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Stefano Guandalini
Flavia Indrio *Editors*

Probiotics and Child Gastrointestinal Health

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Shaping Microbiota During the First 1000 Days of Life

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Abstract

Given that the host-microbe interaction is shaped by the immune system response, it is important to understand the key immune system-microbiota relationship during the period from conception to the first years of life. The present work summarizes the available evidence concerning human reproductive microbiota, and also, the microbial colonization during early life, focusing on the potential impact on infant development and health outcomes. Furthermore, we conclude that some dietary strategies including specific probiotics and other-biotics could become potentially valuable tools to modulate the maternal-neonatal microbiota during this early critical window of opportunity for targeted health outcomes throughout the entire lifespan.

Keywords

Microbiota · Vagina · Diet · Health · Gestation · Antibiotics · Probiotics · Prebiotics

Anna Samarra and Eduard Flores contributed equally with all other contributors.

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1 Perinatal Microbial Environment and Its Relevance for Human Health

The human microbiota is defined as the complex communities of microorganisms that live on, as well as in, the human body, and it is principally composed of bacteria, but also of viruses, fungi, archaea and bacteriophages (Milani et al. 2017; Perez-Muñoz et al. 2017). Microbiota refers to the microorganisms present in one human niche and microbiome refers to the genetic potential of the microbiota. The microbiome is present in different sites of the human body, including the skin, oral, nasopharyngeal and genito-urinary tract (Milani et al. 2017). The differential microbiome compositions depend on the host's genetics as well as the host's environmental characteristics, including humidity, pH, nutrients and oxygen levels (Milani et al. 2017).

The microbiome is responsible for numerous essential functions within the human body, including assisting with digestion and metabolism, the production of vitamins, maintaining the gut barrier and regulating the development of the immune system (Gensollen et al. 2016; Rowland et al. 2018). Microbial alterations, named dysbiosis, has been associated with an increased risk of non-communicable diseases (NCDs), such as asthma, obesity, diabetes and autoimmune conditions (e.g. Crohn's disease) (Huang et al. 2023), all of which are characterized by the over-responsiveness of the immune system,

which in turn leads to an increasing pro-inflammatory status (Clemente et al. 2012; Koleva et al. 2015; Tamburini et al. 2016; Gensollen et al. 2016; Christ et al. 2019).

The first 1000 days of life (i.e. from gestation until the first 2 years of life), are crucial for both the colonization and the establishment of pioneer microorganisms within the human body and, additionally, for the development and maturation of the immune system. Hence, this period is considered to represent a 'window of opportunity' during which any event will have a pivotal impact on the metabolic, immunological and microbiological programming that affects later human health (Agosti et al. 2017). Any alterations and disruptions to the step-wise neonatal microbiota colonization have the potential to increase the risk and predisposition of individuals to developing diseases in the short- and long-term (Verdu et al. 2016). The maternal microbiota represents the most relevant prenatal and postnatal microbial source for the neonate, although external factors, including diet, play an important role on the microbial assembly and evolution during infancy and later, adult life. The present review, therefore, aims to describe the available evidence concerning early microbial (microbiota and their metabolites) exposure during the first 1000 days of life (Fig. 1). The review also provides an overview of the role of microbes and their metabolites in the maternal-neonatal dyad. Additionally, the potential offered by the application of dietary interventions (Mediterranean Diet and /or use of dietry supplements from the -biotics family) during this critical window of opportunity is also discussed.

2 Preconception Microbial Environment: Reproductive Microbiota

In healthy non-pregnant women, the vaginal microbiota is a complex ecosystem populated by more than 200 bacterial species, with *Lactobacillaceae* members representing the dominant species, followed by other less abundant bacteria such as *Prevotella, Streptococcus, Bacteroides,* and *Veillonella* (Mendling 2016; Lebeer et al. 2023). A recent study has described a new specie, *Lactobacillus isalae* sp. nov., isolated from the female reproductive tract (Eilers et al. 2023). It is important to mention that there is a recent reclassification of *Lactobacillus* taxonomy (Zheng et al. 2020) and some names have been changed. Based on the composition of the *Lactobacillus*, five types of microbial communities that differ in terms of both their composition and their abundance have been described (Ravel et al. 2011). Each community, known as the 'community state types' (CSTs), with each group hosting a specific bacteria: *Lactobacillus crispatus* (CSTI), *Lactobacillus gasseri* (CSTII), *Lactobacillus iners* (CSTIII), diverse group (CSTIV; exhibiting a lower presence of *Lactobacillus spp.*) and *Lactobacillus jensenii* (CSTV) (Ravel et al. 2011). The prevalence of the different CSTs differs according to the individual's geographical location, ethnic origin and socio-economic status. For example, a higher abundance of CSTIV among African-American and Hispanic women has been reported in the USA (Stout et al. 2017). The *Lactobacillaceae* members play an important role in both the maintenance of a low pH and the secretion of metabolites in order to prevent pathogenic colonization in the vagina (Aagaard et al. 2012; Ravel et al. 2011). A growing body of scientific evidence has demonstrated the potential use of the *Lactobacillus* species as biomarkers of vaginal health (Petrova et al. 2015). However, the vaginal microbiota does not remain static. Indeed, temporal dynamics have been found in the vaginal microbiota over a two-week period (Gajer et al. 2012), whereby some communities changed and others remained relatively stable, depending on the CST. Recent study involving 3345 women in Belgium (18–98 years) revealed that vaginal microbiota characterized by *Lactobacillus crispatus*, *Lactobacillus jensenii* and

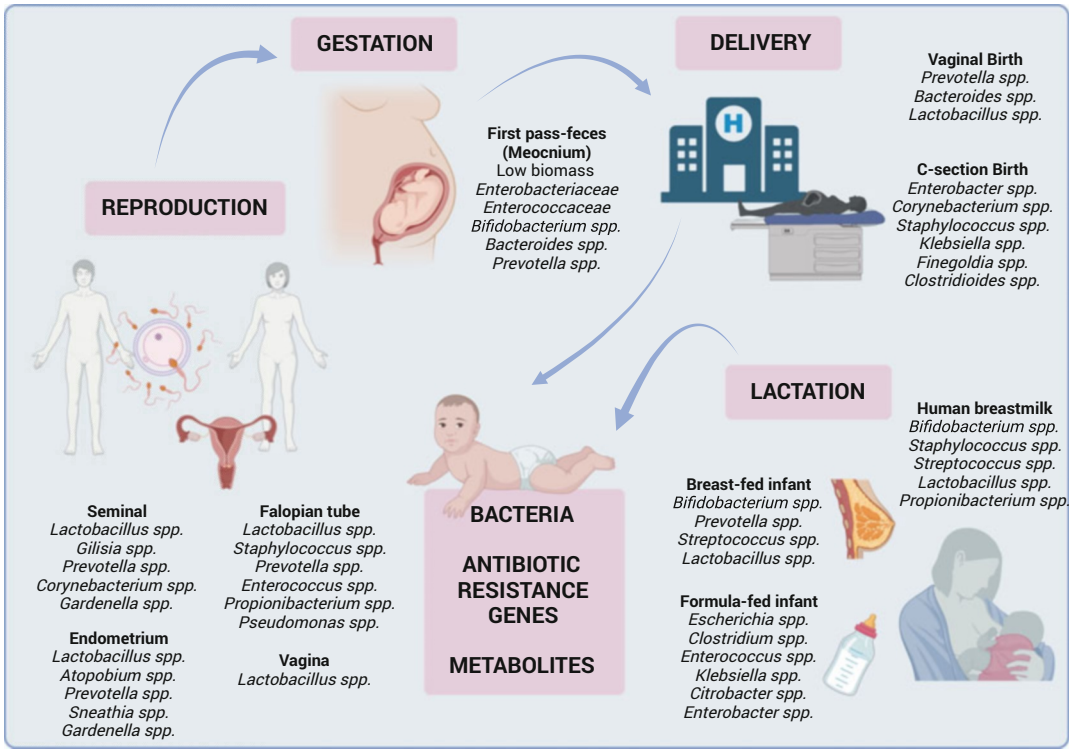

Fig. 1 Early microbiota and its metabolites contact from conception to first years of life. Overview of the microbes present in the male and female reproductive tract. This microbial environment is present before egg fertilisation and would play a key role in fertility, as well as in the fertilised egg and later in fetal development. In addition, changes in the microbiota during pregnancy would support fetal development, and some studies have reported the presence of microbial metabolites and/or microbial-derived extracellular vesicles in the maternal-fetal interphase. The first neonatal feces, meconium, harbours low microbial biomass and higher diversity. These microbial pioneers are influenced by the mode of birth (Caesarean, vaginal) and later modulated by breastfeeding practices (breast milk, mixed and formula milk). It is important to note that breast milk contains bacteria and other organisms, microbial metabolites, extracellular vesicles, immune-related components, among others, which are transferred to the infant and exert potentially beneficial effects. Early life is a critical period where microbial exposure is key to promoting short and long term health outcomes. (*Figure created using BioRender*)

Limosilactobacillus taxa, exhibited a positive association with hormonal changes (oestrogen levels and contraceptive use) (Lebeer et al. 2023). Conversely, this microbiota was negatively linked to factors such as childbirth and breastfeeding. Additionally, the same study (Lebeer et al. 2023) showed that vaginal microbiota enriched in *Gardnerella*, *Prevotella* and *Bacteroides* was associated with menopause, menstrual hygiene and contraceptive use. Other studies have also highlighted the need to analyze the host factors that affect the vaginal microbiota, since little is currently known about the impact it has on the different bacterial communities or the short- and long-term impacts on the individual's overall health status (Witkin 2018).

The female reproductive system contains bacteria that have an impact on women's health also outside of the vagina (Chen et al. 2017; Younes et al. 2018). Several prior studies have demonstrated the presence of microbiota in the upper female reproductive tract (UFRT), including endocervix, uterus, fallopian tubes, and ovaries, characterized by low biomass and also,

linked to both reproductive and uterine health (Benner et al. 2018; Moreno et al. 2016; Chen et al. 2017; Miles et al. 2017; Tao et al. 2017; Gholiof et al. 2022; Canha-Gouveia et al. 2023). The endometrial microbiota is characterized by a high amount of *Lactobacillus*, followed by *Gardnerella*, *Prevotella, Atopobium* and *Sneathia*, which have also been identified in the vagina (Moreno et al. 2016; Moreno and Franasiak 2017). However, it appears that the endometrial bacteria population differs somewhat from that in the vagina, which suggests that the two microbiotas are related, but not identical (Franasiak and Scott 2017). Furthermore, the fallopian tubes are known to be colonized by bacteria such as *Lactobacillus, Staphylococcus, Enterococcus, Prevotella* and *Propionibacterium*, with *Pseudomonas* being the identified genus (Pelzer et al. 2018). Importantly, recent reviews have highlighted the link between microbiota, fertility and pregnancy complications as well as cancer risk and development (Franasiak and Scott 2017; Moreno and Franasiak 2017; Power et al. 2017; Gholiof et al. 2022; Di Simone et al. 2020; Łaniewski et al. 2020).

Yet, very little research has previously been conducted concerning the male microbiota identified in the reproductive tract. Recent studies have demonstrated the presence of microbiota in semen, with the Bacillota representing the most predominant phyla, followed by the Bacteroidota, Pseudomonadota and Actinomycetota, of which *Lactobacillus, Prevotella, Gillisia, Corynebacterium* and *Gardnerella* are the most common genus (Mändar et al. 2015). It has been hypothesized that the predominance of *Gardnerella vaginalis* in the vaginal microbiota is related to inflammation challenges in males, which could be related with colonization/infection from the vagina (Mändar et al. 2017). It has further been suggested that a link exists between the male and female reproductive microbiomes that have an effect on fertility, reproduction, health and gestation. In fact, the majority of prior semen microbiome studies have focused on the issue of infertility, highlighting differences in the microbial profiles and diversity (Weng et al. 2014; Hou et al. 2013; Monteiro et al. 2018). It has been reported that male microbiota is influenced by different factors including environment (Gürsoy et al. 2023). In addition, the seminal microbiota may interfere with both conception and pre-term delivery. It has been reported that a higher presence of typical seminal bacteria, such as *Lactobacillus iners*, during pregnancy is associated with pre-term deliveries, whereas the dominance of *Lactobacillus crispatus,* another common bacterial species in semen, has been identified as protective (Bennett 2017).

Taken together, women's and men's reproductive microbiomes appear to be highly relevant to reproductive medicine, with the studies suggesting the potential of microbes as biomarkers for potentially aiding the development of new tools for diagnosing and treating infertility. In this scenario, the human reproduction takes place under "microbial environment" and those microbes and their metabolites must have greater attention due to their relevance from preconception onwards.

3 Women Microbiota During Pregnancy: What Is Known?

During pregnancy, the women physiology and metabolism are adjusted in order to afford the fetus an optimal intrauterine environment and so promote correct growth (Wahlqvist et al. 2015). Gestational changes, such as endocrine, immune and metabolic alterations provide the fetus with an optimal intrauterine environment to promote a healthy development. Those alterations are also reflected in changes favouring a proinflammatory status, which is reflected in specific microbial shifts seen in the maternal microbiota at different body sites, including the vagina, gut and oral cavity (Nuriel-Ohayon et al. 2016; Calatayud et al. 2019; Turjeman et al. 2021). Thus, further research concerning the association between microbial markers and pregnancy outcomes could prove to be instrumental in preventing undesirable microbial changes during pregnancy, which may be linked to pregnancy complications.

3.1 Vaginal Microbiota

It has been reported that vaginal microbial diversity decreases, while members of *Lactobacillus* members increase, potentially reinforcing their protective function (Romero et al. 2014; MacIntyre et al. 2015). There were significant differences in terms of individual species, in particular, *Lactobacillus crispatus* was associated with samples from a full-term pregnancy, whereas one community state-type was associated with samples from preterm pregnancies (Feehily et al. 2020). It has further been demonstrated that women whose medical history includes repeated urinary tract infections exhibit an increased risk of pre-term delivery. Differences in the vaginal microbiota in terms of the composition, stability and diversity have been observed between full-term deliveries and pre-term deliveries (Nuriel-Ohayon et al. 2016). Presence of *Atopobium*, *Gardnerella* and *Ureaplasma spp*., as well as lower levels of *Lactobacillus* members or a higher presence of *Candida albicans*, have been found to be linked with preterm birth risk (Bretelle et al. 2015; DiGiulio et al. 2010; Farr et al. 2015; Hyman et al. 2014). The detection of abnormalities within the vaginal microbiome during gestation could therefore be used as microbial biomarker for predicting the likelihood of pre-term delivery, particularly when the vaginal microbiota is examined during the first trimester of pregnancy.

A recent study reported that vaginal microbiota transplantation (VMT) without antibiotics with a donor engraftment had a resolution of vaginal dysbiosis and live birth after recurrent pregnancy loss (Wrønding et al. 2023). These studies suggest the VMT as a potential treatment for severe vaginal dysbiosis with adverse fertility and pregnancy outcomes, however, this case report is a proof-of-concept and further studies are needed.

3.2 Gut Microbiota

While during the first trimester of gestation the composition of the gut microbiota remains stable and resembles pre-pregnancy microbiota, from the end of the first trimester onwards its composition changes radically (Magon and Kumar 2012; Calatayud et al. 2019; Koren et al. 2012; Nuriel-Ohayon et al. 2016). These changes reflect a more pro-inflammatory profile and include an increase in Pseudomonadota (*Enterobacteriaceae* family members) but also, Actinomycetota (mainly *Bifidobacterium* members) phyla in late pregnancy as well as a decrease in short chain fatty acid (SCFA) producers. In addition, the gut microbiome has been linked to multiple pregnancy phenotypes including weight gain, low-grade inflammation and insulin resistance (Koren et al. 2012). However, other studies have reported no significant gut microbial changes during pregnancy (DiGiulio et al. 2015), which indicates that further research is needed to clarify the impact of pregnancy on the gut microbiota. In addition, it has been widely demonstrated that antibiotic exposure during pregnancy alters the mother's microbial ecosystem and, consequently, that of the offspring (Stokholm et al. 2014). Antibiotic use during gestation increases the vaginal colonization by the *Staphylococcus* species and, additionally, potential microbial shifts in other areas than the gut could be linked to an increased risk of allergies and obesity (Kuperman and Koren 2016).

Furthermore, the women microbiota is influenced during pregnancy by both the mother's diet and nutritional status also having an impact on the microbiota composition of their offspring (Lundgren et al. 2018; Barrett et al. 2018; Mandal et al. 2016; Garcia-Mantrana et al. 2020). Human studies suggests that changes in the female gut microbiota during pregnancy are vulnerable to modulation by the maternal pre-gestational body max index (BMI), as well as to weight gain over gestation (Collado et al. 2008; Collado et al. 2010; Santacruz et al. 2010). A lower presence of *Bifidobacterium* spp. has been observed in overweight and obese mothers, as well as in

mothers who gained excessive weight during pregnancy, when compared to lean mothers or to those mothers who maintained a weight gain in keeping with recommendations (Collado et al. 2008). Another study reported similar shifts according to weight status during pregnancy and lower levels of *Bacteroides* spp., along with higher abundances of *Staphylococcus* and *Escherichia coli* spp. were identified in overweight pregnant women (Santacruz et al. 2010). Such changes in the maternal gut microbiota could be associated with differences in the intestinal colonization process in neonates born via vaginal delivery (Mueller et al. 2016).

3.3 Oral Microbiota

Pregnancy also induces changes in the oral microbiota (Adriaens et al. 2009; BORGO et al. 2014; Ye and Kapila 2021). In fact, significant differences have been reported when comparing the abundances of several species in the oral cavities of pregnant and non-pregnant women. The oral microbiota during gestation is characterized by an increase in the viable bacterial counts, along with higher levels of pathogenic bacteria, such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans* and *Candida* genus which validates the growing body of evidence proving the link between oral infections and periodontal disease associated with pregnancy complications, including pre-term delivery. There is also evidence of correlation between oral dysbiosis during pregnancy and increased risk of gestational diabetes, pre-eclampsia, and perturbed fetal programming (Offenbacher et al. 2006; Zi et al. 2015; Fujiwara et al. 2017; Nannan et al. 2022). These data suggest that the oral microbiota also plays a key role in the maternal and neonatal outcomes, including pregnancy complications, risk of pre-term birth and early fetal/neonatal microbial colonization.

4 Early Microbiota and Microbial Metabolites Exposition: *Where Are We Now?*

The '*in utero colonization hypothesis*' has been subject to a huge debate, and despite a number of studies having been conducted in the field in recent years, the identification of "microbiota" in the maternal-fetal interphase has not been demonstrated. Critical issues observed in the literature include the low amount of DNA, the potential bias stemming from the contaminant DNA, where its detection, and filtering will increase the risk of false positive results (Lauder et al. 2016; Leiby et al. 2018; Theis et al. 2020a, b; K. M. Kennedy et al. 2021; Kennedy et al. 2023). Due to the amount of false positive results, a significant amount of effort have been expended in identifying new techniques that should reduce the amount of false positive results with regard to the microbiota analysis and also facilitate the contaminant detection in samples with a low microbial biomass (Avershina et al. 2018; de Goffau et al. 2018; Davis et al. 2017).

Despite the debate over the presence of microbial exposure *in utero* (Theis et al. 2019; K. M. Kennedy et al. 2021; Leiby et al. 2018; Kennedy et al. 2023; Theis et al. 2020a, b), there is significant evidence that maternal microorganisms, and specifically their byproducts, including DNA, cell fragments and metabolites, have key roles in fetal immunity. Recent studies have shown the impact of maternal microbiota on the placenta physiology and structure (Pronovost et al. 2023; Lopez-Tello et al. 2022) and also, on the fetal development (Vuong et al. 2020; Husso et al. 2023).

A unique metabolome profile in the fetal gut with an abundance of bacterially derived and host-derived metabolites commonly produced in response to microbiota has been reported (Y. Li et al. 2020). Furthermore, it has been reported that the presence of fetal DNA (Liao et al. 2014) and placenta-derived exosomes (Lai et al. 2018) in the maternal circulation actually evidence their potential association with placenta health, pre-eclampsia and the risk of pre-term birth (Taglauer

et al. 2014; Van Boeckel et al. 2018; Seval et al. 2015). In addition, a recent study has reported the presence of microbial-derived extracellular vesicles in the amniotic fluid of healthy pregnant women, exhibiting similarities to extracellular vesicles found in the maternal gut microbiota and suggesting a new maternal-fetal communication and also, a neonatal immune priming (Kaisanlahti et al. 2023). Human milk oligossachardies (HMO) have been also detected in amniotic fluid (Jantscher-Krenn et al. 2022) and specific changes were reported during pregnancy. The amniotic fluid is continuously swallowed by the fetus, creating contact with the fetal gut throughout pregnancy, so the presence of microbial metabolites, extracellular vesicles and/or HMO would suggest some immune-host interactions and the complex links between the mother-fetus interphase. In addition, it has been reported that maternal gut microbiome and its metabolites are associated to adequate placenta structure and function as well as relevance for fetal growth in animal models (Lopez-Tello et al. 2022; Pronovost et al. 2023).

In medical practice, the presence of bacteria within the amniotic fluid represents an unhealthy condition due to causing an amniotic cavity infection, namely chorioamnionitis, which is the cause of approximately 25% of preterm births (Romero et al. 2014). Indeed, some authors have reported that the bacterial composition of the amniotic fluid in chorioamnionitis patients could be used as a biomarker of the stage of inflammation (Urushiyama et al. 2017). These microbiota is featuring the notable presence of *Ureaplasma* and *Mycoplasma* species (DiGiulio et al. 2008, 2010; Combs et al. 2014) and also, *Fusobacterium* (Prince et al. 2016). Research has identified a relation between inflammatory oral disease and pregnancy complication, such as the existence of periodontitis, and preterm delivery. It has been observed an incremental increase in the oral commensal bacteria in the placental microbiome of preterm subjects with chorioamnionitis (Prince et al. 2016). This suggests the particular relevance of *Fusobacterium nucleatum*, which is a common oral bacterium found in the placenta, in relation to negative pregnancy outcomes (Vander Haar et al. 2018), including preterm birth (Doyle et al. 2014), neonatal sepsis (Wang et al. 2013) and hypertension (Barak et al. 2007). The proposed oral-fetoplacental route has been demonstrated in animal models (Fardini et al. 2010). On the other hand, it has been demonstrated in animal models that microbial translocation from the gastrointestinal (GI) tract is augmented during pregnancy (Donnet-Hughes et al. 2010). This translocation may be mediated by the complex bacteria-immune-system relations that are, in turn, mediated by the dendritic cells in the GI tract (Rescigno et al. 2001). Studies conducted in animal models have reported that orally administered *Enterococcus faecium* was found in both the placenta tissue and meconium (Jiménez et al. 2008). A similar model reported the physiological translocation of ingested *Lactobacillus* strains containing lux genes to the mammary tissue and milk, as well as in the kidney, liver and spleen (de Andrés et al. 2018).

5 Maternal-Neonatal Vertical Transmission: What Is the Scientific Evidence?

A growing body of evidence has demonstrated the vertical bacterial transmission from mothers to their children (Asnicar et al. 2017; Duranti et al. 2017; Dominguez-Bello et al. 2010; Ferretti et al. 2018). Vertical transmission is a remarkable process where bacterial strains are exchanged between mothers and new-borns, sowing the seeds of the infant gut microbiome (Selma-Royo et al. 2019). While mother-infant microbiota transfer is well-documented, specific strain transmission is less understood (Edwards et al. 2022; Mikami et al. 2012). Researchers explore various maternal sources, including feces, breastmilk, the vaginal microbiome, and skin, to uncover the origins of these crucial bacterial strains. Key factors influencing infant gut colonization include gestational age at birth, vaginal delivery, prenatal exposures, genetics, antibiotics usage, and exclusive breastfeeding (Houghteling and Allan Walker 2015).

5.1 Vertical Transmission of Specific *Bifidobacterium* Members

Bifidobacterium stands out as the most frequently transmitted microbe from maternal feces to the infant's gut, where the primary sources for transmission are breastmilk and feces, with variables like gestational age at birth, birthplace, delivery method, and antibiotic use potentially influencing bifidobacterial status (Feehily et al. 2023; Fehr et al. 2020; Ferretti et al. 2018; Asnicar et al. 2017). The early normal colonization of the gastrointestinal tract is known to collaborate in the developing of the immune system, and in maintaining an homeostasis via modulation of the environment to stablish an adequate crosstalk and ecosystem (Pantazi et al. 2023; Xiao and Zhao 2023). A longitudinal study showed that *B. longum* and *B. Brief* strains, seem to persist in the microbial ecosystem of the infant from birth until 4 months (Ferretti et al. 2018). However, as we delve deeper into the narrative, we encounter that the persistence of these strains is not assured (Asnicar et al. 2017). It is also important to highlight the roles of breastmilk and maternal gut as main sources for vertical transmission from mothers to their new-borns (Rodríguez, 2014; Qi et al. 2022a, b). Through the lens of metagenomic sequencing, we gain insight into the maternal gut-to-infant gut transmission (Feehily et al. 2023; Ferretti et al. 2018; Yassour et al. 2018; Qi et al. 2022a, b), denoting the influence of external factors—the location, the mode of birth and the use of antibiotics—each playing a role in shaping the infant microbiota (Feehily et al. 2023; Manara et al. 2023). *Bifidobacterium* species exhibit meaningful persistence and adaptation. Together with bioactive compounds found in breastmilk, such as HMO, they play a crucial role in facilitating cross-feeding, which, in turn, supports the maturation of the infant's gut microbiome and immune system (Arzamasov et al. 2022).

5.2 The Dark Side: Antibiotic Resistance Genes Transfer

Antibiotic resistance occurs when microorganisms develop new strategies to overcome and counteract the effects of antibiotics, so these become less effective to treat infections caused by microorganisms. This resistance acquisition is a natural process, but it's accelerated by the widespread use of antibacterial drugs (Fletcher 2015). Antibiotic resistant microorganisms (ARMs) are widespread and transferred between people, animals, and through animal-based food. They carry antibiotic-resistant genes (ARG), and the set of ARGs in an entire bacterial population is known as the resistome.

Early life, particularly perinatal and postnatal period, is a critical time for the development of the infant's gut microbiome and resistome. Moreover, the immune system of infants is unmatured, so they are particularly vulnerable to acquire and carry resistant pathogens. This leads to an estimated 214,000 neonatal deaths annually due to septic infections caused by antibiotic-resistant pathogens (Levy and Marshall 2004). The main source of antibiotic-resistant microbial strains, the resistome, in an infant's gut is uncertain; although maternal microbiota would be the main source, other individuals and also, environment are relevant and may contribute to the infant resistome (Pärnänen et al. 2018). Microbes are transmitted from mothers to offspring during birth, breastfeeding, and close contact. In this exchange, the oral microbiota of breastfed infants plays a crucial role as it serves as the gateway for milk to reach the gastrointestinal tract (Biagi et al. 2017). Various perinatal factors like C-sections, antibiotic exposure, prematurity, and breastfeeding practices influence the vertical transmission of microbes from mothers to infants (Korpela 2021; Li et al. 2021). However, the impact of these factors on the transmission of antibiotic-resistant genes is not fully understood. Some studies have elucidated the presence of ARGs present in newborns shortly after birth, suggesting initial colonization by ARMs occurs soon after birth, likely from contact with the

mother or the hospital environment (Carvalho et al. 2022). Some studies indicate the transmission of resistant bacteria from mothers to infants, including various antibiotic-resistant strains like *E. coli*, *Streptococcus*, and others (Sáez-López et al. 2016; McDonald et al. 2003).

Breastfeeding is known for its beneficial impact on infant health, providing nutrients and bacteria to the infant. However, human milk appears to be one of the sources of resistant bacteria and ARGs for the developing infant. Resistant strains have been identified in human milk (Pärnänen et al. 2018; Huang et al. 2019). Metagenomic analysis have revealed high levels of ARGs and genetic elements in breast milk, resembling the infant's resistome to that of the mother's milk (Pärnänen et al. 2018). This suggests that the mother plays a role in developing the infant's gut resistome through the transfer of resistant bacteria, although it is important to consider that these genes could also have come from sources other than the mother where horizontal gene transfer also plays a very important role (Samarra et al. 2023). More research is needed to understand the vertical transmission of resistant bacteria at a strain level to comprehend its impact on the initial gut colonization in infants. This deeper understanding will assist in developing strategies to control the spread of antibiotic-resistant genes across the mother-neonate interface.

6 Neonatal Microbiota Establishment and Assembly: What Is Known?

At birth, the neonate encounters an immense quantity and diversity of microbes, as well as other organisms from the vagina and maternal gut, in addition to those from the environment. Neonatal colonization is a sensitive and dynamic step-wise process that is particularly relevant to health. Early and diverse step-wise microbial establishment and specific production of SCFAs are essential for adequate immune mucosal and systemic maturation (West et al. 2015; Fusco et al. 2023; Gensollen et al. 2016). However, the exact steps of microbial colonization—including the order and timing of arrival of specific species/consortia and their impact on the assembly and development of the microbial community–are not fully understood.

6.1 First Pass Neonatal Microbiota: Who Are the Pioneers?

Different studies have reported the presence of bacteria in the first-pass feces, known as meconium, as determined by culture dependent and independent methodologies (Hansen et al. 2015; Gosalbes et al. 2013; Jiménez et al. 2008). Some authors have suggested that microbial contact could start during uterus contraction and membrane rupture (Rehbinder et al. 2018), thereby explaining the presence of bacteria in the meconium.

The first-pass microbiota is composed of species from the Pseudomonadota and Bacillota phylum, such as the *Enterobacteriaceae* and *Enterococcaceae* families (Hansen et al. 2015; Moles et al. 2013), which are typically present in the microbiota during adulthood to a greater degree than during infancy, reflecting the maternal origin of such bacteria.

In addition, other genera were also found, including *Bifidobacterium, Bacteroides* and *Prevotella* (Hansen et al. 2015). First pass meconium microbiota showed higher microbial diversity and richness compared to 7 days neonatal microbiota (Mueller et al. 2017) suggesting that neonates at birth are massively exposed to microbes but just few of them are able to colonize and persist in the gut for later evolve in a step-wise process during first year of life.

Several factors influence meconium microbiota: mode of birth, antibiotic exposure, prematurity, timing of first-pass fecal sample collection as well as different maternal factors including health status (Turunen et al. 2023; Klopp et al. 2022). Infants born via elective Caesarean section showed a similar meconium microbiota profile to those born via vaginal delivery and non-elective Caesarean section (Chu et al. 2017). The intrapartum antibiotic prophylaxis

could alter the intestinal microbiota during the first week of life, including a lower presence of Actinomycetota and an steady increase in the Pseudomonadota and Bacillota proportions (Nogacka et al. 2017; Nogacka et al. 2018). Indeed, another study reported that antibiotics administered during delivery could also modify the oral microbiome in neonates, thereby resulting in a higher abundance of families from the Pseudomonadota phyla following the antibiotic treatment (Gomez-Arango et al. 2017). This could have important consequences in terms of child development. Relatedly, it was observed that children who were exposed to antibiotic treatment during the second and third trimesters of gestation had an 84% higher risk of developing obesity (Mueller et al. 2015). In addition, there are several studies reported that preterm microbiota is unbalanced (Hiltunen et al. 2021; Underwood and Sohn 2017; Drell et al. 2014). For example, it has been shown that the preterm meconium microbiome induced altered immune function, growth restriction and changes in hormonal levels in germ-free mice after fecal transplant from preterm compared to term infants (Hiltunen et al. 2021). However, the preterm neonates had other comorbidities due most of them were born by C-section, were exposed to antibiotics at birth and during hospitalisation; as well as they were fed with donor-milk and spent more time in the neonatal intensive care units. There is also a growing body of evidence that in utero inflammation leads to increased perinatal complications, but whether this is related to the maternal microbiome is unclear (Ansari et al. 2021).

The association between infant health and mother-infant transmission of microbiota has may be driven by factors such as maternal diabetes (Hu et al. 2013), atopic disease (Gosalbes et al. 2013), diet (Chu et al. 2016), BMI (Mueller et al. 2016) and perinatal antibiotic use (Gosalbes et al. 2016; Mueller et al. 2015), where researchers observed that the meconium of infants born to mothers with pre- GDM harboured an enrichment of bacteria normally observed in adult diabetic patients (Call et al. 2013). A high-fat maternal diet influences the meconium microbiota with regard to a notable depletion of the *Bacteroides* genus (Chu et al. 2016). However, it remains unclear which and how long the meconium shifts would be maintained for during the early years of life. Additionally, the potential effects on the immune system, metabolism and health outcomes are currently unknown. Moreover, the molecular mechanisms by which meconium microbiota affect immune system development are not yet fully understood despite the growing body of evidence concerning the immunomodulatory activity of the gut microbiota in disease and health during both childhood and adult life (Nash et al. 2017; Dzidic et al. 2018). It is also important recognized the role of microbial metabolites in the immune training and health outcomes. Recent studies showed that the microbial metabolites in the fetal environment can have a major impact on neonatal development and immune system maturation (Gómez de Agüero et al. 2016; Petersen et al. 2021). It has been established that microbial products and metabolites can reach the systemic circulation and hence arrive in different places. These observations highlight the potential impact of microbes and their metabolites during gestation and, further, suggest the need to increase our knowledge in order to promote infant health.

6.2 What Are the Factors that Contribute to Infant Microbiota Evolution from Birth to the First Years of Life?

The neonatal gut microbiota is characterized by a low microbial diversity and richness as well as a relative higher abundance of Pseudomonadota and Actinomycetota phyla. The initial and Pioneer Microbiota, consisting of members from Bacillota and Bacteroidota phylum, *Enterobacteriaceae* family members, *Veillonella* and especially *Bifidobacterium* genera (Gensollen et al. 2016; Jost et al. 2012; Houghteling and Allan Walker 2015; Tanaka and Nakayama 2017), is responsible for initial immune system training and also, it provides the favorable environment for further microbial species settlement

(Jost et al. 2012). Subsequently, the gut microbiota evolves and it becomes more diverse, resembling the adult composition by between 2 and 5 years of age (Rodríguez et al. 2015). Thus, the neonatal colonization is a fragile, dynamic and step-wise process and it would be affected by a number of maternal-neonatal factors as well as environmental influences. Among them, the most relevant ones include: gestational age, mode of delivery (vaginal versus C-section), place (hospital vs home), and mode of feeding (breast milk, mixed diet and/or infant formula) as well as maternal health status, genetics, medication, environmental exposure to xenobiotics or physicochemical agents, geolocation, siblings, pets, and other factors (Rodríguez et al. 2015; Gomez-Gallego et al. 2016; Stewart et al. 2018) and also, person-to-person horizontal microbial transmission (Valles-Colomer et al. 2023).

It has previously been demonstrated that neonates born vaginally have a microbiota resembling the vaginal and gut maternal microbiome, enriched with *Lactobacillus* and *Prevotella* species (Dominguez-Bello et al. 2010), although other bacteria, such as the *Enterobacteriaceae* family, including *Escherichia* or *Klebsiella* genera, are also present. Neonates born via Caesarean section presented a distinct microbiota similar to the oral, skin and environmental microbiota (Dominguez-Bello et al. 2010). The maternal gut also appears to be an important source of early colonising bacteria, since 72% of gut bacteria in vaginally delivered newborns have been found to be of maternal intestinal origin as compared to 41% in subjects born via Caesarean section (Bäckhed et al. 2015), where the delivery mode has highlighted *Bifidobacterium* members as the pioneering bacteria in the process of neonatal infant gut seeding (Milani et al. 2017), and infants born via Caesarean section have exhibited delayed colonization of the *Bacteroides* genus as well as reduced diversity (Jakobsson et al. 2014). After birth, breastfeeding practices both supports the microbial gut colonization and drives the immune system maturation (Cacho and Lawrence 2017; Riskin et al. 2012; He et al. 2014; Turfkruyer and Verhasselt 2015). Differences between breastfed and formula-fed neonatal microbiota have been reported (Pannaraj et al. 2017; Guaraldi and Salvatori 2012; Madan et al. 2016), moreover the cessation of breast-feeding, rather than the introductions of complementary and solid food, have had a key impact on the infant microbiome (Bäckhed et al. 2015). It is important to mentioned that traditionally, human milk was considered to be sterile. Nonetheless, the evidence has demonstrated the presence of viable microorganisms including *Staphylococcus* and *Streptococcus* as the most abundant microbial groups followed by lactic acid bacteria, *Bifidobacterium* and certain Pseudomonadota (*Pseudomonas* and *Acinetobacter*) in breast milk samples (Selma-Royo et al. 2021). A recent study showed that the infant gut derives 27.7% of its bacteria from breast milk and 10.3% from the areola skin (Pannaraj et al. 2017), thereby highlighting the potential impact of breastfeeding on the infant gut microbiota. In addition, shared microbial and viral species between breast milk and the infant gut have been identified (Martín et al. 2009; Pannaraj et al. 2018; Edwards et al. 2022).

Other factors have also been widely studied to influence infant microbial development, such as antibiotics, gestational age, host genetics and other environmental factors, including geographical location, rural areas, siblings, pet ownership, etc. (Selma-Royo et al. 2021; Chong et al. 2018; Milani et al. 2017). These factors, individually or in combination, have been shown to exert an impact on the microbiota engrafment and immune system maturation (Dzidic et al. 2018).

7 Tools to Modulate the Maternal-Infant Microbiota During Early Life: From Conception to 2 Years of Life

Diet is a key factor in terms of shaping our gut microbiota (Graf et al. 2015). Dietary and nutritional counselling, as well as nutritional supplementation, are hence recommended in order to achieve an adequate nutritional status during the preconception and gestational stages (Scholl 2008). It has recently been reported that the maternal diet during pregnancy as well as during subsequent breast feeding has a key impact on both maternal and infant microbiota (Lundgren et al. 2018; Barrett et al. 2018; Mandal et al. 2016; Garcia-Mantrana et al. 2020). Maternal diet mainly the plant protein and fibers intakes influenced the breast milk composition including microbiota (Cortes-Macías et al. 2021), lipids (Calvo-Lerma et al. 2022), HMO (Selma-Royo et al. 2022) and also, microRNA expression profile (Yeruva et al. 2023). The field is growing and accumulating evidence on the effect of Mediterranean diet (MD) during pregnancy is able to improve maternal-neonatal health outcomes including growth restriction (Crovetto et al. 2022), fetal and neonatal brain development (Nakaki et al. 2023), child neurodevelopment (Crovetto et al. 2023). MD during pregnancy, mainly higher consumption of fruits, vegetables and legumes with reduced intake of red meat, has been associated to *Acidaminoacaeae* family level and also, a higher abundance of SCFAs producers (Miller et al. 2021). Higher intake of vegetable protein, fiber and polyphenols have been associated to higher presence of *Ruminococcus* genus, *Christensellaceae* family, *Dehalobacterium* and *Eubacterium* genus and SCFAs producers (Garcia-Mantrana et al. 2020). On-going studies with 20-year follow-up after a randomized controlled trial of a Mediterranean diet in pregnancy studying maternal-infant cardiovascular risk factors will provide novel data on the diet-health during pregnancy (Troensegaard et al. 2023).

Most of the available data on diet-pregnancy-microbiota is focused on gut microbiota, but it has been reported that increased intake of fruit, vitamin D, fibre and yogurt was associated with *L. crispatus* in vaginal microbiota among black women; fibre intake was associated to higher heterogeneity (Rosen et al. 2022). Limited evidence shows the impact of diet on vaginal microbiota and pregnancy outcomes. In this scenario, it remains to be established which foods, food components, nutrients and other dietary compounds influence the women microbiota (gut, oral and vaginal) during the perinatal period and the potential impact on maternal-neonatal outcomes (García-Mantrana et al. 2016). Taken together, these observations explain the increasing research interest in perinatal dietary interventions, as well as in the use of probiotics, prebiotics and symbiotics during pregnancy in order to modulate the microbiota and promote a 'favourable microbial vertical transmission'. There is some evidence of a protective role for probiotics and less evidence for prebiotics and other -biotics when administered during pregnancy and lactation. It is important to note that vulnerable and sensitive populations require special safety considerations, including safety and potential reporting of adverse effects associated with pregnancy, lactation and infancy. Evidence shows that -biotics are a safe therapeutic tool during pregnancy, as no serious concerns have been reported. (Lindsay et al. 2013; Merenstein et al. 2023). Adverse effects associated with the use of probiotics and prebiotics do not pose a serious health risk to the mother or infant. A recent systematic review and meta-analysis reported the most common potential adverse effects, which include the risk of vaginal discharge, stool consistency and constipation. (Sheyholislami and Connor 2021).

7.1 Impact of Probiotics on Fertility

Vaginal infections affect the *Lactobacillus* equilibrium, favouring the growth of other bacteria that could have an effect on fertility