



A Comparative Study of Oral Microbiome in Neonates – Review

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Abstract

Oral microbiota is observed to play a key role in the health and physiology of humans. The oral microbiome is seen to vary in its colony diversity and composition with age. Various studies have identified factors that affect oral microbiota in neonates most important among which is the mode of delivery (either cesarean or vaginal) and the initial mode of nutrition (either breastfed or formula fed). A thorough studying and reviewing of the literature reveal that the oral microbiota of vaginally delivered neonates are richly diverse, health-inducing and increases the innate immunity of the infant while the oral microbiota of cesarean-delivered neonates is mainly composed of harmful pathogenic microbes. Studies show that vaginal seeding is an effective means to partially restore the vaginal microbiota in cesarean-delivered neonates. Secondly, studies have shown that the enzyme xanthine oxidase present in breast milk reacts with its substrates xanthine and hypoxanthine present in neonatal saliva as a result, antimicrobial hydrogen peroxide is produced which inhibits the growth of pathogenic and cariogenic bacteria in infant oral cavity. On the other hand, the formula milk supports no such reaction. This review describes the above two factors with great detail, reasoning and examples compiling numerous research studies in the past.

Keywords

Oral microbiota, neonates, gut microbiome, parturition.

INTRODUCTION

Microorganisms have been associated with various other organisms as parasites, symbionts, commensals and as various other probiotic interactions. Microbes are an integral part of human physiology [1]. Various body sites act as specific niches to their respective microflora. These colonies may cause disease or adverse effect [2], become beneficial and help in metabolic or physiological functions or remain as commensal probiont. The colonization of microbes depends majorly on mode

of attachment, frequency and type of exposure and the biochemical environment of the niche [1, 3].

Oral microbiome plays key role in allergies, dental caries and immunity. Variation in their composition is observed to cause diseases and infections in early life [4, 5]. Various studies in the past have shown correlation between the oral microbial compositions with age [6]. Oral microbes have been observed to be clearly varying with age and from person to person depending on climate, geography, diet, any diagnosed disease, and many other factors. Adult microbiota is quite consistent, uniform and stable

while infants seem to show many diverse and inconsistently varying colonies. The key factors for this, according to numerous studies, is firstly the mode of delivery, and secondly, mode of nutrition [7, 8].

Mothers undoubtedly play a key role in acquisition of initial colonies of oral microbiota. Various microbes are transmitted from the mother both in-utero [2-4] [6, 7] and during parturition [9-11]. Moreover, a mother or primary caregiver transmit microbes by physical contact during early infancy [12].

It is important to understand whether the oral microflora of neonates is beneficial or harmful. It has been observed that the microbes acquired through vaginal mode of delivery are widely beneficial microbes while that acquired by caesarean mode of delivery are harmful [12-16]. For the same reason in recent times, vaginal seeding has become a common practice performed on neonates born by caesarean delivery to restore the microbiome.

This article deals with a detailed review of literature and recent research studies on oral microbiome of neonates, its role in the infant's early health and various factors affecting the acquisition of neonatal oral microbial colonies.

1.1. Sterile womb hypothesis and its contradictions

Mother is a principle source and a key factor of microbiota of the neonate [1, 2]. Earlier Sterile womb hypothesis was predominantly accepted which stated that the entire in utero environment was assumed to be sterile and the initial microbiome was supposed to be acquired through the birth canal and vaginal exposure along with initial exposure to the environment post parturition [3,4]. Recent studies however, detected the presence of micro flora in utero, the first meconium of neonate (primarily consisting of the amniotic fluid swallowed by the foetus in uterus continuously from mid gestation to late gestation) showed signs of microbes like *Enterococcus spp.*, and *Escherichia spp.*, which are common inhabitants of gastrointestinal tract [4-6]. This along with further studies contradicted the sterile womb hypothesis [7-12]. Molecular sequencing detected similar microbes in the amniotic fluid and hence the origin of neonatal microbiota can be traced back to even before birth itself [13-17].

1.2. Effect of parturition on neonatal oral microflora

On a time line, parturition is the second major event that decides the microbiota of an infant. An immensely large number of reports focus on the various differences in gut microbiome of neonates delivered by vaginal type delivery (VTD) and caesarean type delivery (CTD). CTD is one of the most

common mode of delivery across the world [14] [18,19]. Neonates by CTD have relatively higher flora of microbes, richer and diverse on the skin. In CTD, the first time exposure of the neonate to microbes is by contact of the skin (usually of the mother), hence the oral microbiome of the neonate was found to be similar to that of the skin of the mother where *Staphylococcus spp.*, *Corynebacterium spp.*, *Propionibacterium spp.*, are found to be prevalent [20-22]. The microbiota was also found to be dependent on the environment of the first time exposure which in case of CTD is the hospital and medical equipments. In contrast, the first-time exposure of microbes in case of VTD is the birth canal of the mother. Hence the microbial diversity of the gut and oral cavity is much more diverse than the skin and these microbes resemble the microbiome of the birth canal and vaginal microbes of the mother where *Lactobacillus spp.*, *Prevotella spp.*, *Sneathia spp.*, are found to be prevalent [22].

In fact, studies show that the microbial composition of neonates delivered by VTD is much more diverse in general and specifically the oral microbiota of these infants are much richer than that of infants delivered by CTD but this result has been contradicted in some research [14][20][23-26]. Contradiction to the previous studies were suggested in some studies where in the CTD microbiome was found to be more diverse and richer than the VTD microbiome [25][27-29], mixed responses were observed in some other studies [18], and yet some reported to have found no difference in microbial diversity with respect to the mode of delivery [30,31]. It was also reported that most of the microbes obtained by VTD are beneficial and probiotics. Microbes like *Bifidobacteria spp.*, *Lactobacillus spp.*, *Prevotella spp.*, *Escherichia coli.*, *Sneathia spp.* are predominantly found in the guts of neonates delivered by VTD [14][22][26][32]. Oral cavity of these VTD neonates consisted generally of *Lactobacillus spp.*, *Prevotella spp.*, *Gardnerella spp.*, *Streptococcus salivarius*, *Lactobacillus curvatus*, *Lactobacillus salivarius*, *Lactobacillus casei* [20][26][32]. Although these are beneficial microbes, one cannot ignore the microbes of intra uterine infections which is also found in the vaginal tract and can possibly be transmitted by VTD [33]. On the other hand the neonates of CTD had much less diverse microbiota in both gut and oral cavity; these mostly had colonies of *Staphylococcus spp.*, *Enterococcus spp.*, *Clostridium spp.*, on the skin [14] and *Pseudomonas spp.*, *Bacteroides spp.*, *Desulfovibrio spp.*, *Pseudomonas spp.*, *Staphylococcus spp.*, *Tepidibacillus spp.*, *Bifidobacterium spp.*, in the oral

cavity and gut [20]. Although most of the studies suggest that the mode of delivery is a key factor that shapes oral microbiota of neonates [22][34,35], there are some contradictory studies as well [13,14][30,31][36,37]. Yet some studies suggest that oral, skin and nares microbiota depend on mode of delivery while gut microbiota originates entirely in-utero and is affected by maternal diet and is independent of mode of delivery [13].

1.3. Effect of microbiota on the health of neonates

The diversity of oral microbiota due to the mode of delivery directly affects the health and physiology of the neonate in the first few months of life. Many studies indicate that various diseases like asthma, diabetes, obesity, rhinitis, eczema, various allergies, autoimmune diseases, dental caries are much more likely to affect neonates delivered by CTD than by VTD [14][36] [38-47]. This is because, according to hygiene hypothesis the differential exposure of microbes in case of CTD and VTD play key role in acquiring of various disease by neonates [39][48]. The skin microbiome that is initially exposed to neonates by CTD is mostly pathogenic and very few beneficial microbes are acquired [49, 50]. The vaginal microbiome initially exposed to neonates of VTD is mostly probiotic, beneficial, and health inducing [14]. Secondly, early exposure to the commensal microbes in VTD leads to a stronger and well developed innate and acquired immunity [51]. Another reason is that the most dominant microbe in VTD is *Lactobacilli spp.*, and *Bifidobacterium spp.*, (an anaerobic airborne microbe)[52]. It is well-known that *Bifidobacterium spp.*, balances immunity and provides allergy resistance while *Lactobacilli spp.*, protects intestinal mucosa [53], increase immunity and protect the neonate against allergy and eczema [54-58]. Another microbe obtained in VTD is *Akkermansia spp.*, which is known to protect the metabolic condition and prevent diseases like obesity and diabetes [53]. Clearly the microbes obtained by VTD is more health

inducing. It was believed that *S.mutans*(causative agent of dental caries) can only colonise the oral cavity post tooth eruption [54], but recent detection of *S.mutans* in preterm neonates as young as 2 months has clearly contradicted this belief [59]. It is observed that microbes like *S. mitis* and *S.sanguinis* which are initial colonisers of oral cavities of VTD neonates, are antagonistic to *S.mutans* and protect neonates from early dental caries [60-62]. Studies show that *S.mutans* is acquired almost 1 year earlier in CTD infants than in VTD infants [63], and that *S.mutans* were found in higher abundance in CTD than VTD neonates [64]. *Slackia exigua* was exclusively detected in abundant colonies in CTD, it's a causative organism of root canal infection, periodontitis, extra oral surgical wound and intestinal abscesses [65,66]. This tendency of colonising exclusively in oral cavities of CTD neonates is believed to be because of absence of the vaginally acquired microbe *S.exigua* that suppresses such oral biofilms in VTD [26].

In order for the neonates delivered by CTD to acquire some of these benefits, a new process called "vaginal seeding" may be carried out [36][51]. By this method one can achieve partial restoration of vaginal beneficial microbes by exposing neonates delivered by CTD to vaginal fluids for up to first 30 days of life. A study conducted for microbial restoration of CTD neonates showed that the microbial colony post restoration was similar to vaginal microbiome in oral cavity and skin of infants than in anus or gut [36][51]. Hence vaginal seeding is considered ineffective for gut microbial restoration. The protocol involved exposing the CTD neonate to maternal vaginal microbiome and comparing the changes in the neonate microbiome to the microbiome of a healthy neonate born from VTD. The microbiome of the vaginally seeded CTD infants resembled healthy VTD infants while unexposed CTD infants did not [36][51].

Table 1. Role of various oral bacteria in neonates and their source of transmission.

Sl.no.	Neonatal oral bacterium genus	Mode of transmission from mother to neonate.	Function / role in neonates	References
01	<i>Bifidobacterium spp.</i>	Vaginal delivery	Probiotic gut microbe, balances immunity.	[53]
02	<i>Lactobacillus spp.</i>	Vaginal delivery Breast feeding	Protects intestinal mucosa, increases immunity, prevents allergies and eczema, Inhibits pathogens like <i>E. faecalis</i> , <i>S. enterica</i> , <i>S. aureus</i> , <i>L. Monocytogenes</i> , <i>E. coli</i> .	[54-58] [155]

Sl.no.	Neonatal oral bacterium genus	Mode of transmission from mother to neonate.	Function / role in neonates	References
03	<i>Akkermansia spp.</i>	Vaginal delivery	Protects metabolic conditions, prevents diseases like diabetes and obesity.	[53]
04	<i>Streptococcus spp.</i>	Vaginal delivery	<i>S. mitis</i> and <i>S. sanguinis</i> antagonistic to <i>S. mutans</i> prevents dental caries, <i>S. exigua</i> suppresses oral biofilms formation.	[60-62] [26]
		Breast feeding	Produces bactericidal H ₂ O ₂ that inhibits pathogens like <i>S. aureus</i> and <i>P. aeruginosa</i> .	[154]

1.4. Diversity of neonate microbiome during development.

Neonate microbiome is extremely variable throughout the first year of life [5][67-71]. External factors such as environment at birth, mother's microbiome, mode of nutrition (either breast fed or formula fed) [72] and internal factors like developmental changes play crucial role in shaping the oral and gut microbiota of infants [73-75]. Initial exposure in utero [6] [76-79], followed by exposure through parturition [80] and breast feeding are the key contributors of the microbiome [81, 82]. Mostly genetic and environmental factors are believed to affect the microbiota post birth [83, 84]. Most importantly person to person contact through infant's saliva, mode of nutrition or cross talk usually between mother and neonate affect the oral microbiome during first few months of life [39]. Surprisingly, antibiotic treatments on mother played no role in oral microbiome of neonates up to 3 months of age [39]. Initial oral microbiota is mostly inhabited by *Streptococci spp.*, [34][85]. Anaerobic microbes only start colonising post 2 months [34]. *S. salivaris* and *S. mitis* are the first microbes to colonise oral cavity of neonates predominantly [32]. It's also observed that oral cavity of VTD neonates had higher microbial biofilms than CTD infants [26]. Initial microbiota of infants was undifferentiated across multiple body sites [86], for the first few weeks post birth, microbial distribution is decided by the body sites [13], this is also the period where the microbiota across body sites of VTD and CTD infants are vastly different as discussed so far [36]. But eventually the microbiome uniformly changes and starts resembling the normal adult microbiota specific to their respective body sites [32][51][86]. This process of achieving normal adult microbiome can take from 1 week to as long as 8 months after birth, depending on the body site [32][51]. Studies show that anal and gut microbiome take 1 to 4 months to acquire adult configuration [89], while

skin and oral microbiota can achieve the same in about 1-week post birth [51]. A common trend observed in this transition is the replacing of early facultative anaerobes with the new obligate anaerobe colonies [89]. It is also seen that microbiota across body sites are very dense and rich at the time of birth, they sharply decrease in number within the first week, then gradually increase their colonies to reach the normal adult microflora configuration corresponding to the body sites [89]. The infant salivary microbiota for instance, has microbial colonies many consisting of *Veillonella spp.*, *Streptococcus spp.*, *Neisseria spp.*, *Rothia spp.*, *Haemophilus spp.*, *Gemella spp.*, *Granulicatella spp.*, *Leptotrichia spp.*, *Fusobacterium spp.*, with *Streptococcus spp.*, *Veillonella spp.*, *Neisseria spp.*, being predominant [5]. While adult salivary microbiota has microbial colonies mainly consisting of *Haemophilus spp.*, *Neisseria spp.*, *Veillonella spp.*, *Fusobacterium spp.*, *Oribacterium spp.*, *Rothia spp.*, *Treponema spp.*, and a few *Actinomycetes* [69]. Clearly, the infant salivary microbiota is a lot richer, diverse and undifferentiated than their adult counterparts. Infact the salivary microbiota is found to be much richer before tooth eruption [69]. It has been hypothesised that at the time of birth, there is a rapid influx of microbes to infants; these microbes gradually select their suitable niche post birth and then start functioning to affect the physiology of the neonate at their respective niche (body sites) [89]. Infant's oral cavity is colonised by over 700 bacterial species of which upto 94.4% of the colonies are similar to the mother's oral microbiome or that of the primary care givers [88][90-92]. Dental caries are mainly acquired by colonisation of *S. mutans* [93-95], a high density of this microbe on biofilms cause early childhood caries (ECC) [94-96]. It must be noted that although *S. mutans* are the prime causative agent of ECC, there are various cariogenic bacteria other than *S. mutans* as well like *Veillonella spp.*, *Lactobacilli spp.*, *Bifidobacterium spp.*, *Propionibacterium spp.*,

Streptococci spp., *Atopobium spp.*, and *Actinomyces spp.*, [97-103]. Infact cariogenic microbes like *S. mutans*, *S. sobrinus*, and *L. acidophilus* are found to be present in the oral cavities of both caries affected and caries free infants [104]. Hence, it's believed that caries is not caused by the presence of a specific pathogen but instead by the presence of a pathogenic population in the oral cavity [105]. A study indicated that over 80% of the mothers or primary care givers of the children with ECC had dental caries themselves [106]. There's no doubt that mothers play a crucial role in influencing and contributing to oral health and microbiota of infant's oral cavity [107-109]. A study using strain level metagenomic profiling proved the vertical transmission of microbiota from mother to neonate [110]. It was seen that unique and specific strains of *Bifidobacterium bifidum*, *Coprococcus comes* and *Ruminococcus bromii* obtained from mother's fecal and breast milk samples, accurately matched to the samples obtained from their offspring, the strains matched only with mother-infant pairs and didn't match with the microbiome of other families [110].

1.5. The effect of breast milk on neonatal oral microbiome

It is clear that the first microbial exposure occurs as placental microbiome [111] and due to the swallowed amniotic fluid [90][113], further microbial exposure occurs due to delivery either by VTD or CTD [114-117]. The skin to skin contacts of the neonate and the mother / primary care giver as well as the environment further shapes the microbial colonies of the neonate [118-120]. However, the next major factor that affects the infant oral microbiota is early nutrition [121]. Human breast milk microbiome plays crucial role in oral microbiome of the neonate [122]. It is observed that human breast milk contains many antimicrobial and growth enhancing factors that get transmitted to the neonate through breast feeding [123]. The microbiome of the human breast milk depends on various factors such as backflow of microbes during breast feeding, geography, gestational age and consumption of antibiotics [122][124]. This microbiome of the milk is stable in a breast-feeding mother [125]. Interestingly the process of breast feeding also causes transmission of microbes from the mouth of the neonate to the breast skin because the microbial transmission during breast feeding is bi-directional [126-129]. It was observed that prior to breast feeding, the breast milk microbiome dominantly consisted of *Staphylococcus MCT-2*, but post the first time breast feeding, the microbiome consisted of common neonatal oral microbes like *Streptococcus spp.*,

transmitted due to the back flow of microbes from neonatal saliva to breast milk [130,131].

A study involving deep sequencing of 16s ribosomal RNA of neonatal oral microbiome to compare the oral microbiome between breast fed and formula fed infants revealed major differences in the oral microbial composition [132]. Microbes like *Proteobacteria spp.*, which is abundantly present in breast milk, is found to be abundant in breast fed infants but not in formula fed infants [133]. Microbes like *Prevotella spp.*, which is present in vagina and oral cavity of the mother, is present only in formula fed infants and not breast-fed infants [134]. In fact, same strains of *Prevotella spp.*, is present in the saliva of mother and infant dyad, and this strain is unique to every mother and her offspring [135]. It's observed that the microflora of formula fed infants generally consists of *Firmicutes* and *Bacteroidetes* while that of breast-fed infants generally consists of *Firmicutes*, *Actinobacteria spp.*, *Proteobacteria spp.*, [136,137].

The reason for this difference lies in the composition of breast milk [138]. Breast milk contains enzyme xanthine oxidase (XO) which reacts with its substrates: xanthine and hypoxanthine present in large concentrations in infant's saliva [139,140]. This reaction yields hydrogen peroxide which can activate the lactoperoxidase system (LPS) of the milk, leading to the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which possess antimicrobial activity and affects microbial population drastically [132] [141-143]. Concentration of hydrogen peroxide due to breast feeding during the first week is reported to be 27 μ M [144]. A study involving In-vitro examination of the effect of a mixture of neonatal saliva with maternal breast milk on a culture of various oral microbes showed that over time the growth of the colonies drastically declined except in methicillin resistant *Staphylococcus aureus* [141]. Another mechanism known for this antimicrobial activity is- the superoxide formed when XO is exposed to molecular oxygen, reacts with nitric oxide and forms a bactericidal compound RNS peroxynitrite [145,146]. Hence the XO of the mammalian breast milk not only prevents microbial infestation in the mother's milk and milk glands, but also plays a key role in shaping the innate immunity of the neonate [147]. Other functions of human breast milk are to regulate the functioning and shape the microflora of neonatal gastrointestinal tract.

In mammals, XO is present in liver, small intestine and mammalian milk [141]. The XO of mammalian milk is more active in bovine milk than human breast

milk [148,149]. It is also seen that XO is completely absent in formula milk (manufactured from bovine milk) and pasteurised human breast milk [141]. This is the key reason for variation in oral microbiome of breast fed and formula fed infants.

The most abundant microbial family in the oral cavity of both breast fed and formula fed neonates is *Streptococcaceae* and this abundance is observed to increase with age [150-153]. Some *Streptococcus* spp. also produce antimicrobial hydrogen peroxide that inhibits the colonisation of harmful microbes like *Staphylococcus aureus* and *Pseudomonas aeruginosa* [154]. Breast milk also contains lactic acid bacteria (LAB) that restricts the colonisation of harmful microbes like *Enterococcus faecalis*, *Salmonella enterica*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* [155]. Most of these *Lactobacillus* spp., are found to be present in breast fed infants but not formula fed infants.

Many studies show that unique, beneficial and health inducing microflora of the breast milk gets transmitted from mother to neonate through the process of breast feeding [156]. Similarly, even harmful pathogenic microbes and viruses like HIV, Zika, Cytomegalovirus, *Candida* spp., *Klebsiella* spp., *Acinetobacter* spp., *Staphylococcus* spp., may get transmitted from mother to neonates through breast feeding [157-159]. *Bifidobacterium* spp., which is an important probiotic gut microbe is acquired due to breast feeding [160]. Colonies of *Pseudomonas* spp., which is a harmful oral pathogenic bacterium, is seen to decrease in number due to breast feeding [161].

CONCLUSION

The numerous studies reviewed in this article clearly indicate that the neonatal oral microbiota is greatly affected by the mode of delivery and mode of nutrition in early days of life. Infants delivered by VTD acquire a richer, health inducing oral microbiome while those delivered by CTD are more likely to have acquired pathogenic microbes. According to many studies, a vaginal seeding of CTD infants seems to partially restore the beneficial microbiome of VTD infants. Secondly, breast fed infants seem to have a healthier oral microbiota due to the indirect bactericidal action of XO present in mammalian breast milk but absent in formula milk. It is important to note that the oral microbiota plays a major role in early health and development of an infant. Hence it is crucial to study further the various factors and consequences of neonatal oral microbiome as well as to apply the results of these studies in medical and research fields.

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