101:6176-81.
103. Matarazzo F, Ribeiro AC, Feres M, Faveri M, Mayer MP. Diversity and quantitative analysis of Archaea in aggressive periodontitis and periodontally healthy subjects. J Clin Periodontol 2011;38:621-7.
104. Kleinegger CL, Lockhart SR, Vargas K, Soll DR. Frequency, intensity, species, and strains of oral Candida vary as a function of host age. J Clin Microbiol 1996;34:2246-54.
105. Lay KM, Russel C. Candida species and yeasts in mouths of infants from a special care unit of a maternity hospital. Arch Dis Child 1977;52:794-6.
106. Odds FC. Candida and candidosis. 2nd ed. London: Baillière Tindall; 1988.
107. Hannula J, Saarela M, Jousimies-Somer H, Takala A, Syrjanen R, Kononen E, *et al.* Age-related acquisition of oral and nasopharyngeal yeast species and stability of colonization in young children. Oral Microbiol Immunol 1999;14:176-82.
108. Kadir T, Uygun B, Akyuz S. Prevalence of Candida species in Turkish children: Relationship between dietary intake and carriage. Arch Oral Biol 2005;50:33-7.
109. van Wyk C, Steenkamp V. Host factors affecting oral candidiasis. South Afr J Epidemiol Infect 2011;26:18-21.
110. Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, Naqvi A, *et al.* Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog 2010;6:e1000713.
111. Brambilla E, Strohmenger L, Vogel G. The effect of storage in liquid nitrogen on the isolation of oral yeasts in human saliva. Arch Oral Biol 1992;37:237-9.
112. Darwazeh AM, al-Bashir A. Oral candidal flora in healthy infants. J Oral Pathol Med 1995;24:361-4.
113. Contreras I, Ponton J, Quindos G. Prevalence of Candida parapsilosis in the oral cavities of infants in Spain. Clin Infect Dis 1994;18:480-1.
114. Scully C, el-Kabir M, Samaranayake LP. Candida and oral candidosis: A review. Crit Rev Oral Biol Med 1994;5:125-57.
115. Monteiro-da-Silva F, Sampaio-Maia B, Pereira Mde L, Araujo R. Characterization of the oral fungal microbiota in smokers and non-smokers. Eur J Oral Sci 2013;121:132-5.
116. Ghabanchi J, Zibaei M, Afkar MD, Sarbazie AH. Prevalence of oral Entamoeba gingivalis and Trichomonas tenax in patients with periodontal disease and healthy population in Shiraz, southern Iran. Indian J Dent Res 2010;21:89-91.
117. Bergquist R. Parasitic infections affecting the oral cavity. Periodontol 2000 2009;49:96-105.
118. Wantland WW, Lauer D. Correlation of some oral hygiene variables with age, sex, and incidence of oral protozoa. J Dent Res 1970;49:293-7.
119. Vrablic J, Tomova S, Catar G, Randova L, Suttova S. Morphology and diagnosis of Entamoeba gingivalis and Trichomonas tenax and their occurrence in children and adolescents. Bratisl Lek Listy 1991;92:241-6.
120. Pride DT, Salzman J, Haynes M, Rohwer F, Davis-Long C, White RA 3rd, *et al.* Evidence of a robust resident bacteriophage population revealed through analysis of the human salivary virome. ISME J 2012;6:915-26.
121. Sallberg M. Oral viral infections of children. Periodontol 2000 2009;49:87-95.
122. Consortium THMP. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-14.
123. Tanner AC, Milgrom PM, Kent R Jr, Mokeem SA, Page RC, Riedy CA, *et al.* The microbiota of young children from tooth and tongue samples. J Dent Res 2002;81:53-57.
124. Vaahtontemi LH, Raisanen S, Stenfors LE. The age-dependence of bacterial presence on oral epithelial surfaces in vivo. Oral Microbiol Immunol 1992;7:263-6.
125. Ben-Aryeh H, Fisher M, Szargel R, Laufer D. Composition of whole unstimulated saliva of healthy children: Changes with age. Arch Oral Biol 1990;35:929-31.
126. Rudney JD, Chen R, Sedgewick GJ. Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, and Tannerella forsythensis are components of a polymicrobial intracellular flora within human buccal cells. J Dent Res 2005;84:59-63.

127. Klein MI, Florio FM, Pereira AC, Hofling JF, Goncalves RB. Longitudinal study of transmission, diversity, and stability of Streptococcus mutans and Streptococcus sobrinus genotypes in Brazilian nursery children. J Clin Microbiol 2004;42:4620-6.
128. Li Y, Ismail AI, Ge Y, Tellez M, Sohn W. Similarity of bacterial populations in saliva from African-American mother-child dyads. J Clin Microbiol 2007;45:3082-5.
129. Blaschke-Hellmessen R. Vertical transmission of Candida and its consequences. Mycoses 1998;41(Suppl 2):31-6.
130. Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. Am J Clin Nutr 2012;96:544-51.
131. Collado MC, Delgado S, Maldonado A, Rodriguez JM. Assessment of the bacterial diversity of breast milk of healthy women by quantitative real-time PCR. Lett Appl Microbiol 2009;48:523-8.
132. Rautava S, Luoto R, Salminen S, Isolauri E. Microbial contact during pregnancy, intestinal colonization and human disease. Nat Rev Gastroenterol Hepatol 2012;9:565-76.
133. Alves AC, Nogueira RD, Stipp RN, Pampolini F, Moraes AB, Goncalves RB, *et al.* Prospective study of potential sources of Streptococcus mutans transmission in nursery school children. J Med Microbiol 2009;58:476-81.
134. Asikainen S, Chen C, Slots J. Likelihood of transmitting Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis in families with periodontitis. Oral Microbiol Immunol 1996;11:387-94.
135. Dogan B, Kipalev AS, Okte E, Sultan N, Asikainen SE. Consistent intrafamilial transmission of Actinobacillus actinomycetemcomitans despite clonal diversity. J Periodontol 2008;79:307-15.
136. McDonald R. Dental Caries in the Child and Adolescent. In: McDonald R, Avery D, Dean J, editors. Dentistry for the Child and Adolescent. 8th ed. Missouri: Mosby, Inc; 2004. p. 203-35.
137. Pahkla ER, Jogi E, Nurk A, Pisarev H, Koppel T, Naaber P, *et al.* Periodontal disease in mothers indicates risk in their children. Int J Paediatr Dent 2010;20:24-30.
138. Domejean S, Zhan L, DenBesten PK, Stamper J, Boyce WT, Featherstone JD. Horizontal transmission of mutans streptococci in children. J Dent Res 2010;89:51-5.
139. Kohler B, Lundberg AB, Birkhed D, Papapanou PN. Longitudinal study of intrafamilial mutans streptococci ribotypes. Eur J Oral Sci 2003;111:383-9.
140. Mattos-Graner RO, Li Y, Caufield PW, Duncan M, Smith DJ. Genotypic diversity of mutans streptococci in Brazilian nursery children suggests horizontal transmission. J Clin Microbiol 2001;39:2313-6.
141. Li Y, Wang W, Caufield PW. The fidelity of mutans streptococci transmission and caries status correlate with breast-feeding experience among Chinese families. Caries Res 2000;34:123-32.
142. Tedjosasongko U, Kozai K. Initial acquisition and transmission of mutans streptococci in children at day nursery. ASDC J Dent Child 2002;69:284-8,34-5.
143. Corby PM, Bretz WA, Hart TC, Schork NJ, Wessel J, Lyons-Weiler J, *et al.* Heritability of oral microbial species in caries-active and caries-free twins. Twin Res Hum Genet 2007;10:821-8.

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