Abstract
From epidemiological studies and studies done evaluating microbiomes in infants, there is a strong signal that the infants born by elective cesarean section (C-section) develop microbiota that differs from those babies born by vaginal delivery. Epidemiological studies show increased odds ratios for the development of immunological disorders such as type 1 diabetes, celiac disease, asthma, allergic diseases as well as metabolic diseases such as obesity in babies born by C-section. These are interesting associations, and if supported by additional studies that rigorously control for confounding factors, they will have major public health implications. Such studies represent major challenges because the confounding factors are numerous. The fact that provision of vaginal bacteria to C-section-delivered babies using a mouth swab that may actually transmit these bacteria to the infant is of interest and supports the concept that this can be done to alter the infant microbiota. However, significant caution needs to be taken, and alternative approaches that are safe as well as effective need to be considered; follow-up studies showing efficacy as well as safety need to be evaluated in the long term.

Introduction
Vaginal birth has been the natural and common mode of newborn delivery until the last century, where cesarean sections (C-sections) have become increasingly common. There are many indications for C-section delivery, both maternal and fetal ones. C-sections can be further classified into planned or emergent C-sections. Emergent C-sections are typically performed when the life of the infant or the mother can potentially be saved. States of emergency can be caused
by events such as the prolapse of the umbilical cord and nonreassuring fetal heart rate tracings. Planned C-section deliveries are usually performed prior to the onset of labor and may be either medically indicated or elective. Medically indicated C-sections are generally defined as situations with a significant risk of an adverse outcome for the mother or the baby if the operation is not performed at a given time [1, 2]. Medical indications can be due to situations such as untreated maternal HIV, herpes, a history of prior uterine rupture, fetal anomalies, or abnormal fetal presentation. There are also indications which are clearly not based on medical needs, and these are usually performed secondary to convenience of scheduling for the obstetrician or family.

Although the optimal C-section delivery rate is not clear, in 1985, at a meeting organized by the World Health Organization in Fortaleza, Brazil, a panel of reproductive health experts stated that “there is no justification for any region to have a rate higher than 10–15%” [3]. Despite these recommendations, C-section rates have risen dramatically in the past 2 decades, with rates approaching 50% in some countries, such as Brazil, Iran, and the Dominican Republic. Thus, it is very likely that many of these C-sections may be unnecessary and/or simply based on convenience in countries with a high rate. Although concern should be raised in regard to the effects of these high C-section rates on the mother, their effect on public health as well as the health and maturation of the individual infants should be considered, as will be discussed in this review.

With the advent of the human microbiome project in the past decade, it has become increasingly clear that early microbial colonization will have major effects on the maturing individual. Although the term “dysbiosis” is not clearly defined, it reflects deviations from the normal microbial ecology that result in detrimental health effects on the host. Therefore, early-life perturbations of the microbiota are likely to lead to metabolic, immunological, and epigenetic consequences that have major effects on the developing individual and perhaps may even affect subsequent generations.

In this review, the effects of C-section versus vaginal delivery on the subsequent development of the microbiota, and how this relates to health outcomes, will be discussed. Studies will be presented that suggest that C-section delivery may result in a “dysbiosis” that has detrimental effects on the individual in later life. Various caveats in terms of the interpretation of the results of recent studies suggesting major differences in terms of the microbiota development and associations to subsequent health and disease will be scrutinized. These include the fact that there is considerable evidence suggesting that microbiota exists prior to delivery, and that this may also play a major role in subsequent health and disease. Furthermore, there are various environmental perturbations such as maternal diet, maternal body habitats, such as body mass index, the use of antibiotics as well...
as various other drugs, introduction of human milk versus donor milk versus formula, early stressors, and other factors that may play a role in confounding results of epidemiological studies as well as studies of microbiota in babies who are delivered by C-section versus vaginal delivery. In this review, we will also discuss recent methods to restore vaginal microbes after C-section delivery.

**Mode of Delivery and the Infant Microbiome**

Until recently, the fetal-maternal unit was believed to be sterile and only to become colonized at birth through contact with the microbial community in the vaginal canal. Thus, those infants delivered by C-section would not receive this early colonization from the vagina and required other environmental exposures to begin this colonization. However, there is ample evidence that colonization of the fetal-maternal unit begins earlier than birth since microbes have been identified in the amniotic fluid, umbilical cord blood, fetal membranes, meconium, and placenta [4]. These prenatal microbial conditions are seldom mentioned in studies comparing the effects of C-section versus vaginal delivery. In addition, differences have been found in the microbiota of children born vaginally, or by elective or emergency C-section [5]. Many of these studies suffer from interstudy methodology variability such as PCR bias and other confounding factors. Nevertheless, some of the important trends found in the literature regarding microbial composition differences in vaginally versus C-section-delivered children include: (1) children born by C-section, elective C-section in particular, exhibit diminished diversity in their microbiota; (2) less health-inducing bacterial species such as lactobacilli are seen after C-section delivery; and (3) there appears to be a trend toward more pathogenic bacteria (a possible “dysbiosis”) in the developing microbiome of C-section-delivered infants.

In terms of diversity, a few studies have shown evidence of persistent negative associations between C-section delivery and infant microbial diversity and richness. In a study evaluating 16S rRNA genes in 24 healthy term infants’ stool, infants born by C-section had lower total microbial diversity compared to vaginally delivered infants, and these differences persisted through the first 2 years after birth [6]. Another study in Canada using high-throughput DNA sequencing in term infant fecal samples 4 months after birth found that infants born by elective C-section had lower diversity [7]. Other studies have demonstrated higher richness in vaginally delivered infants when evaluating bacteria found in oral swab samples [8].

In 2010, Dominguez-Bello et al. [9] described the microbial communities of 10 mothers and their infants, 6 of whom were delivered by C-section. Using 16S
rRNA sequencing of samples, it was found that in vaginally delivered infants, stool sample microbes collected within the first 24 h after birth most resembled their own mother’s vaginal microbes. Babies who were born by C-section were colonized by bacteria that most resembled skin flora. It was suggested from this work that infants delivered by C-section lack exposure found in mother’s vaginal or intestinal environment. The most dominant genera of bacteria in vaginally delivered babies when compared to those delivered by C-section were *Lactobacillus*, *Prevotella*, *Atopobium*, or *Sneathia*, whereas the most dominant genera in C-section infants was *Staphylococcus*, a common skin microbe [9]. *Lactobacillus* microbial communities are found as a dominant group in healthy vaginal communities [10]. Other studies have also found that vaginally delivered infants have more lactobacilli in the gastrointestinal tract than those delivered by C-section [11]. However, not all groups have found differences in bacterial communities in C-section versus vaginally delivered infants. In a study performed in different countries in Europe analyzing 606 infants, mode of delivery had no effect on relative proportions of bifidobacteria in 6-week-old infants [12]. In this study, infants born via C-section also had less *Bacteroides* than vaginally delivered infants. This is of interest in that *Bacteroides* may play a beneficial physiological role in the neonatal intestine [13].

There are several confounding factors that need to be taken into account in such studies (Fig. 1). Our group found that the microbiota measured in meconium during the first 48 h after birth was more diverse in preterm infants delivered by C-section [14]. This begs the question of whether meconium or samples derived within the first 24 h after birth are reasonable samples to evaluate the differences between C-section versus vaginal delivery, and whether gestational age matters in terms of the developing microbiome when related to mode of delivery. These early samples theoretically would not yet reflect microbes that are passed to the infant during the voyage through the birth canal, but rather microbes attained in utero. Again, confounding factors such as whether the C-sections were done electively or emergently, whether the mothers received antenatal or immediate postnatal antibiotics, and the length of time that either breast-feeding or formula feeding was initiated after birth are all factors that have not been fully addressed in most of these studies. The fact that many mothers are not able to provide milk for their babies shortly after birth by C-section and that colonization may differ temporally in these individuals are factors that have not been taken into account in most of the previous studies.

In conclusion, there appear to be differences in the microbial communities of infants delivered by C-section when compared to infants delivered vaginally. These differences can be persistent and can be found throughout childhood. Several of the bacteria that are found in those infants in higher quantities when
delivered by vaginal delivery versus C-section appear to have the known beneficial effects. However, there are confounding effects that lessen the clarity of whether the microbial differences were simply due to the process of C-section versus vaginal delivery.

**Epidemiological Studies Suggesting Differences in Health Outcomes**

As for short-term outcomes, there are little data to support that C-section versus vaginal delivery results in increased complications such as necrotizing enterocolitis or infections in the immediate neonatal period. C-sections are clearly associated with increased transient tachypnea of the newborn [15]. The mechanisms for this remain unclear, and hypotheses have ranged from a lack of vaginal squeeze causing more fluid to be retained in the lungs to a lower production of chloride and other channels related to lower glucocorticoid and other hormone levels in babies delivered by C-section when compared to those infants delivered vaginally [15].

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**Fig. 1.** Confounding factors that can affect the establishment of infant gut microbiota in addition to mode of delivery.
Children born by C-section have an increased susceptibility to several immune-related conditions that are seen during the 1st year after birth. These include asthma [16, 17], type I diabetes [17, 18], food allergies [16], allergic rhinitis [16], and celiac disease [19]. Other studies have found long-term associations between C-sections and non-immune-related health outcomes such as increased body mass [17, 20–22], and nervous system abnormalities [23]. Thus, there is a body of epidemiological literature that demonstrates that mode of delivery is associated with long-term health outcomes in infants and children. Nevertheless, it is important to remember that these epidemiological associations do not prove causality.

The results presented here have a high potential for overinterpretation. As illustrated in Figure 1, there are several mitigating factors that are not clearly accounted for in these studies. The initial medical reason for the C-section and the socioeconomic status of the mother and family may play a significant role as to whether the infant is delivered by C-section versus vaginal delivery. Socioeconomic status and race have also been shown to play a role in the development of different microbiomes [24]. Stress and its subsequent effects on lactation [25] experienced by mothers who eventually have C-section instead of vaginal delivery are also not accounted for in these epidemiological studies. Most babies born by C-section in the United States also received a dose of antibiotics either shortly before or after delivery in order to prevent maternal infection [26]. If the mother is breastfeeding these antibiotics may get transferred through the breast milk to the infant and this may alter the microbiota of the newly born infant. Furthermore, those infants who were born by C-section may have mothers who are not able to produce breast milk for 3–5 days versus vaginally born infants whose mothers are able to produce milk usually within the first 24 h after birth [27]. Delayed breastfeeding in the C-section-delivered infants may contribute to the altered development of the microbiota, as well as very early immunological alterations that may affect subsequent health. Such confounding factors need to be accounted for in order to decrease skepticism for studies that suggest later immunological or metabolic diseases that differ because of the mode of delivery and modulation of the infant microbiome.

Strategies for Restoration of the Vaginal Microbes in C-Section-Delivered Infants

If exposure to vaginal microbes during vaginal delivery results in colonization with vaginal microbes and C-section does not, an obvious remedy would be to expose infants born by C-section to vaginal microbes. A recent pilot study sought to recapitulate infants’ initial encounter with vaginal microbiota right
after birth using a technique where gauze that was preincubated in the maternal vagina for 1 h and then applied to the baby’s mouth, face, and body immediately after C-section birth [28]. The composition of the microbiota in infants born by C-section with and without the gauze restoration procedure was compared to vaginally born infants. The gauze treatment restored the presence of vaginal-type bacteria in C-section-delivered infants. Of interest is the fact that anal samples of C-section-delivered exposed babies were not different from unexposed babies. All newborns including those born by vaginal delivery had an abundance of bacteria categorized as gut derived when evaluated immediately after birth. It is unlikely that the meconium samples of C-section-delivered babies, as well as those from the gauze-exposed C-section-delivered babies or vaginally delivered babies, in any way represented the vaginal microbiota since this would more likely represent in utero environment rather than extraterine environmental modification.

This study did have several limitations, one of which was the very small number of subjects with only 4 of the C-section-delivered babies exposed to the gauze microbial restoration technique. All mothers undergoing C-section had cephalosporin antibiotics administered whereas those who delivered vaginally had not. Furthermore, the decision whether or not to deliver by C-section may have been made because of specific medical indications, and this was not clearly delineated in this study. The effect of breastfeeding and length of time to establish breastfeeding in these mothers and infants was also not evaluated. Nevertheless, this study is an important first step for proof of concept that the microbial colonization of the vaginal tract can be transferred to infants who were born by C-section delivery.

Clearly, this is not a technique that is ready for routine use despite many parents already requesting this be done for their infants. Parents and physicians need to recognize inherent safety concerns with this technique. For example, even if the mother’s serology is group B Streptococcus negative, this should not rule out the possibility of exposure because many cases where group B streptococci had led to death were in infants whose mothers’ serologies were negative [29]. Undetected herpes or HIV may also be of concern. If the mother has primary herpes which is undetected, and the infant is born vaginally, the chances of death from fulminant systemic herpes infection in that infant are high.

Breastfeeding confers a set of microbes to the newborn infant. Breast milk is known to contain microbes that may play a role in infant health [30]. The study by Azad et al. [7] shows that by 4 months after birth, breastfeeding leads to a microbial colonization in C-section-delivered babies that is somewhat similar to that of vaginally delivered babies [7]. However, none of these techniques have thus far been demonstrated to provide a short- or long-term subsequent benefit.
Concluding Remarks

From this review, it is obvious that there is a strong signal that infants born by elective C-section develop microbiota that differs from those babies born by vaginal delivery. This is seen in association with epidemiological studies that show increased odds ratios of immunological and metabolic diseases in those babies born by C-section versus vaginal delivery. This may have major public health implications. However, there are major difficulties with the interpretation of these results from both the epidemiological and the microbiota-oriented studies since there are numerous confounding factors that are difficult to control. Studies that tightly control for these factors still need to be done. The fact that provision of vaginal bacteria to C-section-delivered babies using a mouth swab may actually transmit these bacteria to the infant is of interest, but caution is advised, and alternative approaches need to be developed that are safe as well as effective. Follow-up studies showing efficacy as well as safety need to be evaluated in long-term studies.

Acknowledgment

The author was supported by a Scientific Advisory Board and Research Grant from Medela, a Research Grant from Infant Bacterial Therapeutics, and by the Scientific Advisory Board of Nutricia.

Disclosure Statement

The author has no conflict of interest.

References


