An Investigation into Differences in the Infant Intestinal Microbiome

Based on Method of Delivery

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Abstract

The infant intestinal microbiome is greatly influenced by method of delivery in relation to bacterial composition, diversity, and richness. The intestinal tracts of cesarean delivered infants are more likely to be colonized with skin microbiota like *Clostridium, Staphylococcus, Streptococcus,* and *Corynebacterium*, and have less bacterial diversity and richness than their vaginally delivered counterparts, who are more likely to be colonized with *Bacteroides, Bifidobacterium,* and *Lactobacillus*, which are common vaginal bacteria. These microbial differences make cesarean delivered infants more likely to develop allergies, asthma, eczema, gastrointestinal conditions, obesity, and diabetes, among other diseases. Though vaginal seeding has been proposed as a potential solution for microbial dysbiosis, it carries concerns of infection and safety, therefore, probiotics and breastfeeding are recommended as alternatives.

Keywords: Cesarean delivery, microbiome, bacterial colonization, microbial dysbiosis

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Introduction

Cesarean section delivery, though developed to allow birth when vaginal delivery poses danger to the mother or fetus, has been recently shown to have adverse effects on health, placing the cesarean delivered infant at greater risk for allergies, asthma, gastrointestinal diseases, and obesity, among other afflictions. These conditions have been linked to changes in the intestinal microbiome, which is established around the time of the birth process and is related to the mode of delivery. Microbial differences include not only variations in bacterial species but also in bacterial diversity and richness. In order to fully understand the correlation between cesarean section delivery, differences in the infant intestinal microbiome, and later health outcomes, a basic comprehension of bacteria and the human microbiome must first be established.

The Human Microbiome

Importance of the Microbiome in Infancy

The human microbiome consists of all of the microorganisms that live in and on the human body, with the number of microbial cells outnumbering human cells ten to one hundredfold. The human genome numbers around 23,000 genes, but the intestinal microbiome encodes upwards of three million genes (Rinninella et al., 2019). The gut microbiome varies largely between people and has effects ranging from metabolism and biosynthesis to immune system development (Turnbaugh et al., 2007).

The foundation for the immune system and intestinal microbiome is laid within the first 1000 days after conception, which are comprised of pregnancy and the first two years of life

(Dzidic et al., 2018). This "window of opportunity," as it is called by Dzidic et al. (2018), is a crucial time to ensure the healthy development of the infant. Disruptions of the microbiome in this period may cause harmful effects later in life. Studies suggest that the entirety of the gastrointestinal tract is colonized before, during, and immediately after birth; therefore, the birth process and the moments soon after are integral to the formation of a healthy and stable intestinal microbiome (Salas Garcia et al., 2018; Wampach et al., 2018).

A healthy microbiome is essential in maintaining an energy balance and metabolism, resisting the invasion of potentially pathogenic microorganisms, and furthering the development of both the intestines and the immune system, all of which are of utmost importance in the developing infant (Dominguez-Bello et al., 2010). It is hypothesized cesarean delivery may cause microbial dysbiosis, specifically in the infant intestine, which has the potential to cause various pathologies related to metabolism and intestinal and immune system development (Dominguez-Bello et al., 2010). This dysbiosis is likely due to the disruption of normal colonization that occurs when the infant is not exposed to maternal vaginal microbes in cesarean birth, instead becoming exposed to skin and environmental microbes (Salas Garcia et al., 2018). Increasing evidence shows that an infant's initial exposure to microbes is important in defining the protective foundation of the immune system and other, more complex systems for later in life (Dominguez-Bello et al., 2010).

In their 2010 study, Dominguez-Bello et al. found that the entire infant microbiota was homogenously distributed shortly after birth (unlike the mothers, whose microbiota differentiated across body habitats) and that the major determining factor of microbial composition was the mode of delivery. Furthermore, they discovered vaginally delivered infants possessed microbiota

that resembled that of their mothers' vaginal communities, whereas infants delivered via cesarean section possessed microbiota that were most similar to their mothers' skin communities (Dominguez-Bello et al., 2010). This was expected because the vagina and skin are the first environments infants encounter during the vaginal and cesarean birth processes, respectively.

Importance of the Microbiome in Later Life

The intestinal microbiome assists in the maintenance of metabolism, provides protection from invasive microbes, and promotes the proper functioning of the immune system (Di Renzo, Tosto, & Giardina, 2018). By the time an infant reaches one year of age, his intestinal microbiota is moving toward that of an adult and will resemble an adult intestinal microbiota somewhere between the ages of two and five (McCann et al., 2018). The microbial communities of the intestines are integral in fighting opportunistic pathogens by competing with invading bacteria for nutrients, mediating inflammatory responses, and activating the innate and adaptive immune responses, which allows the maintenance of metabolism and overall health throughout life (Hand, 2016).

Deconstructing the Sterile Womb Hypothesis

The sterile womb hypothesis, which dates back to 1885, states the placenta and amniotic fluid are completely sterile, allowing the fetus to develop without microbial contact in utero (Perez-Muñoz et al., 2017). As Perez-Muñoz et al. (2017) describe, it was believed an infant's initial microbial acquisition occurred vertically from its mother during birth and horizontally from other humans and the surrounding environment after birth. According to this hypothesis, no microbial contact occurred before the beginning of the birth process.

Recently, evidence has arisen to the contrary; it is now believed microbial colonization of the infant begins in utero and continues through the birth process and throughout childhood (de Frietas et al., 2018). This new hypothesis stems from the discovery of bacteria both in meconium, the first feces passed by an infant that consists only of what was consumed in utero, and in amniotic fluid (Dzidic et al., 2018; Montoya-Williams et al., 2018). Interestingly, a study done by Bearfield et al. (2002) indicated amniotic fluid bacteria may come from the oral cavity rather than from the intestines or vagina. According to Aagaard et al. (2014), the placenta has a microbiome that is more similar to that of the oral cavity than to vaginal, anal, skin, and nasal microbiomes. Though placental microbes contribute minimally to the bacterial colonization of the infant microbiome, they are likely not the primary determinants of infant intestinal microbial communities.

Methods of Delivery

Cesarean Section

In the United States, cesarean delivery is the most frequent surgical procedure for women (Menacker, Declercq, & Macdorman, 2006). According to Hamilton et al. (2019), the rate of cesarean delivery in the United States was 31.9% in 2018. Although this is the lowest cesarean birth rate the United States has seen since 2009, it still indicates an increase in cesarean births of close to 50% since 1996 (Menacker, Declercq, & Macdorman, 2006). The cesarean birth process involves first making either a transverse or vertical cut through the skin of the abdomen and the abdominal wall; a cut is then made into the uterus, through which the baby is delivered, followed by cutting of the umbilical cord and removal of the placenta (The American College of Obstetricians and Gynecologists, 2018). This procedure, removing the baby through the

abdominal skin of the mother, prevents the infant from contacting the vaginal canal, and, therefore, prevents the infant from being colonized with the bacteria that make up the mother's vaginal microbiome (Stinson, Payne, & Keelan, 2018).

The difference between elective and emergency cesarean section lies in whether the procedure is necessary to preserve the health or safety of the mother or infant; this is determined by the presence of one or more factors indicating a cesarean section as beneficial to either the mother or infant. Some associations between cesarean delivery and certain diseases or conditions are dependent on whether the delivery is emergency or elective whereas other conditions are just as likely to occur as a result of emergency cesarean section as they are to be a result of elective cesarean section (Tribe et al., 2018). For example, Tribe et al. (2018) observed babies born by elective cesarean section were at greater risk of asthma, lower respiratory tract infections, and obesity than babies born by emergency cesarean section.

Elective Cesarean Section

There are conflicting opinions on the morality of performing elective cesarean sections, with the American College of Obstetricians and Gynecologists stating the procedure is ethically justified if the physician believes it will benefit the mother or infant and the International Federation of Gynecology and Obstetrics taking the opposite stance, asserting that elective cesarean section is not medically justified and, therefore, not ethically justified (Menacker, Declercq, & Macdorman, 2006). Some women request cesarean sections for fear of urinary and fecal incontinence, pelvic organ prolapse, or sexual dysfunction, although elective cesarean section is still not medically recommended to prevent any of these indications (Mylonas & Friese, 2015; Minkoff & Chervenak, 2003). Elective cesarean sections are performed before the

rupture of the amniotic sac, avoiding both the stress of natural birth and the microbes received by traveling through the birth canal (Wang et al., 2017).

Emergency Cesarean Section

Indications for emergency cesarean section include, but are not limited to the following: chorioamnionitis, infection of the placenta that may spread to the fetus; maternal pelvic deformity or small size; eclampsia, maternal seizures resulting from high blood pressure; umbilical cord or uterine prolapse; uterine rupture; fetal asphyxia or acidosis; and breech presentation; arrest of labor (Mylonas & Friese, 2015). Most emergency cesarean deliveries are performed once the birth process has already begun, meaning the membranes have already ruptured, and the fetus has likely already been exposed to some vaginal microbiota (Wang et al., 2017). Because of this, some adverse health outcomes associated with elective cesarean delivery do not exhibit the same association when considering emergency cesarean delivery.

Vaginal Delivery/Natural Birth

The natural birth process is one of vaginal delivery. In the first stage of labor, contractions begin, moving the baby down in the uterus toward the pelvis, and the cervix effaces and dilates to prepare for delivery (Office on Women's Health, 2018). At first, contractions are short and far apart, but they become longer, more intense, and more frequent as the baby moves closer to the cervix. The second stage of vaginal delivery is the stage in which the baby's head crowns, appearing visibly at the cervix, and the mother pushes during contractions to deliver the baby (Office on Women's Health, 2018). When the infant is delivered, it passes through the vaginal canal, coming into contact with the mother's vaginal microbiota. Delivery of the placenta follows soon after delivery of the neonate.

Vaginal delivery is associated with a shorter hospital stay after birth, a lower risk of subsequent hysterectomy as a result of postpartum bleeding, and reduced incidence of cardiac arrest in the mother (Mylonas & Friese, 2015). Though there is the risk of vaginal tearing and pain in labor, these prospects are minimal compared to the potential adverse effects that cesarean delivery may have on an infant's microbiome and health. These adverse health outcomes are explained in great detail below.

Microbiome Differences Based on Method of Delivery

Bacterial Diversity and Richness

Bacterial richness is defined as the quantity of distinct bacterial taxa or species in a sample, and bacterial diversity gauges both the number of microbiota and how evenly the various species are distributed in a sample (Montoya-Williams et al., 2018). The Canadian Healthy Infant Longitudinal Development (CHILD) study found infants delivered by elective cesarean section had the lowest intestinal bacterial diversity and richness when compared to infants born vaginally and by emergency cesarean section (Azad et al., 2013). Similarly, Rinninella et al. (2019) observed cesarean delivered infants have less diverse microbiomes when compared to their vaginally delivered counterparts. Richer and more diverse intestinal bacteria are more indicative of the ability of a person to resist threats from pathogenic invaders (Rinninella et al., 2019). It is important to note several studies have observed a dramatic increase in bacterial richness and diversity in both vaginally and cesarean delivered infants after the introduction of solid food (Wampach et al., 2017). These results imply that although delivery mode is not the only determinant of microbial composition, it is one of the primary determinants until solid food is introduced.

Bacterial Differences

Hill et al. (2017) concluded there are significant differences in the infant microbiome that are influenced by the mode of delivery, but these differences diminish after the first 24 weeks of life. A study by Montoya-Williams et al. (2018) disagrees, citing studies that have found higher levels of *Haemophilus* and *Clostridium* species in adults born by cesarean section when compared to those born vaginally. Nagpal and Yamashiro (2018) also found differences in *Bacteroides fragilis* and *Lactobacillus sakei* persisted into early adulthood, with both of these species present in lower levels in cesarean-delivered subjects. Other studies have observed microbial differences attributed to method of delivery that are still present at seven years of age (Rinninella et al., 2019). The extent to which microbial differences last is up for debate; however, it is clear that delivery mode does have an effect on microbial composition.

In their study, Dominguez-Bello et al. (2010) found the microbial communities of vaginally delivered infants were abundant in *Lactobacillus, Prevotella, Atopodium,* and *Sneathia* species, whereas cesarean-delivered infants' microbiotas were more colonized with *Staphylococcus* species. Dzidic et al. (2018) also observed that vaginal bacteria, including *Lactobacillus, Prevotella, Escherichia, Bifidobacterium,* and *Klebsiella* species dominate the intestines of vaginally born infants, whereas the intestines of cesarean-born infants are dominated by *Staphylococcus, Corynebacterium,* and *Propionibacterium*, all of which are skin microbes.

Likewise, Leon et al. (2018) noted the *Lactobacillus* and *Bacteroides* (common vaginal genera) were more abundant in vaginally delivered infants, and *Streptococcus* and *Corynebacterium* (common skin genera) were more abundant in cesarean delivered infants.

Rinninella et al. (2019) observed *Bifidobacterium longus* and *Bifidobacterium catenulatum*

predominated the microbiomes of vaginally delivered infants as well. Other studies have found

increased presence of *Veillonella* in cesarean delivered infants in addition to the previously

observed *Staphylococcus, Streptococcus, Corynebacterium* and *Propionibacterium* (Salas Garcia

et al., 2018). Shi et al. (2018) noted that infants delivered by cesarean section were often

abundantly colonized by *Bacillus licheniformis*, the presence of which is suggested to contribute

to microbial dysbiosis.

Interestingly, Nagpal and Yamashiro (2018) also noticed cesarean delivered infants

possessed higher levels of *Clostridium perfringens* than vaginally born infants. *C. perfringens* is

a toxigenic bacterium that is known to cause gastrointestinal diseases and gangrene (Petit,

Gibert, & Popoff, 1999). A complete list of infant intestinal bacteria and their correlations with

vaginal or cesarean delivery may be found in Table 1.

Differences in Method and Rate of Colonization

Studies by Grönlund et al. (1999) and Adlerberth et al. (2006) found although cesarean

delivered babies were eventually colonized with *Lactobacillus, Bacteroides,* and

Bifidobacterium, colonization with these bacteria was delayed. Makino et al. (2013) noted

similar results specific to *Bifidobacterium*: cesarean delivered infants were eventually colonized

with *Bifidobacterium*, but colonization was slower and bacterial counts did not reach that of

vaginally born infants. Other studies corroborate these findings, with some also noting an

association between cesarean deliveries and an abundance of *Clostridium* species (Dzidic et al.,

2018). Moreover, Dominguez-Bello et al. (2010) recognized evidence showing the direct

mother-to-neonate transfer of bacteria, finding greater similarities between the mother's vaginal

bacteria and her own baby's microbiome than between the mother's vaginal bacteria and the

microbiomes of other babies delivered vaginally in 75% of vaginal deliveries; these findings

suggest vertical transmission of the unique vaginal microbiota.

Similar evidence was not found in infants delivered by cesarean section, which suggests

initial colonization of cesarean delivered infants may occur through both maternal and

nonmaternal skin sources (Dominguez-Bello et al., 2010). Nagpal and Yamashiro (2018)

discovered similar results, concluding that vaginal *Lactobacillus* species were transferred

vertically from mother to infant during vaginal delivery, and seeding of *Lactobacillus* species

was delayed in infants born via cesarean section.

Infant Intestinal Virome

Just as the bacterial microbiome of the infant gut varies based on method of delivery, the

infant intestinal virome exhibits variation as well. The virome is a subset of the complete human

microbiome that is composed of the bacteriophages, viruses, and viral elements that live inside

the human body (Virgin, 2014). Alpha and beta diversity tests, which determine species richness

and variety, have confirmed that there are significant differences between the viromes of

vaginally and cesarean delivered infants (McCann et al., 2018). Variations in viromes are

hypothesized to affect the development of infant intestinal microbiomes, although more research

is necessary to confirm this theory (McCann et al., 2018).

Immune Cell/System Differences

Cesarean-delivered children usually exhibit higher numbers of immunoglobulin-secreting

cells (IgA and IgG) at age one (Dahlen et al., 2018). Brugman et al. (2015) also found vaginally

delivered infants presented with lower peripheral blood levels of IgA-, IgG-, and IgM-secreting

B cells. Sandall et al. (2018) observed that abnormal gut microbial colonization, as is seen in cesarean delivered infants, causes a prolonged increase in natural killer T cells that persists into adulthood.

In their study concerning cytokine concentrations, Malamitsi-Puchner et al. (2005) observed higher blood serum levels of sIL-2R, sIL-4R, INF-γ, IL-1β, IL-6, and TNF-α in vaginally delivered infants when compared to those born via elective cesarean section. All of these cytokines or cytokine receptors are related to inflammation or the labor process: sIL-2R is a receptor for IL-2, which regulates white blood cell activity; sIL-4R is a receptor for IL-4 and performs anti-inflammatory actions; INF-γ combats viral pathogens and regulates immune responses; IL-1β is thought to initiate labor; IL-6 may regulate growth of the placenta and fetus; TNF-α has pro-inflammatory effects (Malamitsi-Puchner et al., 2005).

Wampach et al. (2018) propose the ability of the infant intestinal bacteria to synthesize lipopolysaccharides is one of the primary determinants of immune system functioning. Vaginally delivered infants are colonized by an abundance of Gram-negative bacteria, which include species of *Bacteroides*, *Prevotella*, and *Enterobacteriaceae* (Ly et al., 2006). Lipopolysaccharides are present in the outer membranes of Gram-negative bacteria, so infants with a greater amount of Gram-negative bacteria in their normal flora will have higher levels of lipopolysaccharide biosynthesis; this biosynthesis stimulates the pro-inflammatory cytokine release and primes the immune system for further action against invading pathogens both in infancy and throughout life (Wampach et al., 2018).

Cesarean-delivered newborns are also more prone to developing certain infections, and Dominguez-Bello et al. (2010) hypothesize that this is due to their delivery mode-caused

microbial dysbiosis. One such infection is methicillin-resistant *Staphylococcus aureus* (MRSA). Watson et al. (2006) found that 64-82% of reported MRSA cases in infants occurred in those born via cesarean section. It is thought the vaginal microbiota, which are absent or underdeveloped in cesarean-delivered infants, prevent MRSA and other pathogenic microbes from colonizing in the infant (Dominguez-Bello et al., 2010). In addition, cesarean section delivery exposes infants to skin organisms earlier than vaginal delivery, which may increase the risk of exposure to MRSA; in one study, MRSA was the organism most commonly found in maternal wounds after cesarean delivery (Thurman et al., 2010).

Another component to the infant's ability to fight invading or indigenous pathogens is the intestinal pH. According to Nagpal and Yamashiro (2018), cesarean-born infants have lower fecal levels of short-chain fatty acids and, therefore, higher fecal pH than vaginally delivered infants. Higher short-chain fatty acid levels and lower pH characterize an intestinal habitat that impedes the growth of harmful microorganisms; cesarean-born infants lack these features and are more likely to be infected by opportunistic pathogens like *Clostridium perfringens* (Nagpal & Yamashiro, 2018).

Bacteroides fragilis is considerably important in developing and maintaining immune function due to its production of polysaccharide A (Troy & Kasper, 2010). Polysaccharide A plays an integral role in activating T cell responses, sustaining the T cell balance in the body, and preventing inflammation (Tanaka & Nakayama, 2017; Troy & Kasper, 2010). Nagpal and Yamashiro (2018) found a lower prevalence of *B. fragilis* in cesarean-born infants and propose this bacterium as a potential factor affecting the immune differences between vaginally and cesarean delivered infants.

It is thought a group of bacteria (Firmicutes, Bacteroidetes, Enterobacteriaceae, *Veillonella,* and *Bifidobacterium*), referred to as *pioneer microbiota*, are the microbes responsible for creating and maintaining a favorable environment for future microorganisms (Dzidic et al., 2018). These bacteria are more abundant in vaginally delivered infants, so disruption of this typical colonization is a potential contributor to the immune system dysfunction observed in cesarean delivered infants.

These differences in immune system development and function are thought to have effects reaching beyond infancy and early childhood. According to Wampach et al. (2018), deviations from the normal immune system establishment may cause aberrations in normal physiology throughout life. Thus, it is important to investigate and consider the unfavorable health outcomes that could arise from cesarean delivery.

Adverse Health Outcomes

Dermatologic/Allergic Conditions

In their meta-analysis of 26 epidemiologic studies, Bager, Wohlfahrt, and Westergaard (2008) concluded cesarean-delivered infants were at a moderately increased risk of developing allergic rhinitis and asthma. They did not, however, find the same association between cesarean delivery and inhalant atopy, eczema, or atopic dermatitis (Bager, Wohlfahrt, & Westergaard, 2008). A German study of 2,500 infants found a significant association between cesarean section and the risk of wheezing and allergic sensitization to food allergens (Negele et al., 2004).

Pothoulakis (2000) discovered that *Clostridium difficile* produces two specific exotoxins that damage the integrity of the intestinal epithelial cell barrier. These exotoxins initiate and subsequently intensify the intestinal inflammatory response, which is hypothesized to be a cause

of food hypersensitivity (Pike et al., 1986; Pothoulakis, 2000). In contrast, lactic acid-producing

bacteria, like *Lactobacillus* spp., are thought to aid in preventing respiratory infections (de

Frietas et al., 2018). *Lactobacillus* species are more abundantly colonized in vaginally delivered

infants when compared to their cesarean-born counterparts, and this difference in colonization is

likely to contribute to the higher prevalence of some respiratory conditions and diseases in

cesarean-delivered infants.

A study by Lundgren et al. (2018) found cesarean-delivered infants are more likely to

develop dairy allergies than vaginally delivered infants; they hypothesize this difference is due to

the lower abundance of milk-digesting *Lactobacillus* in cesarean-delivered infants. Wampach et

al. (2017) observed allergic diseases to be associated with lower levels of *Bacteroides* and

Bifidobacterium species; these bacteria are less likely to be colonized in cesarean delivered

infants, therefore, cesarean delivered infants are more likely to develop allergic diseases.

Asthma

A study by Montoya-Williams et al. (2018) suggests the anti-inflammatory effects of

Lactobacillus may prevent the development of asthma. In contrast, species of *Staphylococcus*

and *Clostridium* have been linked to atopic dermatitis and asthma (Montoya-Williams et al.,

2018). Other studies have shown that, when compared to infants born vaginally, cesarean

delivery increases one's risk of developing asthma by 16-20% (Huang et al., 2015;

Thavagnanam et al., 2008).

Eczema

Infants colonized with *Escherichia coli* and *Clostridium difficile,* both of which are

consistently more abundant in cesarean delivered infants, were found to be at significantly higher

risk of developing eczema (Penders et al., 2007). Furthermore, increased risk of eczema was associated with increased concentrations of *E. coli*, whereas the risk of eczema was independent of *C. difficile* concentration (Penders et al., 2007). According to Penders et al. (2007), similar associations were not found between the risk of eczema and *Bifidobacteria* species*, Bacteroides fragilis,* or *Lactobacilli* species.

Gastrointestinal Irregularities

Recent studies considered by Dahlen et al. (2018) revealed the importance of the intestinal microbiota in the development of various gastrointestinal irregularities and diseases. For example, *Bifidobacterium* species influence the functioning of the gut barrier, protecting against necrotizing enterocolitis, and low levels of *Bifidobacterium* are associated with cirrhosis and colorectal cancer (Montoya-Williams et al., 2018).

According to de Frietas et al. (2018), higher levels of *Bifidobacterium, Faecalibacterium,* and *Eubacterium rectale* increase the nourishment of enterocytes, the absorptive cells of the intestines, thereby enhancing general intestinal health. These bacterial species, namely *Bifidobacterium*, are of higher abundance in vaginally delivered infants than they are in cesarean-born infants.

Gastroesophageal reflux/disease

In their study of mothers and their infants in New South Wales, Australia, Dahlen et al. (2018) found being born by cesarean section was significantly associated with an increased risk of developing gastroesophageal reflux or gastroesophageal reflux disease. Gastroesophageal reflux disease occurs when gastric contents and stomach acid rise into the esophagus, and

symptoms in infants include spitting up, vomiting, fussiness, and irritability (Dahlen et al.,

2018).

Irritable Bowel Disease/Irritable Bowel Syndrome

A study by Carroll et al. (2010) found patients with irritable bowel syndrome were

colonized with fewer *Lactobacillus* species than healthy controls. This is consistent with the

finding that cesarean delivered infants, who are less likely to be colonized with *Lactobacillus*,

are more likely to develop irritable bowel syndrome (Rinninella et al., 2019). Another study

observed patients with irritable bowel disease typically exhibited higher colonization with

Proteobacteria and *Firmicutes* but had fewer *Actinobacteria* and *Bacteroidetes* (Krogius-

Kurikka et al., 2009).

Obesity

According to Mueller et al. (2018), rates of cesarean delivery and childhood obesity have

both increased over the last thirty years, and although correlation does not necessarily equate to

causation, several studies have found associations between cesarean delivery and risk of obesity.

One study found cesarean-delivered infants were more likely to show differences in adiposity at

as young as three months old and gain excess weight throughout the first year of life (Mueller et

al., 2018). Montoya-Williams et al. (2018) observed a cesarean born infant is at higher risk of

becoming overweight throughout his childhood, teenage years, and early adulthood.

In a study by Mueller et al. (2018), cesarean-delivered infants had an almost 30% higher

growth rate than their vaginally delivered counterparts in addition to being of higher birth weight

based on gestational age. Likewise, Salas Garcia et al. (2018) found that cesarean delivered

infants were at 46% more likely to develop childhood obesity, regardless of antibiotic use or

necessity of cesarean section. Another study makes a distinction between elective and emergency cesarean section, finding birth via elective cesarean section placed an infant at a 30% greater risk of developing obesity, whereas the risk from cesarean section, in general, was 15% (Yuan et al., 2016). Tun et al. (2018) also found cesarean birth yielded a higher risk for childhood obesity than vaginal delivery.

In addition, maternal obesity was more likely to be associated with childhood obesity in offspring if children were born via cesarean delivery; this was attributed to an abundance of *Lachnospiraceae* microbiota, specifically *Coprococcus*, present in the intestinal microbiomes of cesarean-born infants (Tun et al., 2018). Wampach et al. (2017) found similar results with *Staphylococcus*: lower levels of *Bifidobacterium* and higher levels of *Staphylococcus*, as seen in cesarean delivered infants, were linked to a higher likelihood of developing obesity.

Interestingly, though the presence of *Lactobacillus* species is more common in vaginally delivered infants, some species, like *Lactobacillus reuteri*, have been specifically associated with obesity (Montoya-Williams et al., 2018). In contrast, high levels of *Bifidobacterium* are thought to have anti-obesity effects, reducing body fat and improving lipid levels (Montoya-Williams et al., 2018). Rinninella et al. (2019) also found that lower bacterial species diversity was associated with a higher risk of obesity.

Type 1/Type 2 Diabetes

Surprisingly, *Prevotella* and *Bacteroides*, bacteria commonly found in vaginally delivered infants, have both been linked to increased incidence of type 1 diabetes in children (Montoya-Williams et al., 2018). However, Cardwell et al. (2008) observed that being born via

cesarean delivery puts an infant at a 20% increased risk of developing type 1 diabetes mellitus during childhood.

In contrast, Larsen et al. (2010) found, when compared to healthy patients, that sufferers of type 2 diabetes often had reduced intestinal colonization of *Firmicutes* and *Clostridium*; lower levels of both of these groups of bacteria are more common in cesarean delivered infants, indicating that infants delivered by cesarean section are more likely to develop type 2 diabetes in their lifetime.

Acute Lymphoblastic Leukemia

Wang et al. (2017) investigated the connection between mode of delivery and risk of developing acute lymphoblastic leukemia, finding a significant association between elective cesarean delivery and incidence of acute lymphoblastic leukemia between the ages of two and four years. They did not, however, observe any correlation between emergency cesarean section and an incidence of acute lymphoblastic leukemia at all ages (Wang et al., 2017). Similarly, Thomopoulos et al. (2016) noted an association between elective cesarean delivery and early-onset acute lymphoblastic leukemia, which is defined as the development of leukemia before the age of three. A meta-analysis of 13 studies from the Childhood Leukemia International Consortium came to the same conclusion: there is a significant association between elective cesarean section and the risk of developing acute lymphoblastic leukemia (Marcotte et al., 2016).

Potential for Reversal/Treatment and Global Implications

Prevalence of Cesarean Section

Although cesarean delivery is sometimes a necessary procedure to preserve the health of the mother and/or child, rates of cesarean delivery have increased over time. It is now estimated

that globally, 1 in 5 women will have a cesarean birth (Tribe et al., 2018). According to Sandall et al., a study showed that in 2015, 29.7 million cesarean deliveries were performed worldwide, comprising 21.1% of total births, an almost two-fold increase in cesarean deliveries since the year 2000 (Sandall et al., 2018). In Europe, the proportion increased to 25%, and in the United States, the proportion is about 30% (Gibbons et al., 2010; Wampach et al., 2018). The American College of Obstetricians and Gynecologists found the rate of cesarean section in the United States was even higher, at 33% in the year 2011 (ACOG/SMFM et al., 2014). Generally, rates of cesarean delivery, both necessary and unnecessary, are higher in more developed, highly modernized countries with higher average incomes, whereas fewer cesarean deliveries occur in less developed areas with lower incomes and lower levels of modernization (Nagpal & Yamashiro, 2018).

Options for Treatment

Several treatment options have been developed to confront the issue of microbial dysbiosis caused by cesarean delivery. These include procedures like vaginal seeding, pharmacologic alternatives like probiotics and prebiotics, and natural options like breastfeeding. As expected, the natural options and those which employ intrinsic bacteria as treatments are more likely to be recommended.

Vaginal seeding

To perform vaginal seeding, sterile gauze is placed inside the mother's vagina and left to incubate during the cesarean birth process. Once the child is born, the gauze is removed from the vagina and applied to the skin of the neonate, inoculating the neonate with maternal vaginal bacteria (Tribe et al., 2018). The long-term success of this technique has yet to be determined,

but there are questions concerning its associated risks as well as the efficacy of bacterial

colonization (Salas Garcia et al., 2018).

Vaginal seeding carries the risk of transferring a number of viral, fungal, and bacterial

pathogens that may not cause symptoms in the mother (Stinson, Payne, & Keelan, 2018).

Maternal-to-neonate transfer of group B streptococcus (GBS) is of particular interest. GBS is

known to infect newborns as they pass through the birth canal, causing early-onset neonatal

sepsis and meningitis, and though this transfer is prevented by cesarean delivery, infant

inoculation with vaginal bacteria re-introduces this risk (Schuchat, 1999). Mothers infected with

Chlamydia trachomatis, Neisseria gonorrhoeae, and Herpes simplex virus 1 or 2 often deliver

via cesarean section as well and are also at risk of transferring these organisms to their babies

through the vaginal seeding process (Lee et al., 2017). There is also concern about performing

this procedure without the guidance or oversight of a trained healthcare professional.

In addition, a study by Matsumiya et al. found that although vaginal lactobacilli (VLB)

are passed from the mother to the infant during vaginal birth, VLB last only temporarily in the

infant intestine; therefore, they concluded probiotics are more effective than VLB in adhering to

the infant intestinal epithelium (Tobita, Watanbe, & Saito, 2017). Duar, Kyle, and Tribe (2020)

also suggest one-time inoculation of vaginal bacteria would not be effective in colonizing the

infant gut; rather, constant re-application of vaginal bacteria would be necessary to cultivate

stable populations of these bacteria in the infant intestine. This doubt of effectiveness coupled

with the potential risks to the neonate is cause to consider other methods of colonizing the infant

with maternal vaginal bacteria.

Probiotics

Clinical trials have proven probiotics to be effective in the treatment or prevention of eczema and other atopic diseases, especially when the disease occurs in conjunction with intestinal microbial disruption (Penders et al., 2007). A late 2010s trial in the United Kingdom examined daily probiotic supplements with *Bifidobacterium infantis* as a method of promoting healthy gut microbiota in breastfeeding infants who had not yet been introduced to solid foods (Salas Garcia et al., 2018). This supplementation resulted in increased *B. infantis* levels that persisted after cessation of supplementation, as well as decreased fecal pH, decreased bacterial endotoxin, fewer stools, and increased fecal acetate and lactate, all indicators of a healthier intestinal microbiome (Tribe et al., 2018). Duar, Kyle, and Tribe (2020) also encourage probiotic supplementation with *B. infantis*, suggesting supplemented *B. infantis* and breast milk work together to promote infection-fighting and homeostatic conditions.

The U.S. Food and Drug Administration is considering similar treatment options; one study focused specifically on supplementing infants at risk of developing asthma with *Lactobacillus rhamnosus GG* (Salas Garcia et al., 2018). Though the final results from these studies have not yet been determined and no therapies of this variety are approved to specifically treat cesarean delivery-associated microbial dysbiosis at this time, these supplements are viable treatment options worth pursuing. In other studies, infants have responded favorably to probiotic treatments, proving effective in reducing episodes of reflux, increasing blood flow to the intestines, and improving growth and feeding tolerance (Dahlen et al., 2018). A 2009 study by Kuitunen et al. showed supplementation of cesarean-delivered infants with probiotics led to reduced incidence of IgE-associated allergic diseases, including eczema, asthma, and rhinitis.

Probiotics are safer and more easily controlled than vaginal seeding, and some vaginal bacteria have already been formulated into available probiotics (Stinson, Payne, & Keelan, 2018). Studies not particularly focused on cesarean-delivered infants have demonstrated optimal results through probiotic treatments jointly with breastfeeding; this combination has been shown to return disturbed infant intestinal microbiota to a more normal state (Salas Garcia et al., 2018). These favorable results encourage further research and eventual use of probiotic supplements to aid the healthy colonization of the infant intestine.

Breastfeeding

Breastfeeding has also been proposed as a potential method of effecting the colonization of healthy infant fecal microbiota. Some studies have shown breastfeeding can only promote the growth of healthy gut bacteria if those bacteria are present in the intestine when the infant is born; therefore, in cesarean-delivered infants, breastfeeding is commonly recommended alongside probiotic supplementation, as previously discussed (Sandall et al., 2018). This is certainly an area in which future research should occur.

It is suggested some adverse health outcomes related to cesarean delivery may be more closely tied to breastfeeding behavior than microbial transfer. This is because cesarean-delivered infants typically begin breastfeeding later, breastfeed for a shorter duration, and consume less breast milk during the first five days of life (Stinson, Payne, & Keelan, 2018; Tribe et al., 2018). Nevertheless, breastfeeding is clearly connected to the health of the infant intestinal microbiome, especially alongside probiotic supplementation. Cesarean delivery coupled with suboptimal breastfeeding is hypothesized to worsen infant gut dysbiosis (Nagpal & Yamashiro, 2018).

Human milk oligosaccharides (HMOs) are abundant in breast milk and promote the growth of *Bifidobacterium infantis*, which is typically low in cesarean-born infants (Tribe et al., 2018). Although infants cannot digest HMOs, these oligosaccharides are important in facilitating the maturation of the intestinal mucosa and immune system and nourishing the infant intestinal microbiota, enhancing and supporting colonization (Akkerman, Faas, & de Vos, 2019; Seppo et al., 2019). Long-term supplementation of single HMOs or HMO blends with breastfeeding is suggested as another potential method of treating intestinal microbial dysbiosis in infants, as short-term supplementation produced only marginal short-term effects in a pig model (Rasmussen et al., 2017).

Global Implications

The World Health Organization recommends the rate of cesarean delivery in a specific country be between 10-15% (Gibbons et al., 2010). In their analysis of cesarean delivery worldwide, Gibbons et al. (2010) found:

> "Excess" CS could thus potentially finance the "needed" ones 5 times over; in other words, if all the resources currently devoted to "excess" CS could be directed towards countries where additional procedures are "needed," the "needed" procedures could be fully financed and there would in addition be a surplus of resources with a value of nearly US$ 2 billion (p. 8).

Discouraging unnecessary, elective cesarean sections will not only improve infant health and health later in life, but it is also proposed to help close the gap in the distribution of medical resources. The goal of this ideal redistribution is to increase access to necessary medical

procedures in low-income areas, again reducing the incidence of adverse health outcomes

(Gibbons et al., 2010).

Unfortunately, this redistribution of both wealth and medical resources is largely

impractical. The most effective course of action is to discourage elective cesarean sections where

possible. One way to do this is through educating mothers, focusing on dispelling

misconceptions about vaginal birth and cesarean section, preparing mothers for the birth process,

and acknowledging the psychosocial and emotional aspects of childbirth (Betrán et al., 2018).

Other options include providing support of doulas or midwives during the birth process,

increasing the availability of pain relief, and reconsidering government policies on healthcare

(Betrán et al., 2018).

Conclusion

The foundation for the infant intestinal microbiome is largely laid through the birth

process, and proper development and maturation of this microbiome is imperative to ensure

effectiveness in fighting opportunistic pathogens, activating immune responses, and promoting

overall health and wellbeing both in infancy and throughout life (Salas Garcia et al., 2018; Hand,

2016). Cesarean section delivery is shown to disrupt intestinal microbial colonization, causing

microbial dysbiosis. Cesarean-born babies have lower intestinal microbial loads, less microbial

diversity, and colonization with fewer common vaginal bacteria, like *Lactobacillus* and

Bifidobacterium species (Dominguez-Bello et al., 2010). Instead, children delivered by cesarean

section exhibit greater colonization with skin bacteria, such as *Staphylococcus* and

Corynebacterium species (Leon et al., 2018). These microbial differences are correlated with an

increased incidence of allergic diseases, obesity, and diabetes, among others (Wampach et al.,

2017; Montoya-Williams et al., 2018).

Though many of the proposed relationships between cesarean section delivery,

differences in microbial composition, and adverse health outcomes are correlative, it is important

to remember that they have not yet been proven causative. Nevertheless, the impact of cesarean

section delivery on health later in life is worthy of future research and consideration when

implementing new procedures and examining current policies. Large health benefits may be

reaped from not only lessening the occurrence of elective cesarean sections but also developing

therapies and treatments to reverse the microbial dysbiosis caused by the mode of delivery. In

addition, there is certainly potential for global impact, especially when evaluating the prevalence

of cesarean section deliveries worldwide, the disparity of economics and medical interventions,

and the opportunity for ministry through medicine.

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Table 1

Meta-List of Infant Intestinal Microbiota

Genus	Adlerberth et al.	Azad et al.	Brugman et al.	Dominguez-Bello et al.	Dzidic et al.	Grönlund et al.	Hill et al.	Lee et al.	Leon et al.	Lundgren et al.	Makino et al.	Montoya-Williams et al.[1]	Nagpal et al.	Penders et al.	Rinninella et al.	Salas Garcia et al.	Shi et al.	Stinson et al.	Tanaka et al.	Tribe et al.	Tun et al.	Wampach et al. (2018)	Wampach et al. (2017)
Actinomyces																		VD					
Aeromonas																CS							
Aggregatibacter																		CS					
Alioicoccus									CS														
Anaerococcus																		CS					
Anaerostipes																		CS					
Atopobium				VD																			
Bacillus																CS		CS					
Bacteroides		VD			VD	VD	VD	VD	VD			VD	VD	VD		VD	VD/CS	VD	VD		VD	VD	
Bifidobacterium	VD	CS	VD		VD	VD	VD	VD		VD	VD	VD	VD	VD	VD	VD		VD	VD	VD			VD
Bilophila																		VD					
Blautia		CS																CS					
Brevundimonas																		CS					
Butyrivibrio																		CS					
Capnocytophaga																		CS					
Citrobacter												CS						CS					
Clostridium	CS	VD	CS		CS	CS	CS			VD		CS		CS				CS	CS				
Coccidiodes												CS											
Collinsella																		VD					
Comamonas																	VD	VD					
Coprococcus		VD																					
Coriobacteriaceae		VD																					
Corynebacterium			CS	CS	CS			CS				CS				CS		CS					

Genus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Cronobacter																CS				
Deinococcus															CS	CS				
Delftia																CS				
Enterobacter	CS		CS													CS				CS
Enterococcus		VD	CS							CS						CS				
Erwinia									VD											
Escherichia	VD				VD					CS		CS	VD							
Escherichia-Shigella		VD												VD		VD				
Eubacterium															CS	CS				
Faecalibacterium															CS	CS				
Firmicutes																		CS		
Fusobacterium									CS											
Granulicatella																CS				
Haemophilus		CS		CS						CS						CS				CS
Klebsiella	CS		CS		VD					CS						CS				
Lactobacillus			VD	VD	VD	VD		VD	VD	VD	VD		VD	VD	VD		VD			CS
Macrococcus																CS				
Megamonas														VD						
Parabacteroides							VD							VD		VD			VD	VD
Paraprevotella																CS				
Prevotella			VD	VD	VD				VD	VD			VD	VD	CS		VD			
Propionibacterium			CS	CS	CS					CS			CS	CS	VD	VD	CS			CS
Proteus																CS				
Providencia																CS				
Pseudomonas									CS							VD				
Rothia																CS				
Shigella													VD							
Sneathia			VD	VD						VD			VD				VD			
Staphylococcus			CS		CS					CS			VD/CS	CS		VD/CS	CS		CS	CS
Stenotrophomonas																CS				
Streptococcus		CS	CS						CS	CS			VD	CS	CS	CS				CS
Tepidimonas									CS											

Trabulsiella				CS	
Ureaplasma		VD			
Veillonella	VD			CS	CS

Note: VD indicates a greater abundance in vaginally delivered infants compared to cesarean delivered infants; CS indicates a greater abundance in cesarean delivered infants compared to vaginally delivered infants. Multiple data entries listed under one article indicate the article is a meta-analysis reviewing multiple studies.