Duration of labour and its impact on the infant gut microbial composition in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort

by

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Abstract

Background: Balanced development of infant gut microbiota is pivotal for immune maturation and energy homeostasis, and infant gut dysbiosis is associated with increased risk of childhood atopy, allergy and excess weight gain. Shifts in abundance of gut Bacteroidetes and Firmicutes during infancy, along with reduction of probiotic organisms such as *Bifidobacterium* and *Lactobacillus*, has been linked to higher risk of childhood allergy and excess adiposity. Evidence shows that mode of delivery profoundly affects infant gut microbiota development. Yet, information on effect of duration of labour, an inherent component of natural birth, on microbial colonization of infant gut is scarce.

Objectives: To examine the influence of duration of labour on the infant gut microbiota composition and diversity at 3 to 4 months of age.

Methods: A subset of 1028 infants from the Edmonton, Winnipeg and Vancouver sites of the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort were included in the study. Data on duration of labour, other birth characteristics and maternal pre-pregnancy body mass index (BMI) was obtained from hospital birth charts. Infant gut microbiota was characterized using Illumina MiSeq 16S rRNA gene sequencing of fecal samples collected at 3-4 months of age. Microbial relative abundance, Chao1 richness and Shannon diversity were determined.

Results: Longer duration of labour was associated with reduced gut colonization with genera *Bifidobacterium* and *Lactobacillus* in infants at 3-4 months of age. Odds of colonization with *Bifidobacterium* reduced significantly with active first stage longer than 13 hours [aOR = 0.56 (95%CI = 0.34-0.95); p =0.030] and second stage longer than 2 hours [aOR = 0.48 (95%CI = 0.32-0.73); p =0.001]. Likewise, odds of colonization with *Lactobacillus* also reduced with active first

stage longer than 13 hours [aOR = 0.53 (95%CI = 0.30-0.95); p=0.032] and second stage longer than 2 hours [aOR = 0.63 (95%CI = 0.41-0.98); p =0.041]. Infants born to obese mothers showed more severe reduction in *Bifidobacterium* and *Lactobacillus* colonization in association with longer labour durations. In addition, *Veillionellaceae* tended to increase with longer labour in infants of normal weight mothers where as an inverse trend was observed among infants of obese mothers.

Conclusion: The findings provide evidence of infant gut microbiota dysbiosis associated with longer durations of labour. Elevation of maternal pre-pregnancy BMI further accentuates the observed changes in infant gut microbial profile. The long-term consequences of these compositional changes on immune maturation and metabolic homeostasis and risk of childhood allergy and obesity requires further study.

Preface

This thesis is an original work by Usha Rai. The thesis was written in journal-article format according to the guidelines of the Faculty of Graduate Studies and Research at the University of Alberta.

This thesis consists of a literature review (Chapter 1), followed by two studies (Chapter 2 and Chapter 3) designed to address specific objectives, and a concluding chapter (Chapter 4).

Chapter 1 is the introduction that consists of literature review on contemporary patterns of duration of labour, discussion of how labour duration may influence the development of infant gut microbiota, study objectives, hypotheses, sample size calculation, and overview of study design and analyses.

In Chapter 2, the findings of first research question are presented. In this chapter, the associations between duration of active first stage and second stage of labour and changes in infant gut microbiota composition at 3-4 months were investigated in a subsample from the CHILD (Canadian Healthy Infant Longitudinal Development) longitudinal birth cohort.

In Chapter 3, the findings of second research question are presented. In this chapter, the associations between duration of labour and changes in infant gut microbiota composition at 3-4 months among infants born to women with different pre-pregnancy body mass index (BMI) were studied in the CHILD (Canadian Healthy Infant Longitudinal Development) birth cohort.

In Chapter 4, the final chapter, general discussion of results and conclusions are presented. This chapter highlights the main findings from the two studies, significance and clinical relevance of the findings, strength and limitations of the studies, discussion of bias and confounding, and implications for future research.

Dedication

Dedicated to my parents and my husband.

For your support, faith and love, I am forever grateful.

Acknowledgments

I wish to express my sincerest gratitude to my mentor and supervisor, Dr. Anita Kozyrskyj, for her continuous support, guidance and motivation throughout my journey as a graduate student. I am deeply indebted to her for giving me the opportunity to learn from her immense bounty of knowledge, and for her advice and patience with me during the process of completion of my thesis.

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And, for my husband, I reserve all my gratitude, respect and love for being my foundation, core and shield during this journey.

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CHAPTER 1

Introduction

1.1 Infant gut microbiota development and pediatric health

The infant gut microbiome is integrally linked to long-term child health. The earliest microbial colonizers of the gut lay the foundation for immune programming and energy-homeostasis. Balanced development of infant gut microbiota facilitates immune programming and immune maturation by enhancing gut mucosal barrier function, inducing immune tolerance to the normal gut commensals, modulating gut-associated lymphoid tissue (GALT) associated immune responses, balancing T-helper cells subsets, inducing regulatory T cells that guide host Th1/Th2 balance, and aiding the regulation of anti-inflammatory stimuli (1) (2) (3). Additionally, the gut microbiota has metabolic activity which is not only renewable and compliant (4), but can also influence on both the harvest of energy from dietary components and regulation of energy storage in the host through fermentation of dietary fiber into short chain fatty acids (5) (6). Therefore, the development gut microbiota in infant has received much scientific scrutiny in the recent years.

The infant receives its first microbial inoculum from the mother. Evidence suggests that gut microbial seeding may begin *in utero*, even before birth. Isolation of viable bacteria such as *Propionibacterium* (of phylum Actinobacterium) and *Staphylococcus* (of phylum Firmicutes) from placenta and amniotic fluid (7), along with similarity between placental and maternal-oral microbiota (8), suggests a hematogenous transfer of maternal oral microbiota to the intrauterine environment and possible microbial exposure to fetus *in utero*. In addition, maternal fecal and vaginal microbiota are primary sources for 'microbial seeding' of newborn gut during birth.

The gut microbiota of expectant women changes throughout pregnancy. By third trimester, healthy pregnant women possess higher gut bacterial load, and a higher abundance of Actinobacteria and Proteobacteria phyla. Additionally, a reduction in *Faecalibacterium* (of phylum Firmicutes), a gut commensal with anti-inflammatory effects, and in microbial richness (alpha diversity) also accompanies the third trimester (9). The vaginal microbiota also undergoes

significant pregnancy-related changes, which includes higher abundance of *Lactobacillus* species (of phylum Firmicutes) and decreased overall diversity (8). During vaginal delivery, the newborn encounters maternal vaginal and fecal microbes that form the pioneering colonizers of its gut. C-section delivered infants, on the other hand, possess gut microbiota that bear resemblance to maternal cutaneous microbiota (10) (11).

Following birth, the newborn gut is an oxygen-rich environment that supports facultative anaerobes such as members of *Enterobacteriaceae, E.coli* (both of phylum Proteobacteria) and *Enterococci* (of phylum Firmicutes) (12). These first colonizers consume oxygen, converting the gut to an anaerobic environment within days after births, and giving way to proliferation of obligate anaerobes such as *Bifidobacterium* (of phylum Actinobacteria), *Bacteroides* (of phylum Bacteroidetes) and *Clostridium* (of phylum Firmicutes) (12). The neonate gut is dominated by Actinobacteria and Proteobacteria, and is characterized by low diversity. Breastfeeding further fuels the abundance of *Bifidobacterium* (of phylum Actinobacteria) by providing human milk oligosaccharides (HMOs) as their feeding substrate (8) (13). Human breastmilk also contains a unique milk microbiome (8) including *Bifidobacterium* and *Lactobacillus*, albeit in low abundances, and these may be transferred to the gut of breastfed infants (13). In contrast, formula-fed infants show more dominance of *Enterococci*, *Clostridium, Bacteroides fragilis* and *E.coli* along with higher bacterial diversity (8) (13).

As infancy progresses, Firmicutes and Bacteroidetes increase in abundance accompanied by increase in gut microbial diversity. Weaning and introduction of solid food leads to further increase in rise in microbial diversity with elevation of *Clostridium, Ruminococcus* (both of phylum Firmicutes) and *Bacteroides* (of phylum Bacteroidetes) while reducing the abundance of *Bifidobacterium* (of phylum Actinobacteria) and *Enterobacter* (of phylum Proteobacteria) (14). By end of the first year of life, the infant gut microbiota profile approximates that of an adult, and complete maturation takes place by age 3 years (8) (13).

The following table summarizes the relevant bacteria found in the infant gut, displayed by their taxonomic classification:

	Enterobacter	*Bifidobacterium*	*Bacteroides*	*Clostridium*	*Lactobacillus*
Phylum	Proteobacteria	Actinobacteria	Bacteroidetes	Firmicutes	Firmicutes
Class	Gammaproteobacteria	Actinobacteria	Bacteroidia	Clostridia	Bacilli
Order	Enterobacteriales	Bifidobacteriales	Bacteroidales	Clostridiales	Lactobacillales
Family	*Enterobacteriaceae*	*Bifidobacteriaceae*	*Bacteroidaceae*	*Clostridiaceae*	*Lactobacillaceae*
Genus	*Enterobacter*	*Bifidobacterium*	*Bacteroides*	*Clostridium*	*Lactobacillus*

Balanced development of the infant gut microbiota is crucial for child health. C-section (mode of delivery), perinatal antibiotic exposure, formula feeding (infant diet) and other early life environmental exposures perturbs the development of infant gut microbiota (8) (13). Mounting evidence demonstrates that infant gut dysbiosis, i.e. an imbalance in the gut microbial composition of infant, is associated with higher risk of childhood atopy, asthma and excessive weight gain (2) (15) (16) (17) (18). Therefore, the potential benefits of mitigating or possibly preventing these long-term pediatric health challenges by understanding the early-life factors that modulate the development of infant gut microbiota cannot be overstated. As such, many recent research efforts seeking to identify factors that influence the composition of infant gut microbiota have established that besides gestational age, antibiotic exposure and infant diet, the mode of delivery profoundly influences gut microbiota development (2) (18) (19). Compared to vaginally delivered counterparts, C-section born infants show reduced overall gut microbial diversity, and divergent microbial colonization (20). Despite the pivotal role of mode of delivery in the microbial seeding of newborn gut, a knowledge void exists on whether other elements of birth, such as duration of labour, influence the materno-fetal microbial transmission.

1.2 Labour and its role in dictating birth mode

"Whenever a woman is in labor she has pain, because her hour has come; but when she gives birth to the child, she no longer remembers the anguish because of the joy that a child has been born into the world."(21) Since the dawn of creation, labour and childbirth has been deemed both a curse and a boon for women - with the fear of excruciating labour pains often overshadowed by the anticipation of new life. And, although vaginal delivery was the only option for viable birth

in the biblical times, introduction of safe Cesarean delivery practices has led to major changes in the recent delivery trends.

The World Health Organization (WHO) recommends an ideal CS rate of 10-15%. However, the rate of C-section globally has escalated well beyond this mark. Latin America and the Caribbean region currently have the highest C-section rate (40.5%) while Northern America (32.3%), Oceania (31.1%), Europe (25%) and Asia (19.2%) follow at a steadily rising pace (22). The gravity of the situation is worsened by the fact that a woman's chance for a vaginal birth for subsequent deliveries after a primary C-section reduces to just about 11.5% (23). In a report published by ACOG in 2014 (and reaffirmed in 2016), the leading indication for primary CS is "failure to progress"; fetal distress is the second common indication (24) (25). Failure to progress, also known as 'labour dystocia', is diagnosed when progression of labour is unduly slow and abnormal. Since many repeat cesarean deliveries are performed after primary C-sections for labor dystocia, the overall burden of C-section attributable to the diagnosis of 'failure to progress' is substantial. Consequently, many researchers have endeavored to re-examine the *duration of normal labour* patterns in recent years.

1.3 Conventional definition of 'normal' labour

Birth is a dynamic process - an intricate interplay between fetal and maternal components confined by constraints of time and duration of labour. Labour is conventionally divided into three stages: The *latent phase* of first stage begins with maternal perception of labour pains and is accompanied by regular uterine contractions. During the latent phase, the cervix softens and effaces while cervical *os* dilates to 3 to 5 cm. The length of latent phase is variable and may last several hours. The *active phase* of first stage of labour begins with cervical dilatation of 3 to 5 cm in presence of more powerful uterine contractions and ends with full cervical dilation (26). The second stage of labour begins at full dilation of cervix (10 cm) and lasts until expulsion of the fetus. Expulsion of placenta and membrane completes the third stage of labour.

Thus far, the acceptable 'normal' duration of labour during childbirth has been guided by the Friedman's curve. Introduced in 1955, the Friedman's curve is a popular *obstetric gold*

standard used to evaluate the progress of labor. Friedman observed 500 Caucasian labouring primigravidas and documented the dilatation of cervix plotted against duration of elapsed time in a graphical representation. The resultant S-shaped curve of labour pattern is known as the 'Friedman's curve'. For the latent phase, the calculated mean was 8.6 hours (mean plus two standard deviations = 20.6 hours), and for the active phase, the calculated mean was **4.9 hours** (mean plus two standard deviations = **11.7 hours**) (27). A protracted active phase, per Friedman's curve, is diagnosed when the rate of cervical dilatation in the active phase is less than 1.2 cm/hour for nulliparous women and less than 1.5 cm/hour for multiparous women (27). However, new evidence indicates that pattern of labor progression in contemporary practice is significantly slower than depicted in the Friedman's curve.

1.4 Contemporary labour patterns

In 2002, Zhang et al undertook the examination labour patterns in contemporary obstetric practice. Using data from 1329 nulliparous parturients with a term, singleton fetus with vertex presentation, normal birth weight and spontaneous onset of labor, this study showed that the Friedman curve might not be accurate in depicting contemporary labour patterns. Zhang et al found that the dilation of cervix from 4 cm to 10 cm took approximately 5.5 hours (28). This is slower than the active phase duration under the Friedman curve. Over the recent years, other studies have also challenged Friedman's findings. A recent systematic review of 18 studies reporting on mean duration of *active labour* also affirmed that normal labour progression is slower than previously believed. Among low risk nulliparas with spontaneous onset of labour, the weighted mean duration of *active labour* was **6.0 hours** with statistical limit of **13. 4 hours** (mean plus two standard deviation) (29).

The second stage of labor is thought to last approximately 50 minutes in nulliparas and 20 minutes in multiparas. Kilpatrick found the mean second stage of labour was 54 minutes (mean plus two standard deviation = 132 minutes) in nulliparas with spontaneous onset of labour and no regional anesthesia (30). Use of regional anesthesia prolongs duration of second stage of labour. Zhang et al found that the statistical limit for duration of second stage for nulliparas could last up to 2.8 hours without epidural and up to 3.6 hours with epidural analgesia (31).

The findings of these new studies indicate that normal labour progresses much slower than conventionally accepted. This would in turn suggest that that majority of C-section that were done for 'failure to progress' in the past half a century were in fact done prematurely. Thus, duration of labour played a major role in misguided decision-making with regards to mode of delivery worldwide. Based on the new evidence, the new 2014 ACOG guidelines now recommend longer duration of labour for parturients provided that labour progression is monitored, and perinatal outcomes for both mother and the newborn are protected (24). However, it is yet to be determined how the labour, longer or otherwise, may affect the long-term health outcomes of the baby.

1.5 Duration of labour and the infant gut microbial colonization: possible mechanisms

After some degree of initial microbial exposure *in utero* (7) (8), the fetus experiences its first major microbial exposure upon encountering the maternal vaginal and fecal microbiota during its passage through the birth canal. Vaginally delivered infants acquire their gut microbial seeding from the maternal vaginal and colonic commensals, whereas C-section born infants are deprived of this opportunity and are more likely to be colonized by maternal cutaneous commensals and bacteria in the hospital environment (10). C-section born infants have delayed gut microbial colonization and lower abundance of phylum Bacteroidetes as compared to vaginally delivered infants, with the dysbiotic change persisting up to 1 year of age (32) (20). C-section born infants are also found to possess lower abundance of *Bifidobacterium* (of phylum Actinobacteria) and higher abundance of *Clostridium* (of phylum Firmicutes) (33) (34). These findings indicate that vaginal delivery provides unique gut microbial seeding opportunities, which are absent in C-section deliveries.

Since labour is an innate component of vaginal delivery, it is likely that duration of labour may influence the in the microbial colonization of the newborn gut, and consequently the development infant gut microbiota, through number of possible mechanisms as illustrated in the conceptual framework below. First, protracted labour frequently leads to exhaustion of uterine myometrial glycogen stores and ATP resulting accumulation of lactate. Longer labour duration is not only associated with upsurge in maternal lactate (35) but also with increased fetal lactate

concentrations (36). Since certain gut microbes such as *Veillonella and Megasphaera* (of family Veillionellaceae, and phylum Firmicutes) are lactate-utilizers (37), it is conceivable elevated availability of lactate may favor overgrowth of these bacterial communities among the maternal fecal commensals, and alter the composition of microbial inoculum for infant gut. Second, longer labour is often accompanied by amniorrhexis (either spontaneous or artificial). With a gaping conduit open between the *in utero* environment and the cervico-vaginal canal, continuous leakage of alkaline amniotic fluid could discourage the abundance of the habitual vaginal microbiota that normally thrive in slightly acidic vaginal pH. As illustrated in the conceptual framework, a newborn born after prolonged duration of labour could therefore receive 'sub-optimal' microbial seeding of maternal vaginal commensals as it passes through birth canal. Indeed, decreased vertical transmission of *Lactobacillus* (of phylum Firmicutes) from mother to newborn has been documented in vaginal deliveries after prolonged duration of membrane rupture (28). Besides, prolonged duration of labour significantly increases risk of chorioamnionitis (38) . Ascension of pathogenic bacteria into the uterine environment could result *in utero dysbiosis* that could subsequently lead to dysbiotic microbial seeding of fetal gut.

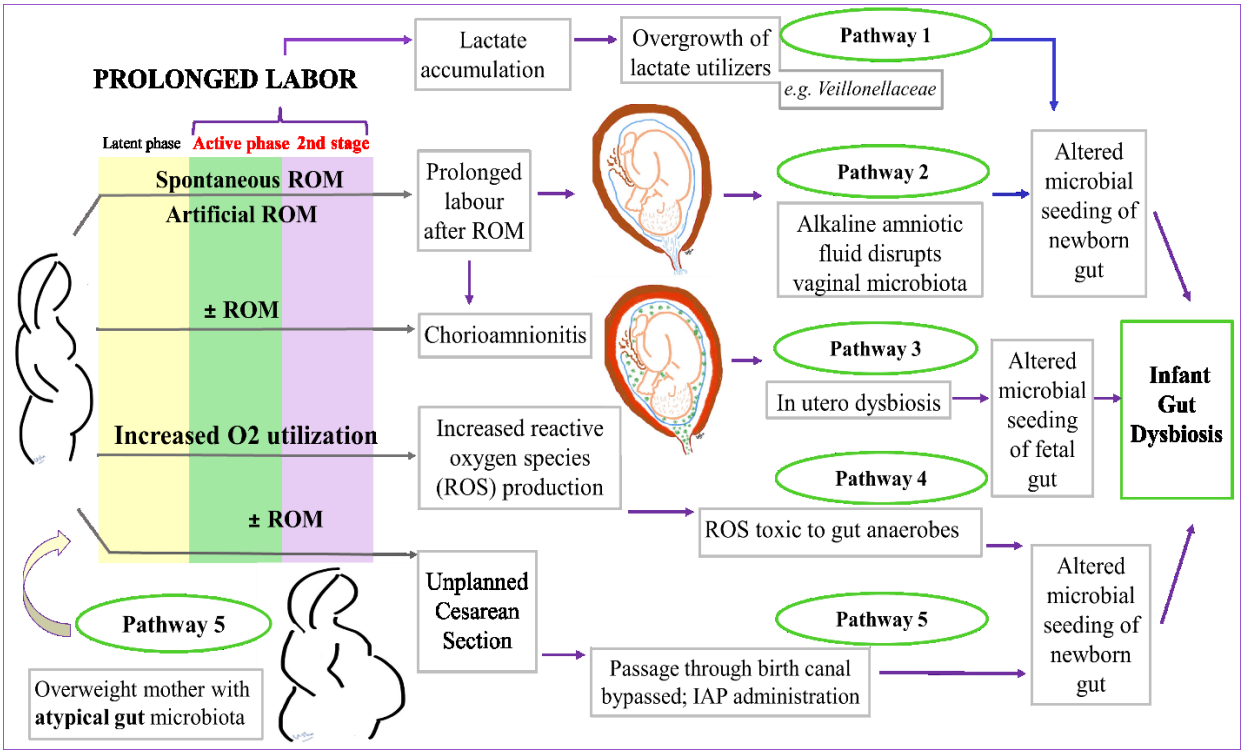
Another possible mechanism through which duration of labour may influence infant gut microbiota is via generation of tremendous oxidative stress during protracted labour states (i.e. 'failure to progress'). Enduring repetitive bouts of powerful myometrial contractions for prolonged periods of time is physically demanding for parturients, and necessitates massive consumption of oxygen (39). The subsequent increased production of reactive oxygen species (ROS) could be potentially toxic to some anaerobic members of gut microbial community because not all gut anaerobes are well-equipped to tolerate prolonged oxygen exposure and oxidative stress (40)(41)(42). Therefore, the maternal 'microbial seeding' received by infants delivered after prolonged labour durations could have subpar representation of these essential fecal anaerobes.

Labour duration can also influence the infant gut microbiota by dictating the mode of delivery. With evidence to indicate that more than two-thirds (68%) of *unplanned*, vertex C-sections are performed due to 'failure to progress' (25), prolonged duration of labour demonstrates a strong association with increased rate of emergency C-section. Interestingly, evidence shows more persistently divergent infant gut microbial colonization after emergency C-section as

compared elective C-section. In a recent Canadian longitudinal cohort study, Azad et al sampled fecal microbial composition of 198 healthy infants at 4 months and 12 months of age. Among infants delivered via emergency C-section, this study documented under-representation of the beneficial Bacteroidetes and over-representation of Firmicutes and Proteobacteria at 3 months of age, and persisting to 1 year. In contrast, there were no persisting microbiota differences at one year of age among infants delivered by elective C-section (19).

Figure 1.1 CONCEPTUAL FRAMEWORK

Possible mechanisms of influence of duration of labour on infant gut microbiota



ROM = Rupture of membranes

IAP = Intrapartum antibiotic prophylaxis

Finally, slow and abnormal progression of labour with protracted first stage, often culminating into unplanned C-section, is common obstetric course for many overweight and obese nulliparas (43). To add, pregnant women with elevated BMI (body mass index) possess distinctly atypical gut microbiota with significantly altered abundance of *Bacteroides* (of phylum

Bacteroidetes) (44) (45), higher *Clostridium*, *Staphylococcus* (both of Phylum Firmicutes) (44) and *Enterobacteriaceae* (of phylum Proteobacteria), and lower *Bifidobacterium* (of phylum Actinobacteria) (45). To what extent the prolonged duration of labour experienced by overweight pregnant women affect vertical transmission of atypical gut microbiota to their newborns remains to be investigated.

1.6 Studies on labour and long-term disease risks in children

Literature on the direct influence of duration of labour on long-term health outcomes for children is scarce and conflicting. Among older studies, Vonk et al found that delivery duration of longer than 12 hours was associated with the development of atopy in adult life [OR 2.24; 95% CI: 1.30-3.86] (46). Dik et al also observed a slight increase in risk of childhood asthma in children born after prolonged labour [HR=1.10, 95% CI=1.08-1.15] (47). In another study, Keski -Nisula et al documented that the risk of allergic sensitization, but not doctor-diagnosed childhood wheezing, tended to increase with the longer duration of labour (48).

In a more recent study, Black et al examined 3,21,287 term singletons and found that children born by scheduled Caesarean section are at higher risk of asthma as compared to children born vaginally [adjusted HR, 1.22 (95% CI, 1.11-1.34)] (49), which may suggest a possible role of labour in decreasing asthma risk in vaginally delivered infants. In contrast, a Swedish study involving 87,500 sibling pairs investigated the effect of labour in different types of C-section with regards to childhood asthma risk, and defined C-section before onset of labour as elective C-section (n= 4.2% of total) and C-section after onset of labour as emergency C-section (n= 5.4% of total). They found that emergency C-section [aOR = 1.14 (1.04–1.25)], but not elective C-section [aOR=1.06 (0.95–1.18)], was associated with increased risk of asthma medication use (50). Further, they also did not find any difference in the association between birth by elective (non-laboured) Caesarean section and asthma medication in children aged 10-12 years when compared to children born vaginally(50), suggesting no protective role of labour in long-term of asthma risk in children.

1.7 Summary

With the growing appreciation of the infant gut microbiome and its role in maintaining health or predisposing to disease(s), scientists and health-professionals seek better understanding of factors that influence development of infant gut microbiome. Since the gut microbiota is more variable and vulnerable to alterations in early life, better insight of factors that influence the microbial seeding and development of infant gut microbiome may allow for innovative ways to reduce disease risks. Although mode of delivery is a key factor to determine infant gut microbiota, a knowledge gap exists about the influence of duration of labour, an inherent element of mode of delivery, on the vertical transmission of gut microbiota.

In light of conflicting available evidence on the association between labour and long-term disease risk in children, and more recent evidence linking the infant gut microbiota to long-term disease outcomes in children, a deeper examination of whether duration of labour influences the infant gut microbiota is not only relevant in filling the knowledge gap but could also provide new insight regarding early life exposures that impact the development of infant gut microbiota. Hence, this study aimed to find out the effects of duration of labour on infant gut microbiota composition and diversity.

1.8 Hypothesis and objectives

This thesis aimed to test two hypotheses that duration of labour may influence the gut microbial composition and diversity of infants at three months of age, and that maternal pre-pregnancy overweight may affect this association. The primary objective of this study was to investigate the effect of duration of labour on infant gut microbial composition and diversity in the Canadian Healthy Infant Longitudinal Development (CHILD) national birth cohort. The specific objectives of this study were:

a) Is duration of labour associated with changes in gut microbial composition of infants at 3-4 months of age?

b) Does maternal overweight during pregnancy affect the above association?

1.9 Sample size calculation

The sample size was calculated based on Shannon diversity index mean and standard deviation (at genus level) for infant gut microbiota at three months of age from a previous CHILD study conducted by Azad et al (11). An α level of 0.05 and power of 80% is aimed.

Sample size (n) = 2 (Power Index * Standard deviation/ Difference in Means) ^{2}

$= 2 [(1.96+ 0.84) * 0.63/ 2.16- 2.00]^2$

$= 2 (2.8 * 0.63/ 0.16]^2$

Sample size (n) = 243.23 (approximately 244)

Allowing for 10% attrition rate:

Desired sample size = N (number to enroll) * (% retained)
Therefore, N (number to enroll) = Desired sample size / (% retained)
Final sample size (N) = 244/0.90 = 271.11

Thus, samples with **271** subjects in each group is required to ensure that the test hypothesis will have power of 80% to detect significant change in difference of means of the study groups.

1.10 Overview of study design

This is a secondary study based on data from the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort. The CHILD is a longitudinal, population-representative birth cohort study of 3624 pregnant mothers recruited from four provinces of Canada: British Columbia (Vancouver, urban), Alberta (Edmonton, urban), Manitoba (Winnipeg, urban; Mordern and Winker, rural), and Ontario (Toronto, urban) between 2008 and 2012 (Moraes et al., 2014). Approximately 85% of pregnant mothers were enrolled during their second trimester at health care locations with the following inclusion criteria: pregnant women 18 years or older, lives in residence in reasonable proximity to the delivery hospital, able to read, write and speak English, provides informed consent, consents to cord blood collection, plans to give birth at a designated recruitment center participating hospital, infants born at or after 35 weeks, and families able to provide name, address and telephone numbers of two alternate contact individuals. Children are

clinically assessed at birth, at three months, and at 1-, 3- and 5 -year visits. The exclusion criteria are children born with major congenital abnormalities or respiratory distress syndrome, expectation of moving away within one year, children of multiple births, children resulting from in vitro fertilization, children who will not spend at least 80% of nights in the home, and children born before 35 weeks.

For this particular study, data from subset of infants (N=1028) recruited in the three sites of the CHILD birth cohort (Edmonton, Winnipeg and Vancouver) was utilized. CHILD recruited the mothers of these infants as consecutive enrollments during their pre-natal visit in the second trimester as per the inclusion and exclusion criteria listed above. To avoid selection bias, all infants from the three CHILD sites with complete fecal microbial sequencing and taxonomic assignment were included in this study, and no other additional criteria or deliberate selection of infants was implemented to define our study sample. After exclusion of home births, a total of 999 infants remained in the study. Hospital birth charts have provided information on duration of labour, labour characteristics, mode of delivery and some covariates of interest. Complete information on *active* first stage of labour (n= 918) and second stage of labour (n=955) was documented. In the CHILD study cohort, the onset of active first stage of labour is defined as cervical dilation of 4 cm in presence of regular uterine contractions and ends at cervical dilatation of 10 cm. The second stage of labour begins from full dilatation of cervix (10 cm) and ends with complete expulsion of the fetus. The 3rd stage of labour, i.e. duration after delivery of newborn to the expulsion of placenta, was not included in this study since the third stage of labour has limited relevance with regards to microbial transmission opportunity from mother to newborn. Likewise, *latent* phase of first stage was also not studied due to the very 'subjective' nature of the perception of its onset.

For vaginally delivered infants with the active 1st stage of labour, a labour length variable denoting three mutually exclusive categories was created as follows: (a) Duration of active 1st stage of labour $\leq$ 6 hours [Reference category: Group 1] (b) Duration of active 1st stage of labour $>$ 6 to $\leq$ 13 hours [Group 2] (c) Duration of active 1st stage of labour $>$ 13 hours [Group 3]. These cut-offs were based on a recent systematic review of eighteen studies by Neal et al that found that weighted mean duration of active labour in nulliparas was 6.0 hours with mean plus two standard

deviation of 13.4 hours (21). For second stage of labour, a labour length variable denoting three mutually exclusive categories was created based on cutoffs described by Kilpatrick et al (30) and are as follows: (a) Duration of 2nd stage of labour ≤ 1 hours [Reference category: Group 1] (b) Duration of 2nd stage of labour > 1 to ≤ 2 hours [Group 2] (c) Duration of 2nd stage of labour > 2 hours [Group3].

For infants delivered by C-section after onset of labour, a labour length variable for active 1st stage of labour denoting two mutually exclusive categories was created as follows: (a) C-section with duration of active 1st stage of labour ≤ 6 hours [Reference category: Group 1] and (b) C-section with duration of active 1st stage of labour > 6 hours [Group 2]. For 2nd stage of labour, a labour length variable denoting two mutually exclusive categories was created as follows: (a) C-section with duration of 2nd stage of labour ≤ 1 hour [Reference category: Group 1] and (b) C-section with duration of 2nd stage of labour > 1 hour [Group 2].

Fecal samples for microbiota analysis were collected from infants at 3–4 months of age. Data on covariates that had capacity to affect the either the exposure variable or outcome variable, or both, were obtained from hospital records (mode of delivery, intrapartum antibiotic prophylaxis (IAP), parity, duration after rupture of membranes, epidural administration, medical induction of labour, length of infant's hospital stay, maternal pre-pregnancy body mass index (BMI), maternal age etc.) or from standardized questionnaires completed by mothers (breastfeeding status, maternal ethnicity, maternal smoking, maternal asthma), and were included in the study. Written informed consent was obtained from parents at enrollment. This study was approved by the ethics board at the University of Alberta.

1.11 Fecal sample collection, DNA extraction and PCR amplification

Faecal samples of infants were collected at 3-4 months of age using a standard protocol during a scheduled home visit. Samples were refrigerated immediately after collection and during transport, and stored at -80 °C until analysis. Genomic DNA was isolated with QIAamp DNA stool Mini Kit (Qiagen, Venlo, the Netherlands), and the hypervariable V4 region of the bacterial 16S

rRNA gene was amplified by polymerase chain reaction (PCR) using universal bacterial primers. For sample multiplexing, reverse primers were barcoded uniquely for each sample (barcoded sequence was denoted in the primer sequence by Xs). PCR amplification consisted of an initial denaturation step for 3 min at 94 °C, followed by 20 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 50 °C and an extension step for 30 s at 72 °C. PCR reactions for each sample were performed in triplicate with a negative control in each run. One hundred nanograms of pooled PCR product from each sample was concentrated using an Amicon Ultra-4 30K centrifugal filter.

1.12 Sequencing and taxonomic nomenclature

Pooled PCR amplicons were sequenced using the MiSeq Illumina Sequencing at the University of Toronto Centre for the Analysis of Genome Evolution & Function (CAGEF). Using a QIIME pipeline, forward and reverse reads were assembled for a final length of 144 bp demultiplexed and filtered against the GREENGENES reference database (v13.8) to discard all sequences with <60% similarity. Remaining sequences were clustered at 97% sequence similarity against the GREENGENES database (using closed picking algorithm in QIIME), and taxonomic assignment was achieved using the RDP classifier. After taxonomic assignment, operational taxonomic units (OTUs) representing bacterial origin were selected, and bacterial OTUs with overall relative abundance below 0.0001 were excluded from subsequence for downstream analyses. Microbiota diversity within samples (α diversity) was calculated using two standard metrics: the Chao1 estimator of OTU richness (which estimates the number of different OTUs present) and the Shannon diversity index (which evaluates both the number of OTUs and the evenness of their distribution). Those metrics were calculated at OTU and family levels.

1.13 Statistical analyses

Statistical analyses were performed in SPPS version 22.0 (SPSS, Inc., Chicago, IL, USA). Chi-square test was used to examine the distribution of potential confounders according to exposure to differential duration of labour. The gut microbial composition (median relative

abundance) of infants with duration of active first stage of labour ≤ 6 hours (reference group) was compared to the gut microbiota composition of infants with first stage of labour > 6 to ≤13 hours and > 13 hours. Similarly, the gut microbial composition of infants with duration of second stage of labour ≤ 1 hours (reference group) was compared to that of infants with first stage of labour > 1 to ≤ 2 hours and > 2 hours. Median richness, diversity and relative abundance of dominant taxa were compared by non-parametric Mann-Whitney U-test. A p-value of <0.05 was defined as statistically significant, and 95% confidence intervals (CIs) were calculated.

Regression analysis was used to determine the relationships between the measured between exposure parameters and gut microbiota outcome Univariate analysis and multivariate logistic regression were used to identify variables independently associated with the outcome variables. Variables with a p-value of <0.25 in univariate analyses and clinically significant covariates were included in multivariable analyses. Microbiota measures were classified in two groups (below vs. above median). The following variables were tested in the multivariable models as potential confounders: mode of delivery, maternal intrapartum antibiotic exposure, infant diet, parity, duration after rupture of membrane, epidural use, medical induction of labour, length of hospital stay and age of stool collection.

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CHAPTER 2

Duration of labour and changes in infant gut microbiota composition at 3- 4 months of life

2.1 Introduction

Balanced development of the infant gut microbiota is crucial for health. The immune-modulatory properties of the early gut colonizers in infant can govern long-term disease risks in children (1) (2) (3) (4). The mode of delivery (vaginal versus Caesarean) is a major determinant of infant gut microbiota development. Compared to vaginally delivered infants, those born by C-section have divergent gut microbial colonization (5) (6). Since labour is intrinsic component of natural birth, investigating the influence of labour and its duration on gut microbial seeding may shed new light on development of infant gut microbiota.

The process of labor is deemed beneficial in aiding the transition of newborn from intrauterine environment to postnatal life. Changes in feto-maternal hormonal milieu near term and during labour helps in clearing fetal lung fluid (7) (8), offers neuroprotection to the newborn from anoxic-aglycaemic episodes during delivery(9), proffers newborn analgesia (10), and contributes to immune maturation (11) (12). However, evidence on the longer-term benefits of labour is limited and conflicting. Compared to vaginal deliveries, children born by scheduled C-section (no labour) were found to be at higher risk of asthma by Black et al [adjusted HR= 1.22; (95% CI, 1.11-1.34)] (13) and Rusconi et al [aRR=1.33 (95% CI: 1.02,1.75)] (14). While these findings may suggest a protective role of labour for childhood asthma risk, no significant difference in asthma risk was found while comparing elective C-section (no labour) to emergency C-section (likely some labour) in both studies. Besides, a study of 87,500 sibling pairs found no significant difference between scheduled C-section (non-laboured) and vaginal delivery for asthma medication in children aged 10-12 years(15), suggesting no protective role of labour. Duration of labour is another consideration for risk modification. Earlier studies observed that longer duration of labour was associated with the higher risk of pediatric atopy [OR 2.24; 95% CI: 1.30-3.86] (16) and physician diagnosed asthma [HR=1.10, 95%CI 1.08-1.15] (17). In contrast, a newer study found no evidence of longer labour and increased risk of doctor diagnosed wheezing (18). With such conflicting evidence at hand, a closer examination of whether labour duration affects infant

gut microbial colonization, and thereby the long-term disease risk in children, may guide accurate conclusions.

Labour is inherent component of natural birth. After some degree of microbial exposure *in utero* (2)(19), the first major microbial exposure of fetus occurs upon encountering the maternal vaginal and fecal microbiota during the time-span of its egress from birth canal. Thus, it stands to reason that duration of labour, especially if prolonged, could affect the magnitude of microbial exposure to infants born vaginally. Protracted labour is also associated with longer duration after rupture of membrane and higher risk of chorioamnionitis (20), and delivery by unplanned C-section (21) (22) (23), all of which could alter the microbial inoculum to the fetus. On the other hand, infants born by elective C-section or emergency C-section with no labour and intact membranes are likely to be deprived of any significant exposure to maternal vaginal and fecal microbes, whereas those born by emergency (unplanned) C-section performed after some length of labour (and rupture of membranes) may experience some degree of interaction with maternal vaginal/fecal microbes. In a study of 198 healthy term infants, under-representation of Bacteroidetes and over-representation of Firmicutes and Proteobacteria phyla observed in fecal samples at 3-4 months persisted to samples obtained at 1 year of age in infants born by emergency C-section (majority of cases with labour), but not in infants born by elective C-section (no labour) (5). Another recent study also observed that the meconium microbiota of neonates born by laboured C-section were similar to maternal fecal microbiota whereas the meconium microbiota of infants born by unlaboured C-section were more similar to maternal cutaneous microbiota (24).

Unfortunately, the literature on the direct influence of duration of labour on the microbial colonization of newborn gut is scarce. Among older studies, Cornelison et al found that the percentage of *E. coli* (of phylum Proteobacteria) in the oronasal cavity of newborns is increased during longer deliveries, suggesting role of duration of labour in microbial transfer to newborns (25). Likewise, Brook et al documented a significant positive correlation between prolonged duration of labour and isolation of anaerobes from newborns' gastric aspirates (26). However, these findings are inconsistent with a more recent study that examined the transmission of *Lactobacillus* (of phylum Firmicutes) dominant mixed vaginal flora to newborns' oral cavity and found that it was not significantly associated with duration of labour ($p=0.216$) (27).

The influence of duration of labour to infant gut microbiota composition is unknown. The duration of labour, its absence or prolongation has real potential to impact microbial seeding of newborn gut thereby affecting the development of infant gut microbiota and future disease risks. Therefore, the aim of this study to examine whether duration of labour influences the composition of infant gut microbiota at 3 to 4 months of life.

2.2 Materials and Methods

2.2.1 Study design

This study involved a subsample of 999 infants from three study sites (Edmonton, Vancouver and Winnipeg) of the CHILD cohort (www.childstudy.ca). Mothers of the studied infants were recruited during pregnancy between 2009 and 2012. Information on duration of *active* first stage of labour and second stage of labour, accompanying labour characteristics, mode of delivery and some covariates were obtained for hospital charts. In the CHILD cohort, the onset of active first stage of labour is defined as cervical dilation of 4 cm in presence of regular uterine contractions and ends at cervical dilatation of 10 cm. The second stage of labour begins from full dilatation of cervix (10 cm) and ends with expulsion of the fetus. The 3rd stage of labour, i.e. duration after delivery of newborn to the expulsion of placenta, was not included in this study since the third stage of labour has limited relevance with regards to microbial transmission opportunity from mother to newborn.

For vaginally delivered infants, a labour length variable for active first stage of labour denoting three mutually exclusive categories was created as follows: (1) Duration of active 1st stage of labour $\leq$ 6 hours [Reference category: Group 1] (2) Duration of active 1st stage of labour $>$ 6 to $\leq$ 13 hours [Group 2] (3) Duration of active 1st stage of labour $>$ 13 hours [Group 3]. These cut-offs were based on a recent systematic review of eighteen studies that found that weighted mean duration of active labour was 6.0 hours with mean plus two standard deviation of 13.4 hours (28). For second stage of labour, a labour length variable denoting three mutually exclusive

categories was created (29) as follows: (1) Duration of 2nd stage of labour ≤ 1 hours (Reference category: Group 1] (2) Duration of 2nd stage of labour > 1 to ≤ 2 hours [Group 2] (3) Duration of 2nd stage of labour > 2 hours [Group3].

For infants delivered by C-section after onset of labour, a labour length variable for active 1st stage of labour denoting two mutually exclusive categories was created as follows: (1) C-section with duration of active 1st stage of labour ≤ 6 hours [Reference category: Group 1] and (2) C-section with duration of active 1st stage of labour > 6 hours [Group 2]. For 2nd stage of labour, a labour length variable denoting two mutually exclusive categories was created as follows: (1) C-section with duration of 2nd stage of labour ≤ 1 hour [Reference category: Group 1] and (2) C-section with duration of 2nd stage of labour > 1 hour [Group 2]. 'Elective C-section births' and 'Emergency C-section without labour' were excluded from the non-parametric analyses for microbial median relative abundance on account of absence of labour.

Data on covariates that could potentially affect the exposure variable or outcome variable, or both, were obtained from hospital charts (mode of delivery, intrapartum antibiotic prophylaxis (IAP), parity, duration after rupture of membranes, infant sex, gestational age ,epidural use, medical induction of labour, length of infant's hospital stay, maternal pre-pregnancy body mass index (BMI), maternal age etc.) or from standardized questionnaires completed by mothers (breastfeeding status, maternal ethnicity, maternal smoking, maternal asthma, furry pet ownership etc.), and were considered in the study. Written informed consent was obtained from parents at enrollment. This study was approved by the ethics board at the University of Alberta.

2.2.2 Fecal sample collection, DNA extraction and PCR amplification

Faecal samples of infants were collected at 3-4 months of age using a standard protocol during a scheduled home visit. Samples were refrigerated immediately after collection and during transport, and stored at -80 °C until analysis. Genomic DNA was isolated with QIAamp DNA stool Mini Kit (Qiagen, Venlo, the Netherlands), and the hypervariable V4 region of the bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal bacterial primers.

For sample multiplexing, reverse primers were barcoded uniquely for each sample (barcoded sequence was denoted in the primer sequence by Xs). PCR amplification consisted of an initial denaturation step for 3 min at 94 °C, followed by 20 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 50 °C and an extension step for 30 s at 72 °C. PCR reactions for each sample were performed in triplicate with a negative control in each run. One hundred nanograms of pooled PCR product from each sample was concentrated using an Amicon Ultra-4 30K centrifugal filter.

2.2.3 Sequencing and taxonomic nomenclature

The MiSeq Illumina Sequencing was employed to sequence the pooled PCR amplicons at the University of Toronto Centre for the Analysis of Genome Evolution & Function (CAGEF). Using a QIIME pipeline, forward and reverse reads were assembled for a final length of 144 bp demultiplexed and filtered against the GREENGENES reference database (v13.8) to discard all sequences with <60% similarity. Remaining sequences were clustered at 97% sequence similarity against the GREENGENES database (using closed picking algorithm in QIIME), and taxonomic assignment was achieved using the RDP classifier. After taxonomic assignment, operational taxonomic units (OTUs) representing bacterial origin were selected, and bacterial OTUs with overall relative abundance below 0.0001 were excluded from subsequence for downstream analyses. Microbiota diversity within samples (α diversity) was calculated using two standard metrics: the Chao1 estimator of OTU richness (which estimates the number of different OTUs present) and the Shannon diversity index (which evaluates both the number of OTUs and the evenness of their distribution). Those metrics were calculated at OTU and family levels.

2.2.4 Statistical analyses

Statistical analyses were performed in SPPS version 22.0 (SPSS, Inc., Chicago, IL, USA). The distribution of potential confounders according to exposure to differential duration of labour was investigated using Chi-square test. The gut microbial profile of infants with duration

of active first stage of labour ≤ 6 hours (reference group) was compared to the gut microbiota profile of infants with first stage of labour > 6 to ≤ 13 hours and > 13 hours. Similarly, the gut microbial profile of infants with second stage of labour ≤ 1 hours (reference group) was compared to the gut microbiota profile of infants with second stage > 1 to ≤ 2 hours and > 2 hours. Median relative abundance, Chao1 richness and Shannon diversity of dominant taxa were compared using non-parametric Mann-Whitney U-test. 'Elective C-section births' and 'Emergency C-section without labour' were excluded from the non-parametric analyses for microbial median relative abundance. A p-value of <0.05 was defined as statistically significant, and 95% confidence intervals (CIs) were calculated. Univariate analysis and multivariate logistic regression were performed to identify variables independently associated with the outcome. Variables with a p-value of <0.25 in univariate analyses and clinically significant covariates were included in multivariable analyses. Microbiota measures were classified in two groups (below vs. above median). The following variables were included in the multivariable models as potential confounders: mode of delivery, maternal intrapartum antibiotic exposure, infant diet (exclusive breastfeeding status), gestational age, parity, duration after rupture of membrane, maternal pre-pregnancy BMI, infant's length of hospital stay and age of stool collection.

2.3 Results

2.3.1 Study population

Of the 999 infants in this general population, 918 (91.9%) infants had complete information on duration of active 1st stage of labour and 955 (95.6%) infants had complete information on duration of 2nd stage of labour.

Of the 918 infants with information on duration of active 1st stage of labour, 564 (61.4%) were born after active 1st Stage duration ≤ 6 hours [Group 1 = Reference group], 267 (29.1%) were born after active 1st stage duration greater than 6 hours and ≤ 13 hours [Group 2 infants], and 87 (9.5%) of infants were born after active 1st stage duration greater than 13 hours [Group 3 infants]. Table 2.1 describes the characteristics of mother-infant pairs according to the three categories of duration of active 1st stage. There were significant differences between the three groups with

respect to mode of delivery by intrapartum antibiotic prophylaxis ($p<0.001$), parity ($p<0.001$), duration after rupture of membranes ($p= 0.003$) and length of baby's hospital stay ($p= 0.001$). No significant differences were detected in the direct antibiotic exposure ($p= 0.790$), infant diet at three months of age ($p=0.979$), maternal age ($p=0.450$), maternal ethnicity (0.992), maternal pre-pregnancy overweight ($p=0.874$), pre-natal smoke exposure ($p=0.083$), maternal asthma ($p=0.260$) and exposure to furry pets at home ($p=0.551$) according to duration of active 1st stage of labour categories.

Of the 955 infants with complete information on duration of 2nd stage of labour, 667 (69.8%) were born after 2nd stage duration ≤ 1 hour [Group 1 infants = Reference group], 125 (13.1%) were born after 2nd stage duration >1 hour and ≤ 2 hours [Group 2 infants], and 163 (17.1%) of infants were born after 2nd stage duration > 2 hours [Group 3 infants]. Table 2.2 describes the characteristics of mother-infant pairs according to the three categories of duration of 2nd stage. There were significant differences between the three groups with respect to mode of delivery by intrapartum antibiotic prophylaxis status ($p<0.001$), parity ($p<0.001$) and duration after rupture of membranes ($p< 0.001$). No significant differences were detected in the direct antibiotic exposure ($p= 0.573$), infant diet at three months of age ($p=0.440$), length of baby's hospital stay ($p= 0.609$), maternal age ($p=0.291$), maternal ethnicity (0.883), maternal pre-pregnancy overweight ($p=0.209$), pre-natal smoke exposure ($p=0.249$) and maternal asthma ($p=0.533$) according to duration of 2nd stage of labour categories.

2.3.2 Fecal microbiota composition, richness and diversity

i) Effect of duration of active 1st stage of labour

Table 2.3 outlines the summary of the significant ($p<0.05$) changes in median relative abundance of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the **duration of active first stage of labour**, and following different levels of stratifications.

Among all delivery modes (vaginal and C-section), we observed underrepresentation of Actinobacteria ($p<0.05$) and over-representation of Bacteroidetes ($p<0.05$) with increasing

duration of active 1st stage of labour at the phyla level [Table 2.4] [Fig.2.2]. Firmicutes appeared to decrease with longer active 1st stage, but this change was not statistically significant. At family level, longer active 1st stage (>13 hours) was associated with significantly lower abundance of *Bifidobacteriaceae* (p=0.042), *Coriobacteriaceae* (p=0.022) and *Lactobacillaceae* (p=0.004) but higher abundance of *Bacteroidaceae* (p=0.009) [Table 2.4]. *Ruminococcaceae* significantly reduced with active 1st stage ≥ 6 to < 13 hours (p=0.018), but not when active 1st stage was > 13 hours [Table 2.4]. At genus level, abundance of *Bifidobacterium* decreased with active 1st stage ≥ 6 to < 13 hours (p=0.001), and reduced further with active 1st stage > 13 hours (p=0.039) [Table 2.4]. In contrast, abundance of *Bacteroides* showed directly proportional increase with increasing durations of active first stage (p <0.05) [Table 2.4]. Additionally, *Lactobacillus* (p=0.004) and *Citrobacter* (p=0.050) reduced in abundance when active first stage was longer than 13 hours [Table 2.4].

Stratified analyses results: Microbial abundance adjusted for delivery mode, intrapartum antibiotic prophylaxis and exclusive breastfeeding

When stratified by delivery mode, vaginally delivered infants who were not exposed to intrapartum antibiotic prophylaxis (IAP) showed significantly decremental abundance of phylum Actinobacteria whereas changes in other phyla were not statistically significant [Table 2.5]. At family level, abundance of *Bifidobacteriaceae* reduced with 1st stage of labour ≥ 6 to < 13 hours (p=0.010) and decreased further with active 1st stage > 13 hours (p=0.015) [Table 2.5] [Fig 2.3]. Further, active 1st stage longer than 13 hours was associated with decreased abundance of *Coriobacteriaceae* (p=0.017), *Enterococcaceae* (p=0.021), *Lactobacillaceae* (p=0.049) and a increased abundance of *Clostridiaceae* (p=0.006) [Table 2.5]. At genus level, *Bifidobacterium* showed reduction with 1st stage of labour ≥ 6 to < 13 hours (p=0.010) and a further decrease with active 1st stage > 13 hours (p=0.016) [Table 2.5]. Upon further stratification by exclusive breastfeeding status, vaginally born IAP-free infants who were deprived of exclusive breastfeeding showed significant reduction in abundance of *Bifidobacterium* (at genus level) in association with active 1st stage of labour > 13 hours (p= 0.038) [Table 2.6a]. Additionally, reduction in genera *Streptococcus* (p=0.010) and *Ruminococcus* (p=0.029) were also observed with active 1st stage > 13 hours among these infants. In contrast, vaginally born IAP-free infants who were exclusively

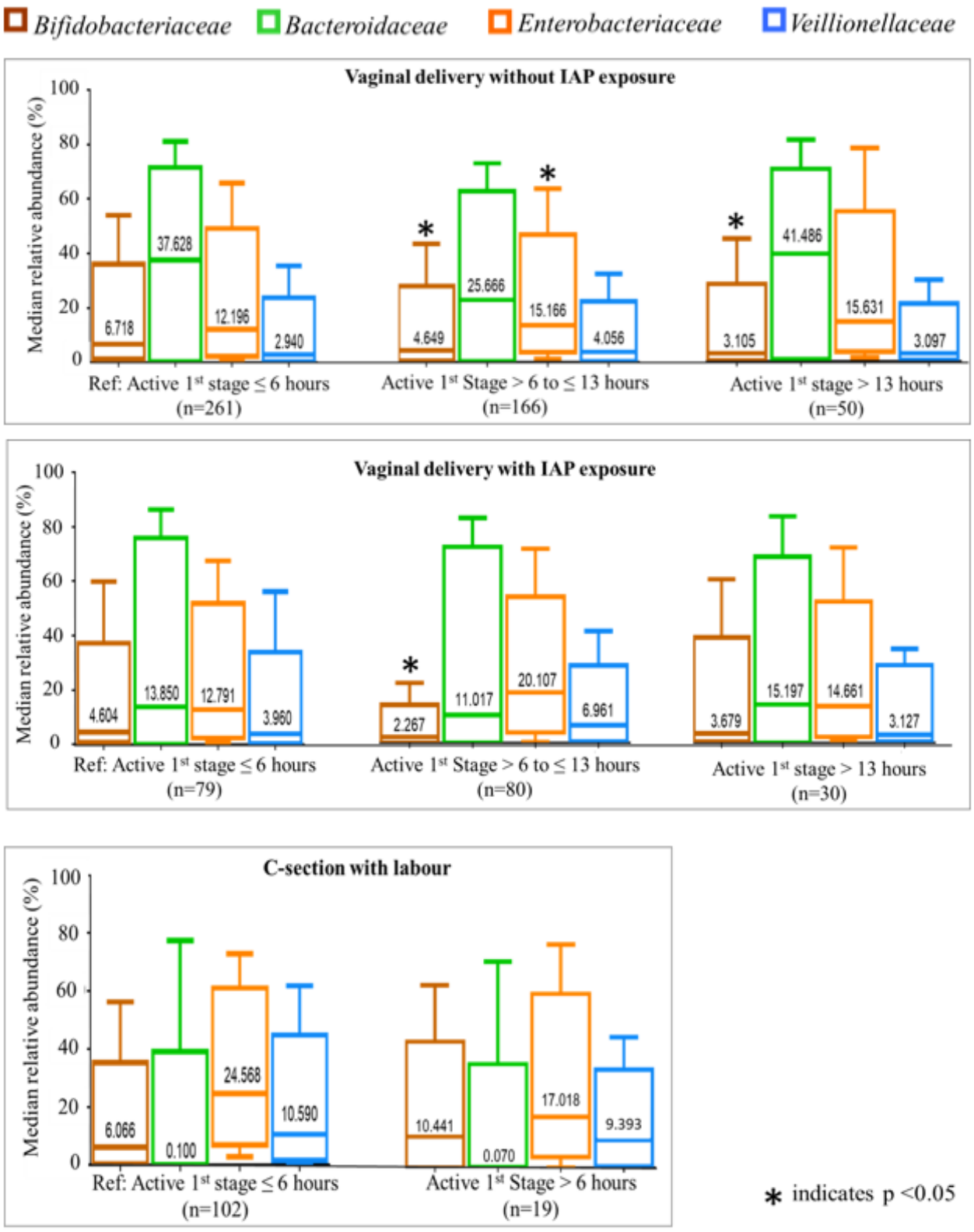
breastfed showed no significant reduction in *Bifidobacterium*, *Streptococcus* or *Ruminococcus* with 1st stage of labour > 13 hours [Table 2.6b].

Among infants delivered vaginally with positive IAP exposure, at phylum level, abundance of Actinobacteria was reduced with 1st stage of labour ≥ 6 to < 13 hours (p=0.003) but not when active 1st stage was longer than 13 hours [Table 2.7]. At family level, *Bifidobacteriaceae* (p=0.003) and *Ruminococcaceae* (p=0.016) were decreased whereas *Streptococcaceae* (p=0.032) was increased in infants born with 1st stage of labour ≥ 6 to < 13 hours. Among infants born after active 1st stage > 13 hours, changes in these microbial families were not significant [Table 2.7]. At genus level, *Bifidobacterium* was significantly reduced with 1st stage of labour ≥ 6 to < 13 hours (p=0.008) but not when active 1st stage was longer than 13 hours [Table 2.7]. Upon further stratification by exclusive breastfeeding status, vaginally born IAP-exposed infants who were not exclusive breastfed showed significant reduction in abundance of genera *Bifidobacterium* (p=0.016) and *Streptococcus* (p=0.035) in association with active 1st stage of labour ≥ 6 to < 13 hours, but not when active 1st stage was > 13 hours [Table 2.8a]. Likewise, for exclusively breastfed infants (vaginally born and IAP-exposed), a reduction in *Bifidobacterium* (p=0.027) was observed with active 1st stage of labour ≥ 6 to < 13 hours (p=0.027), but not when active 1st stage was >13 hours [Table 2.8b]. Changes in other genera were not statistically significant [Table 2.8b].

Among infants delivered by C-section with labour, as compared to infants born by C-section with active 1st stage duration ≤ 6hours, those with active 1st stage > 6 hours appeared to show an increased abundance of *Bifidobacterium* (of phylum Actinobacteria), and decreased abundance *Bacteroides* (of phylum Bacteroidetes) along with decreased abundance C*lostridium* (of phylum Firmicutes) [Fig. 2.5] [Table 2.9]. However, none of these changes were statistically significant. We also performed a sensitivity analysis of the infant gut microbiota comparing infants born by C-section without labour versus C-section with labour and did not find significant associated changes [Table 2.10].

The following figure summarizes the results discussed thus far for active 1st stage:

Fig. 2.1a Summary figure showing changes in microbiota abundance at family level according to duration of active first stage, stratified by mode of delivery



Regression analyses results: Microbial abundance adjusted for all potential confounders

We conducted multivariate logistic regression to further explore the association of duration of active 1st stage of labour and gut microbiota composition and diversity. At phyla level, likelihood of gut colonization with Actinobacteria decreased significantly in infants born after active first stage > 6 to ≤ 13 hours [Group 2 infants versus Group 1: aOR = 0.53 (95%CI = 0.38-0.44); p = 0.001], but not among infants after active first stage > 13 hours [Group 3 infants versus Group 1: aOR = 0.63 (95%CI = 0.38-1.06); p=0.080] [Table 2.11]. At family level, longer active 1st stage durations were associated with progressive reduction in likelihood of colonization with *Bifidobacteriaceae* {[Group 2 infants versus Group 1: aOR = 0.57 (95% CI = 0.41-0.81), p = 0.001]; Group 3 infants versus Group 1: [aOR = 0.56 (95%CI = 0.34-0.95), p = 0.030]} [Table 2.11]. In addition, likelihood of colonization with *Ruminococcaceae* reduced with active 1st stage > 6 to ≤ 13 hours but not when 1st stage was > 13 hours {[Group 2 infants versus Group 1: aOR = 0.66 (95% CI = 0.45-095), p = 0.027]; Group 3 infants versus Group 1: [aOR = 0.90 (95%CI = 0.51-1.59), p = 0.711]}. Likewise, likelihood of colonization with *Lactobacillaceae* reduced with active 1st stage > 13 hours {[Group 2 infants versus Group 1: aOR = 0.78 (95% CI = 0.55-1.10), p = 0.155]; Group 3 infants versus Group 1: [aOR = 0.53 (95%CI = 0.30-0.95), p = 0.032]} [Table 2.13a].

At genus level, longer active 1st stage of labour was associated with higher risk of reduced *Bifidobacterium* and *Lactobacillus* colonization. Likelihood of gut colonization with *Bifidobacterium* decreased by 43% in infants born after active first stage > 6 to ≤ 13 hours [aOR = 0.57 (95%CI = 0.41-0.81), p = 0.001], and decreased by 44% after active first stage > 13 hours [aOR = 0.56 (95%CI = 0.34-0.95), p = 0.030] [Table 2.11] [Fig. 2.6]. In addition, infants born after active first stage longer than 13 hours also showed a 47% reduced likelihood of colonization with *Lactobacillus* [aOR = 0.53 (95%CI = 0.30-0.95), p = 0.032] [Table 2.13b] [Fig. 2.6]. These associations were independent of mode of delivery, intrapartum antibiotic prophylaxis (IAP) use, breastfeeding status, gestational age, parity, membrane rupture duration greater than 18 hours, length of infant's hospital stay, age of fecal sample collection and maternal pre-pregnancy overweight. As for the other genera that showed significant changes in stratified analyses, statistical significance was not retained after adjusting for all potential cofounders in the regression analyses. Finally, we noted a reduced trend for microbial diversity with longer active 1st stage

although it was only significant for infants born with active 1st stage > 6 to ≤ 13 hours {[Group 2 infants versus Group 1: aOR = 0.64 (95% CI = 0.45-0.90), p = 0.011]; Group 3 infants versus Group 1: [aOR = 0.74 (95%CI = 0.44-1.126, p = 0.274]} [Table 2.15].

ii) Effect of duration of 2nd stage of labour

Table 2.16 outlines the summary of the significant ($p<0.05$) changes in median relative abundance of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the **duration of second stage of labour**, and following different levels of stratifications.

Among all delivery modes (vaginal and C-section), at phyla level, we observed underrepresentation of phylum Actinobacteria ($p<0.010$) with 2nd stage longer than 2 hours [Table 2.17] [Fig.2.7]. At family level, *Bifidobacteriaceae* (p=0.006), *Coriobacteriaceae* (p=0.005), *Lactobacillaceae* (p=0.008) and *Ruminococcaceae* (p=0.051) decreased in abundance where as *Clostridiaceae* (p=0.003) increased in abundance when 2nd stage of labour was longer than 2 hours [Table 2.17]. At the genus level, abundance of *Bifidobacterium* (p=0.005) and *Lactobacillus* (p=0.008) decreased when 2nd stage was longer than 2 hours [Table 2.17].

Stratified analyses results: Microbial abundance adjusted for delivery mode, intrapartum antibiotic prophylaxis and exclusive breastfeeding

When stratified by delivery mode, vaginally born IAP-free infants showed underrepresentation (at phyla level) of Actinobacteria when 2nd stage was longer than 2 hours (p=0.012) [Table 2.18]. At family level, these infants showed underrepresentation of *Bifidobacteriaceae* (p=0.005) and *Coriobacteriaceae* (p=0.050), and overrepresentation of *Clostridiaceae* (p=0.000) associated with 2nd stage longer than 2 hours [Table 2.18] [Fig. 2.8]. At genus level, abundance of *Bifidobacterium* (p=0.005) and *Actinomyces* (p=0.032) was reduced when 2nd stage was longer than 2 hours [Table 2.18]. In contrast, an increase in abundance of *Clostridium* (p=0.006,) *Veillionella* (p=0.053) and *Citrobacter* (p=0.016) was seen with 2nd stage longer than 2 hours [Table 2.18]. Upon further stratification by infant diet, vaginally born IAP-

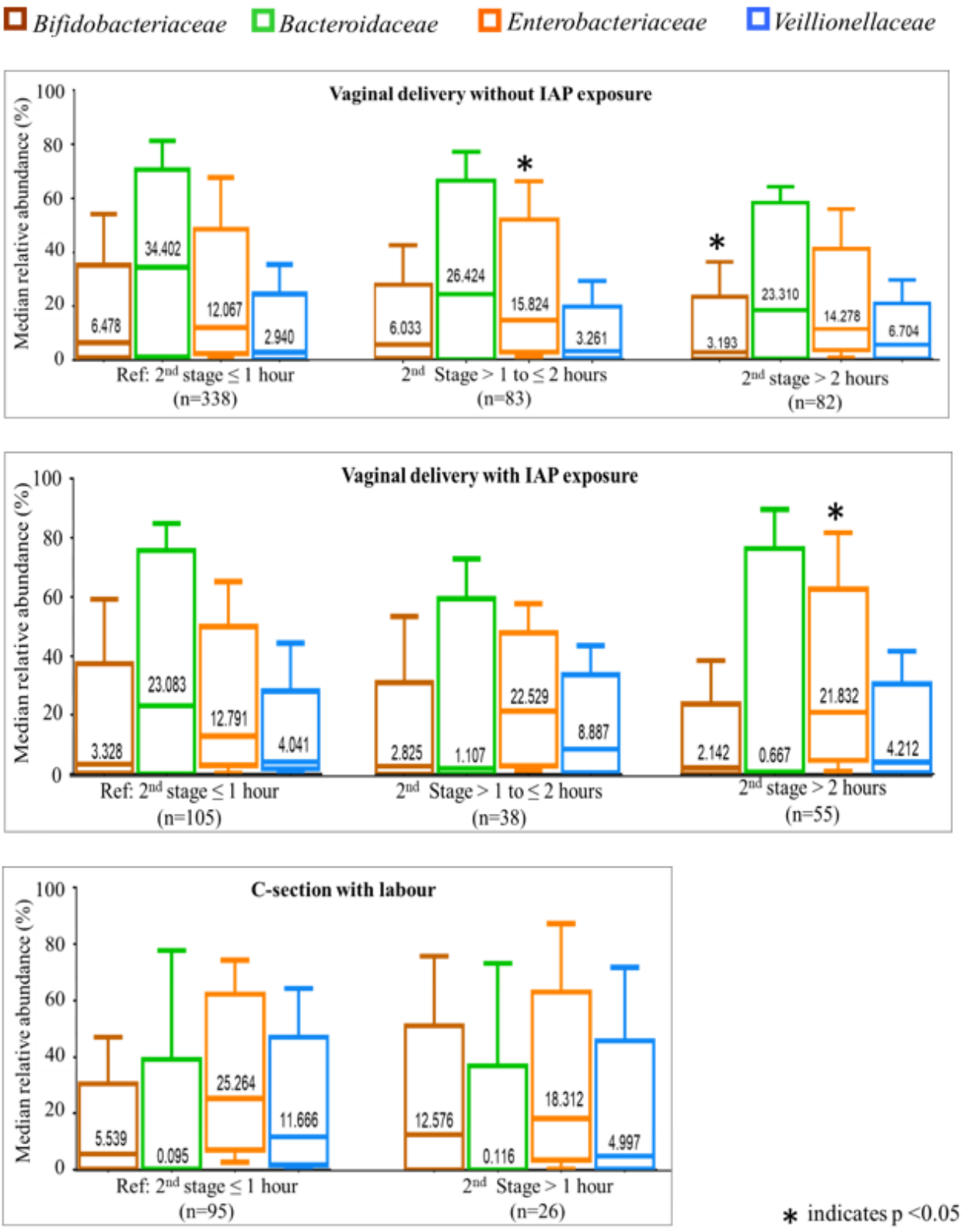
free infants who were not exclusively breastfed showed a reduction in *Bifidobacteriaceae* (p=0.025) and *Actinomycetaceae* (p=0.040) (both of phylum Actinobacteria), and *Lactobacillaceae* (p=0.018) (of phylum Firmicutes), when 2nd stage was longer than 2 hours [Table 2.19a]. In addition, these infants also showed a reduction in *Bacteroidaceae* when 2nd stage was > 1 to ≤ 2 hours (p=0.048) but not when 2nd stage was longer than 2 hours(p=0.383) [Table 2.19a]. At genus level, vaginally born IAP-free infants without exclusive breastfeeding were observed to possess reduced abundance of *Bifidobacterium* (p=0.025), *Actinomyce*s (p=0.031) and *Lactobacillus* (p=0.018) with 2nd stage longer than 2 hours [Table. 2.19a]. On the other hand, when exclusively breastfed, vaginally born IAP-free infants showed lowered abundance of *Bifidobacterium* (of family Bifidobacteriaceae, and phylum Actinobacteria) (p=0.046) and higher abundance of *Clostridium* (p=0.003) when 2nd stage was longer than 2 hours [Table 2.19b].

Among vaginally delivered infants with positive IAP exposure, at phyla level, significant over-representation of Firmicutes with 2nd stage > 1 to ≤ 2 hours (p=0.009) but not with 2nd stage > 2 hours, along with increase in Proteobacteria with 2nd stage > 2 hours (p-0.039) was observed [Table 2.20]. At family level, *Enterobacteriaceae* was increased after 2nd stage longer than 2 hours (p=0.014) where as *Clostridiaceae* was increased significantly (p=0.0.45) only with 2nd stage > 1 to ≤ 2 hours [Table 2.20] [Fig. 2.9]. At genus level, *Enterobacter_unclassified* (of phylum Proteobacteria) showed higher abundance (p=0.016) when 2nd stage was longer than 2 hours [Table 2.20]. When further stratified by infant diet, 2nd stage longer than 2 hours was associated with higher abundance of genus *Clostridium* (p=0.047) (of phylum Firmicutes) among infants who were **not** exclusively breastfed [Table 2.21a] whereas vaginally-delivered IAP-exposed and exclusively breastfed infants showed higher abundance of genus *Enterobacter_unclassified* (p=0021) (of phylum Proteobacteria) [Table 2.21b].

Among infants born by C-section after labour, we observed infants born by C-section after second stage > 1 hour had higher abundance of genus *Enterococcus* (p=0.008) (of Phylum Firmicutes) [Table 2.22]. Infants born by C-section after 2nd stage > 1 hour also appeared to possess increased abundance of *Bifidobacterium*, *Bacteroides* and C*lostridium*, but these changes did not reach statistical significance [Table 2.22].

The following figure summarizes the results discussed thus far for duration of 2nd stage:

Fig.2.1b Summary figure showing changes in microbiota abundance at family level according to duration of second stage, stratified by mode of delivery



∗ indicates p <0.05

Regression analyses results: Microbial abundance adjusted for all potential confounders

Multivariate logistic regression was conducted to further explore the association of duration of 2nd stage of labour and infant gut microbiota profile. At phyla level, infants born after 2nd stage of labour > 2 hours had reduced likelihood of colonization with Actinobacteria [aOR = 0.51, (95 %C1: 0.34-0.77), p = 0.001] [Table 2.23]. At family level, the likelihood of colonization with *Bifidobacteriaceae* [aOR = 0.48, (95 %C1: 0.32-0.73), p = 0.001] and *Lactobacillaceae* [aOR = 0.63, (95 %C1: 0.41-0.98), p = 0.041] when 2nd stage was longer than 2 hours [Table 2.23 and Table 2.25a].

At genus level, compared to infants born after 2nd stage ≤ 1 hours, infants born after 2nd stage > 2 hours showed a 52% decreased likelihood of colonization with *Bifidobacterium* [aOR = 0.48 (95%CI = 0.32- 0.73), p = 0.001] [Table 2.23] [Fig. 2.30]. In addition, infants born after 2nd stage > 2 hours also showed a 37% reduced likelihood of colonization with genus *Lactobacillus* [aOR = 0.63 (95%CI = 0.41-0.98), p =0.041] [Table 2.25b] [Fig. 2.11]. These associations were independent of mode of delivery, IAP use, breastfeeding, gestational age, parity, membrane rupture duration greater than 18 hours, length of baby's hospital stay, age of fecal sample collection and maternal pre-pregnancy overweight.

As for the other genera that showed significant changes in stratified analyses, statistical significance was not retained after adjusting for all potential cofounders in the regression analyses. Finally, infants born after 2nd stage duration >2 hours had a 40% reduced likelihood of higher Shannon diversity [aOR 0.60, (95%CI = 0.39-0.91; p =0.016] [Table 2.27].

Table 2.28 Summary of significant associations between active 1st and 2nd stage labour durations and infant gut microbial composition among infants:

	Active 1st stage (Hours) Ref: ≤ 6		2nd stage (Hours) Ref: ≤ 1	
Microbiota measure	>6 to≤13 aOR* (95% CI); p-value	> 13 aOR* (95% CI); p-value	>1 to≤2 aOR* (95% CI); p-value	> 2 aOR* (95% CI); p-value
Phylum Actinobacteria	**0.53 (0.38-0.74); p=0.000**	0.63 (0.38-1.06); p=0.080	0.74 (0.48-1.15); p=0.178	**0.51 (0.34-0.77); p=0.001**
g_Bifido-bacterium	**0.57 (0.41-0.81); p=0.001**	**0.56 (0.34-0.95); p=0.030**	0.78 (0.51-1.21); p=0.270	**0.48 (0.32-0.73); p=0.001**
g_Lactobacillus	0.78 (0.55-1.10); p=0.155	**0.53 (0.30-0.95); p=0.032**	0.75 (0.48-1.19); p=0.227	**0.63 (0.41-0.98); p=0.041**
Chao 1 Richness	0.86 (0.61-1.22); p=0.863	0.74 (0.43-1.26); p=0.738	0.64 (0.41-1.00); p=0.050	0.76 (0.050-1.16); p=0.211
Shannon diversity	**0.64 (0.45-0.90); p=0.011**	0.74 (0.44-1.26); p=0.274	0.80 (0.51-1.24); p=0.314	**0.60 (0.39-0.91); p=0.016**

aOR = adjusted odds ratio; CI =Confidence Interval

* Odd ratios adjusted for mode of delivery by intrapartum antibiotic prophylaxis, exclusive breastfeeding, parity, duration after membrane rupture > 18 hours, infant's hospital stay length and infant's age at stool collection. Significant associations are **bold-faced.**

2.4 Discussion

In this study cohort of 999 Canadian infants, longer duration of labour was associated with reduced colonization with Actinobacteria, *Bifidobacteriaceae, Bifidobacterium* and decreased diversity of gut microbiota at 3-4 months. After its first discovery in feces of breast-fed infants by Tissier in 1899 (30), *Bifidobacterium* colonization in human gut has been widely studied

due to its immune-modulatory properties affecting both innate and adaptive immune processes (31) (32). As a probiotic, gut *Bifidobacteria* offer protection against risks of childhood allergic diseases (33). Lower abundance of gut *Bifidobacteria* at 3 weeks and 3 months of age was found to be associated with higher risk of atopic diseases at 1 year of age (34), and its underrepresentation in infant gut at 3 months of age is associated with higher incidence of atopy (at age 2 years) and doctor-diagnosed asthma (at age 4 years) (35). Vertical transmission of maternal fecal and vaginal *Bifidobacterium* provides microbial seeding of *Bifidobacterium* for the infant gut (30) (36) (37). C-section delivered infants have significantly pronounced reduction of *Bifidobacterium* colonization as compared to vaginally delivered infants (37) (38) (39).The importance of laboured birth in vertical transmission of *Bifidobacterium* is also highlighted by recent evidence that showed significantly lower *Bifidobacterium* counts in infants born by *elective* C-section when compared to vaginal births (40).

Till date, this is the first study to examine the association between duration of labour and infant gut microbiota at 3-4 months of age. We found that the odds of infant gut colonization by *Bifidobacterium* was reduced with longer durations of the active 1st stage of labour [Group 2 vs Group 1 infants: aOR = 0.57 (95%CI = 0.41-0.81); Group 3 vs Group 1 infants: aOR = 0.56 (95%CI = 0.34-0.95)] and 2nd stage longer than 2 hours [Group 3 vs Group 1 infants: aOR = 0.48 (95%CI = 0.32- 0.73)]. These associations were independent of mode of delivery, IAP use, breastfeeding, gestational age, parity, membrane rupture duration greater than 18 hours, length of baby's hospital stay, age of fecal sample collection and maternal pre-pregnancy overweight. Our results suggest that the time duration of fetal passage through the birth canal is an important influential aspect of birth that determines the *Bifidobacterium* seeding and development of infant gut microbiota.

Bifidobacterium are obligate anaerobes whose optimum survival is regulated by narrow range of pH, temperature and oxygen. Barring few strains, most *Bifidobacterium* species thrive best in an anaerobic, pH neutral environment (pH 6.5 to 7.0) at temperature ranges of 36-38°C (41). We believe that longer labour duration may affect the abundance and viability of maternal Bifidobacteria communities by altering these conditions. Labour and parturition requires tremendous amounts of maternal oxygen utilization with increased minute ventilation and oxygen consumption (42) (43), that leads to increased production of reactive oxygen species (ROS). This

oxidative stress during labour is further intensified with cycles of myometrial ischemia and reperfusion caused by periodic suppression of utero-placental blood flow during powerful myometrial contractions (44), and increased concentrations of labour-associated pro-inflammatory mediators (44) that stimulate increased ROS production (45). To add, longer durations of labour has been previously shown to aggravate the oxidative stress (44) not only in the mother but also in the fetus (46). Although some species and strains of *Bifidobacteria* are thought to possess some level of oxygen tolerance, its anaerobic nature renders most *Bifidobacteria* poorly equipped to handle reactive oxygen species (ROS) produced during oxidative stress. Prolonged exposure to ROS results *Bifidobacterial* cell death (47) (48). Thus, we theorize that higher oxidative stress experienced by mothers undergoing longer duration of labour could lead to attenuated viability of maternal colonic *Bifidobacteria* abundance. Consequently, the microbial inoculum ingested by the fetus during birth after prolonged labour durations could be wanting in *Bifidobacteria* representation and this may influence the gut microbiota colonization at 3-4 months of age.

In addition, we also observed reduced odds of infant gut colonization with *Lactobacillus* when active 1st stage of labor was longer than 13 hours [aOR = 0.53 (95%CI = 0.30-0.95)] and when the 2nd stage longer than 2 hours [aOR = 0.63 (95%CI = 0.41-0.98)]. *Lactobacilli* are anaerobic or microaerobic Gram-positive rods that dominantly colonize the healthy human vagina (49), and are also important constituents of the human gastrointestinal microbiota (50). As members of lactic acid bacteria (LAB), they contribute to generation of ATP (Adenosine triphosphate) through fermentation of carbohydrate to lactic acid (51). In recent years, their role as probiotics and their immune-modulatory effects on gut epithelium (52) (53) has renewed scientific interest in their potential as disease-modifying organisms. Within the gastrointestinal lumen, *Lactobacilli* serve to enhance both innate and adaptive cellular immune responses through induction of mucosal immunity, aiding maturation of epithelial dendritic cells which in turn stimulate the T cells, and modulating T-helper cells responsiveness (50) (54). Evidence shows that gut co-colonization of *Bifidobacterium* and *Lactobacilli* during neonatal period is critical for immune maturation and immune homeostasis (53), and maternal probiotic supplementation with *Bifidobacterium* and *Lactobacillu*s during pregnancy and breastfeeding has been shown to reduce the risk of atopic dermatitis in infants (55). Our results show that prolonged labour is associated with reduced odds infant gut colonization with both *Bifidobacterium* and *Lactobacillus*. However,

further research needed on whether this association influences the risk of pediatric allergic diseases.

Among other phyla, increasing duration of active 1st stage of labour was associated with relatively preserved abundance of phylum Bacteroidetes among infants who were born vaginally and *without* intrapartum antibiotic prophylaxis [Table 2.4] whereas it was under-represented in infants born vaginally who received intrapartum antibiotic prophylaxis [Table 2.9], and was lowest in infants born by C-section [Table 2.12]. Although none of the changes were significant for increasing length of labour, these results demonstrate the vulnerability of Bacteroidetes with respect to delivery mode and antibiotic exposure. Further, the crude odds for infant gut colonization with Bacteroidetes showed an increasing trend with increasing length of active first stage [Group 2 vs Group 1 infants: aOR = 1.50 (95%CI = 1.12-2.01); Group 3 vs Group 1 infants: aOR = 1.60 (95%CI = 1.02-2.53)] [Table 2.16], but not with duration of 2nd stage of labour [Table 2.31]. However, the significance of observed change with active 1st stage was lost when mode of delivery by intrapartum antibiotic prophylaxis was introduced in the model [Table 2.16]. This indicates that delivery mode (vaginal versus C-section) and antibiotic exposure profoundly influence the gut colonization with Bacteroidetes infants, and this finding is in agreement with previous studies reaching the same conclusions (5) (6).

2.5 Strengths and limitations

Our study was conducted in a large population based longitudinal cohort that recruited mothers in their third trimester and followed the children up to early life years. Therefore, the results are generalized to the population and temporality of results in ascertainable. The use of high throughput gene sequencing technique imparts high degree of accuracy and reliability to our gut microbiota measures.

Home births were excluded from our study. Therefore, our study is unable to characterize the association between duration of labour and infant gut microbiota in the infants delivered at home, which is likely to be different from hospital delivered infants. Another major limitation is that our study did not study the influence of duration of labour on infant gut microbiota at an older

age. It would be interesting to see whether the changes seen in infant gut microbiota at 3-4 months of age in association with labour duration would persist at 6 months or one year of age. Thus, future studies could be directed towards these efforts.

2.6 Conclusion

This study highlights the association between exposure to longer durations of labour at birth and changes to infant gut microbial composition at the first 3-4 months of life. The role of these changes in relation to the development of gut immunity and long-term disease risks in later life requires further investigation.

The beneficial influence of probiotics such as *Bifidobacterium* and *Lactobacillus* in promoting immune maturity emphasize their role in the early gut microbiota in reducing risks for atopy, allergy and asthma in children. Recognizing the early life factors, such as prolongation of labour that can influence infant gut microbial composition is crucial for deeper understanding of balanced development of infant gut microbiota and possibly widening the opportunity for early life counteractive measures. Further, finding of this study can be implied in favor of healthy pregnancy, informed decision making during difficult birth or labour dystocia, and to target increment of probiotics to reduce pediatric disease risks.

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Table 2.1

Population characteristics by duration of active 1st stage of labour (n = 918)				
	1st stage <= 6 hours [Reference: Group 1]	**1st stage > 6 to <= 13 hours** [Group 2]	**1st stage > 13 hours** [Group 3]	p-value (x^2)
	N (%)	N (%)	N (%)	
Row percentages	564 (61.4%overall)	267(29.1 % overall)	87 (9.5% overall)	
Baby's gender (n =918)				0.679
Male	297 (61.1%)	146 (30.0%)	43 (8.8%)	
Female	267 (61.8%)	121 (28.0%)	44 (10.2%)	
Delivery mode (n =903)				**<0.001**
Vaginal without IAP	261 (54.7%)	166 (34.8%)	50 (10.5%)	
Vaginal with IAP	79 (41.8%)	80 (42.3%)	30 (15.9%)	
Elective C-section	104 (100.0%)	0.00%	0.00%	
C-section with labour	102 (84.3%)	15 (12.4%)	4 (3.3%)	
Term gestation (n= 918)				0.080
No	21 (80.8%)	5 (19.2%)	0 (0.0%)	
Yes	543 (60.9%)	262 (29.4%)	87 (9.5%)	
Infant diet 3 months (n= 911)				0.979
EBF = Yes	292 (60.7%)	144 (29.9%)	45 (9.4%)	
EBF= Partial	171 (62.4%)	77 (28.1%)	26 (9.5%)	
EBF= Zero	96 (61.5%)	44 (28.2%)	16 (10.3%)	
Parity (n=918)				**<0.001**
Primipara	396 (67.7%)	149 (25.5%)	40 (6.8%)	
Multipara	168 (50.5%)	118 (35.4%)	47 (14.1%)	
Membrane rupture >18 Hours (n=897)				**0.003**
No	488 (62.8%)	225 (29.0%)	64 (8.2%)	
Yes	60 (50.0%)	40 (33.3%)	20 (16.7%)	
Length of hospital stay (n=883)				**0.001**
24 hours of less	120 (56.6%)	69 (32.5%)	23 (10.8%)	
2-3 days	334 (60.1%)	168 (30.2%)	54 (9.7%)	
4 days or more	91 (79.1%)	18 (15.7%)	6 (5.2%)	
Maternal ethnicity (n=910)				0.992
Caucasian	428 (61.8%)	199 (28.7%)	66 (9.5%)	
Other	67 (59.8%)	34 (30.4%)	11 (9.8%)	
Asian	65 (61.9%)	31 (29.5%)	9 (8.6%)	
Maternal pre-pregnancy overweight(n=884)				0.874
No	322 (60.6%)	156 (29.4%)	53 (10.0%)	
Yes	219 (62.0%)	102 (289%)	32 (9.1%)	
Prenatal smoke exposure (n=896)				0.083
No	518 (60.6%)	257 (30.1%)	80 (9.4%)	
Yes	29 (70.7%)	6 (14.6%)	6 (14.6%)	
Maternal asthma (n= 918)				0.260
No	428 (62.1%)	202 (29.3%)	59 (8.6%)	
Yes	136 (59.4%)	65 (28.4%)	28 (12.2%)	

IAP = Intrapartum Antibiotic Prophylaxis; EBF = Exclusive breastfeeding
Comparison made by Chi square test. p-value <0.05 are in boldface type.

Table 2.2

Population characteristics by duration of 2nd stage of labour (n = 955)				
Row percentages	Duration of 2nd stage <= 1 hour [Reference: Group 1]	Duration of 2nd stage > 1 to <= 2 hours [Group 2]	Duration of 2nd stage > 2 hours [Group 3]	p-value (x^2)
	N (%)	N (%)	N (%)	
	667 (69.8%)	125 (13.1%)	163 (17.1%)	
Baby's gender (n =955)				0.119
Male	344 (67.7%)	77 (15.2%)	87 (17.1%)	
Female	323 (67.7%)	48 (10.7%)	76 (17.0%)	
Delivery mode (n = 955)				**<0.001**
Vaginal without IAP	338 (67.2%)	83 (16.5%)	82 (16.3%)	
Vaginal with IAP	105 (53.0%)	38 (19.2%)	55 (27.8%)	
Elective C-section	104 (100.0%)	0 (0.00%)	0 (0.00%)	
C-section with labour	95 (78.5%)	1 (0.8%)	25 (20.7%)	
Term gestation (n= 955)				0.246
No	22 (84.6%)	2 (7.7%)	2 (7.7%)	
Yes	645 (69.4%)	123 (13.2%)	161 (17.1%)	
Infant diet 3 months (n= 948)				0.440
EBF = Yes	338 (68.0%)	74 (14.9%)	85 (17.1%)	
EBF= Partial	206 (71.5%)	33 (11.5%)	49 (17.0%)	
EBF= Zero	119 (73.0%)	16 (9.8%)	28 (17.2%)	
Parity (n=955)				**<0.001**
Primipara	171 (49.0%)	71 (20.3%)	107 (30.7%)	
Multipara	496 (81.8%)	54 (8.9%)	56 (9.2%)	
Membrane rupture >18 Hours (n= 931)				**<0.001**
No	588 (73.2%)	106 (13.2%)	109 (13.6%)	
Yes	61 (47.7%)	17 (13.3%)	50 (39.1%)	
Length of hospital stay (n=918)				0.609
24 hours of less	152 (69.7%)	33 (15.1%)	33 (15.1%)	
2-3 days	402 (69.1%)	79 (13.6%)	101 (17.4%)	
4 days or more	86 (72.9%)	11 (9.3%)	21 (17.8%)	
Maternal ethnicity (n=944)				0.833
Caucasian	504 (70.4%)	90 (12.6%)	122 (17.0%)	
Other	86 (70.5%)	16 (13.1%)	20 (16.4%)	
Asian	69 (65.1%)	17 (16.0%)	20 (18.9%)	
Maternal pre-pregnancy overweight (n= 921)				0.209
No	379 (68.2%)	75 (13.5%)	102 (18.3%)	
Yes	268 (73.4%)	44 (12.1%)	53 (14.5%)	
Pre-natal smoke exposure (n= 928)				0.249
No	612 (69.1%)	117 (13.2%)	157 (17.7%)	
Yes	34 (81.0%)	4 (9.5%)	4 (9.5%)	
Maternal asthma (n= 955)				0.533
No	507 (70.6%)	94 (13.1%)	117 (16.3%)	
Yes	160 (67.5%)	31 (13.1%)	46 (19.4%)	

IAP = Intrapartum Antibiotic Prophylaxis; EBF = Exclusive breastfeeding
Comparison made by Chi square test. p-value <0.05 are in boldface type.

Table 2.3 Summary table showing **significant** ($p<0.05$) changes in median relative abundance of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the **duration of active first stage of labour**, and following different levels of stratifications:

ALL MODES OF BIRTHS (n=918)		
Ref. group: 1st Stage ≤ 6 hours	1st Stage > 6 to ≤13 hours	1st Stage > 13 hours
Phylum Actinobacteria	↓	↓
Bifidobacteriaceae	↓	↓
Coriobacteriaceae	--	↓
g_Bifidobacterium	↓	↓
Phylum Bacteroidetes	↑	↑
Bacteroidaceae	↑	↑
g_Bacteroides	↑	↑
Phylum Firmicutes	--	--
Lactobacillaceae	--	↓
Ruminococcaceae	↓	--
g_Lactobacillus	--	↓
Phylum Proteobacteria	--	--
g_Citrobacter	--	↓

VAGINAL BIRTHS WITHOUT IAP (n=477)		
Reference group: 1st Stage of labour <= 6 hours	1st Stage > 6 to ≤13hrs	1st Stage >13 hrs
Phylum Actinobacteria	↓	↓
Coriobacteriaceae	↓	↓
Bifidobacteriaceae	↓	↓
genus_Bifidobacterium	↓	↓
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Enterococcaceae	--	↓
Clostridiaceae	--	↑
Lactobacillaceae	--	↓
genus_Lactobacillus	--	↓
Phylum Proteobacteria	--	--
genus_Citrobacter	↑	--

VAGINAL BIRTHS WITH IAP (n=189)		
Reference group: 1st Stage of labour <= 6 hours	1st Stage >6 to ≤13 hrs	1st Stage >13 hrs
Phylum Actinobacteria	↓	--
Coriobacteriaceae	–	--
Bifidobacteriaceae	↓	--
genus_Bifidobacterium	↓	↓
Phylum Bacteroidetes	–	--
Phylum Firmicutes	–	--
Enterococcaceae	–	--
Ruminococcaceae	↓	--
Lactobacillaceae	–	--
genus_Lactobacillus	–	--
Phylum Proteobacteria	–	--
genus_Citrobacter	–	--

VAGINAL BIRTHS WITHOUT IAP WITHOUT EXCLUSIVELY BREASTFEEDING (n=216)		
Reference group: 1st Stage of labour <= 6 hours	1st Stage >6 to ≤13 hrs	1st Stage > 13 hrs
Phylum Actinobacteria	–	–
Bifidobacteriaceae	–	↓
genus_Bifidobacterium	–	↓

VAGINAL BIRTHS WITHOUT IAP WITH EXCLUSIVEL BREASTFEEDING (n=257)		
Reference group: 1st Stage of labour <= 6 hours	1st Stage >6 to ≤13 hrs	1st Stage >13 hrs
Phylum Actinobacteria	–	–
Bifidobacteriaceae	↓	–
genus_Bifidobacterium	↓	–

VAGINAL BIRTHS WITH IAP WITHOUT EXCLUSIVELY BREASTFEEDING (n=84)		
Reference group: 1st Stage of labour <= 6 hours	1st Stage >6 to ≤13 hrs	1st Stage >13 hrs
Phylum Actinobacteria	--	–
Bifidobacteriaceae	↓	–
genus_Bifidobacterium	↓	–

VAGINAL BIRTHS WITH IAP WITH EXCLUSIVEL BREASTFEEDING (n=102)		
Reference group: 1st Stage of labour <= 6 hours	1st Stage >6 to ≤13 hrs	1st Stage >13 hrs
Phylum Actinobacteria	--	--
Bifidobacteriaceae	↓	--
genus_Bifidobacterium	↓	--

Table 2.4
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among *all modes of delivery*, according to the duration of active first stage of labour (n=918)

Bacterial Taxa	1st Stage of labour ≤ 6 hours [Reference: Group 1]	1st Stage of labour > 6 to ≤ 13 hours [Group 2]	p-value	1st Stage of labour > 13 hours [Group 3]	p-value
	(n=564; 61.4%0	(n=267; 29.1%)		(n=87; 9.5%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	6.775 (2.051-17.361)	3.599 (0.750-13.093)	**0.000**	3.797 (0.719-13.693)	**0.039**
Family					
Actinomycetaceae	0.024 (0.000-0.111)	0.023 (0.000-0.085)	0.471	0.016 (0.000-0.078)	0.394
Bifidobacteriaceae	5.989 (1.674-16.329)	3.427 (0.458-12.853)	**0.001**	3.164 (0.411-13.424)	**0.042**
Coriobacteriaceae	0.047 (0.008-0.187)	0.031 (0.008-0.139)	0.091	0.016 (0.000-0.095)	**0.022**
Genus					
Bifidobacterium	5.989 (1.674-16.315)	3.376 (0.458-12.807	**0.001**	3.164 (0.411-13.424)	**0.039**
Phylum					
Bacteroidetes	7.009 (0.093-58.176)	26.144 (0.148-66.773)	**0.010**	35.395 (0.287-68.287)	**0.012**
Family					
Bacteroidaceae	2.346 (0.062-52.331)	21.745 (0.086-60.701)	**0.013**	34.334 (0.124-62.712)	**0.009**
Genus					
Bacteroides	2.346 (0.062-52.331)	21.745 (0.086-60.701)	**0.013**	34.334 (0.124-62.712)	**0.009**
Phylum					
Firmicutes	23.201 (10.075-46.661)	21.250 (8.103-43.814)	0.234	18.718 (7.205-44.531)	0.145
Family					
Enterococcaceae	0.023 (0.000-0.117)	0.016 (0.000-0.093)	0.127	0.015 (0.000-0.085)	0.067
Lactobacillaceae	0.000 (0.000-0.045)	0.000 (0.000-0.015)	0.066	0.000 (0.000-0.008)	**0.004**
Streptoccocaceae	0.690 (0.217-1.940)	0.534 (0.208-1.584)	0.286	0.448 (0.170-1.169)	0.102
Clostridiaceae	0.411 (0.031-2.682)	0.358 (0.031-1.972)	0.450	0.541 (0.101-2.411)	0.418
Lachnospiraceae	2.900 (0.039-10.090)	2.282 (0.070-9.405)	0.933	2.302 (0.054-8.256)	0.445
Ruminococcaceae	0.140 (0.000-2.182)	0.047 (0.000-0.924)	**0.018**	0.116 (0.000-2.326)	0.901
Veillionellaceae	4.803 (0.819-16.114)	4.494 (0.650-15.064)	0.401	3.177 (0.820-17.793)	0.563

Genus					
Enterococcus	0.023 (0.000-0.116)	0.016 (0.000-0.085)	0.210	0.015 (0.000-0.085)	0.126
Lactobacillus	0.000 (0.000-0.045)	0.000 (0.000-0.015)	0.066	0.000 (0.000-0.008)	**0.004**
Streptococcus	0.665 (0.217-1.940)	0.534 (0.208-1.584)	0.292	0.448 (0.170-1.169)	0.106
Clostridium	0.023 (0.000-0.782)	0.023 (0.000-0.380)	0.180	0.085 (0.000-0.858)	0.728
Ruminococcus	0.023 (0.000-1.951)	0.031 (0.000-2.042)	0.479	0.031 (0.000-1.049)	0.385
Veillionella	3.333 (0.470-14.629)	2.614 (0.317-13.009)	0.187	2.214 (0.335-13.114)	0.405
Phylum					
Proteobacteria	17.980 (7.703-39.925)	18.923 (8.217-40.127)	0.940	17.024 (6.588-35.676)	0.369
Family					
Enterobacteriaceae	16.352 (5.199-38.648)	16.364 (6.982-37.483)	0.769	14.281 (4.677-34.265)	0.382
Genus					
Citrobacter	0.031 (0.000-0.257)	0.039 (0.000-0.234)	0.610	0.015 (0.000-0.147)	**0.050**
Enterobacter_ unclassified	15.884 (5.005-36.850)	16.258 (6.786-37.258)	0.709	13.926 (4.607-34.181)	0.427

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.5
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births without intrapartum antibiotic prophylaxis (IAP), according to the duration of active first stage of labour (n=477)

Bacterial Taxa	1st Stage of labour ≤ 6 hours [Reference: Group 1]	1st Stage of labour > 6 to ≤ 13 hours [Group 2]	p-value	1st Stage of labour > 13 hours [Group 3]	p-value
	(n=261; 54.7%)	(n=166; 34.8%)		(n=50; 10.5%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	7.648 (2.932-19.450)	5.114 (1.255-14.420)	**0.005**	4.538 (0.684-12.506)	**0.018**
Family					
Actinomycetaceae	0.016 (0.000-0.090)	0.027 (0.000-0.087)	0.520	0.016 (0.000-0.078)	0.953
Bifidobacteriaceae	6.718 (2.432-18.274)	4.649 (0.773-13.915)	**0.010**	3.105 (0.323-12.491)	**0.015**
Coriobacteriaceae	0.054 (0.008-0.195)	0.035 (0.000-0.134)	**0.042**	0.015 (0.000-0.093)	**0.017**
Genus					
Bifidobacterium	6.718 (2.432-18.224)	4.649 (0.773-13.915)	**0.010**	3.105 (0.323-12.491)	**0.016**
Actinomyces	0.016 (0.000-0.070)	0.023 (0.000-0.072)	0.574	0.016 (0.000-0.066)	0.979
Phylum					
Bacteroidetes	41.701 (2.096-65.490)	29.946 (0.985-67.063)	0.553	45.964(1.657-69.808)	0.773
Family					
Bacteroidaceae	37.628 (0.622-61.854)	25.666 (0.292-59.070)	0.338	41.486(1.620-64.403)	0.590
Genus					
Bacteroides	37.628 (0.622-61.854)	25.666 (0.292-59.070)	0.338	41.486(1.620-64.403)	0.590
Parabacteroides	0.008 (0.000-0.887)	0.008 (0.000-0.447)	0.934	0.008 (0.000-0.085)	0.370
Phylum					
Firmicutes	15.625 (7.380-31.026)	19.902 (7.780-39.158)	0.232	15.514(5.560-38.374)	0.727
Family					
Enterococcaceae	0.016 (0.000-0.078)	0.016 (0.000-0.063)	0.670	0.008 (0.000-0.033)	**0.021**
Lactobacillaceae	0.008(0.000-0.033)	0.000 (0.000-0.039)	0.441	0.000 (0.000-0.016)	**0.049**
Streptoccocaceae	0.717 (0.194-1.885)	0.462 (0.155-1.211)	0.090	0.360 (0.099-1.061)	0.074
Clostridiaceae	0.163 (0.016-1.427)	0.195 (0.023-1.347)	0.410	0.672 (0.146-5.115)	**0.006**
Lachnospiraceae	2.103 (0.039-8.497)	2.333 (0.128-8.772)	0.286	2.403 (0.037-7.600)	0.720
Ruminococcaceae	0.117 (0.000-2.115)	0.062 (0.000-0.974)	0.300	0.101 (0.000-1.749)	0.771
Veillionellaceae	2.940 (0.619-12.154)	4.056 (0.500-13.574)	0.477	3.097 (0.830-13.459)	0.749

Genus					
Enterococcus	0.015 (0.000-0.062)	0.015(0.000-0.062)	0.831	0.008 (0.000-0.031)	**0.038**
Lactobacillus	0.000 (0.000-0.039)	0.000 (0.000-0.016)	0.441	0.000 (0.000-0.008)	**0.049**
Streptococcus	0.714 (0.194-1.885)	0.462 (0.155-1.211)	0.094	0.360 (0.099-1.061)	0.076
Clostridium	0.008 (0.000-0.236)	0.008 (0.000-0.308)	0.833	0.082 (0.000-3.320)	0.137
Ruminococcus	0.117 (0.000-2.397)	0.062 (0.000-2.431)	0.994	0.035 (0.000-0.480)	0.195
Veillionella	1.808 (0.264-10.351)	1.984 (0.233-9.588	0.751	2.053 (0.289-9.811)	0.715
Phylum					
Proteobacteria	14.569 (6.458-33.333)	16.931 (8.089-35.888)	0.107	16.692(7.128-35.209)	0.621
Family					
Enterobacteriaceae	12.196 (3.961-32.543)	15.166 (6.569-33.751)	0.054	15.63 (6.056-34.219)	0.480
Genus					
Citrobacter	0.015 (0.000-0.101)	0.039 (0.000-0.212)	**0.004**	0.008 (0.000-0.093)	0.627
Enterobacter_ unclassified	12.048 (3.694-31.535)	14.833 (6.556-32.390)	0.057	15.403(5.898-34.194)	0.447

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.6a
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births without intrapartum antibiotics prophylaxis (IAP) without exclusive breastfeeding, according to the duration of active first stage of labour (n=216)

Bacterial Taxa	1st Stage of labour ≤ 6 hours [Reference: Group 1]	1st Stage of labour > 6 to ≤ 13 hours [Group 2]	p-value	1st Stage of labour > 13 hours [Group 3]	p-value
	(n=115; 53.2%)	(n= 77; 35.6%)		(n=24; 11.1%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	6.796 (2.636-14.780)	3.514 (1.103-14.668)	**0.042**	3.655 (1.119-7.717)	**0.047**
Family					
Actinomycetaceae	0.031 (0.000-0.123)	0.039 (0.000-0.125)	0.948	0.023 (0.008-0.084)	0.623
Bifidobacteriaceae	5.532 (2.345-13.936)	3.016 (0.666-14.241)	0.076	3.020 (0.389-7.327)	**0.038**
Coriobacteriaceae	0.085 (0.016-0.404)	0.077 (0.008-0.466)	0.660	0.027 (0.008-0.189)	0.156
Genus					
Bifidobacterium	5.532 (2.345-13.936)	3.016 (0.666-14.241)	0.077	3.020 (0.389-7.327)	**0.038**
Actinomyces	0.031 (0.000-0.123)	0.039 (0.000-0.125)	0.948	0.023 (0.008-0.084)	0.623
Phylum					
Bacteroidetes	46.125 (9.206-70.690)	41.220 (15.540-71.373)	0.726	51.387(8.278-77.392)	0.597
Family					
Bacteroidaceae	38.575 (6.701-62.820)	32.004(8.558-64.655)	0.749	45.340(8.113-77.257)	0.533
Genus					
Bacteroides	38.575 (6.701-62.820)	32.004 (8.558-64.655)	0.749	45.340(8.113-77.257)	0.533
Phylum					
Firmicutes	17.267 (8.130-32.331)	17.720 (7.977-38.599)	0.969	15.159(5.655-37.285)	0.452
Family					
Enterococcaceae	0.023 (0.000-0.070)	0.023 (0.000-0.077)	0.849	0.008 (0.000-0.031)	0.128
Lactobacillaceae	0.000 (0.000-0.008)	0.000 (0.000-0.004)	0.258	0.000 (0.000-0.000)	0.225
Streptoccocaceae	0.808 (0.201-1.841)	0.488 (0.161-1.259)	0.139	0.164 (0.072-0.787)	**0.010**
Clostridiaceae	0.333 (0.054-1.262)	0.180 (0.035-1.198)	0.592	0.263 (0.150-2.309)	0.290
Lachnospiraceae	3.336 (0.985-9.977)	3.936 (1.070-9.751)	0.792	3.974 (0.429-8.493)	0.585
Ruminococcaceae	1.621 (0.046-3.334)	0.449 (0.015-1.691)	**0.029**	0.221 (0.002-2.272)	0.059
Veillionellaceae	3.866 (1.048-14.614)	4.585 (1.393-11.636)	0.993	3.587 (1.023-14.358)	0.824

Genus					
Enterococcus	0.023 (0.000-0.070)	0.023 (0.000-0.077)	0.849	0.008 (0.000-0.031)	0.128
Lactobacillus	0.000 (0.000-0.008)	0.000 (0.000-0.004)	0.258	0.000 (0.000-0.000)	0.225
Streptococcus	0.806 (0.201-1.841)	0.488 (0.161-1.259)	0.139	0.164 (0.072-0.787)	**0.010**
Clostridium	0.008 (0.000-0.147)	0.008 (0.000-0.125)	0.244	0.008 (0.000-1.777)	0.838
Ruminococcus	0.434 (0.008-2.554)	0.596 (0.008-2.818)	0.637	0.031 (0.000-0.760)	**0.029**
Veillionella	2.447 (0.425-12.770)	2.130 (0.345-8.154)	0.483	1.471 (0.381-8.390)	0.585
Phylum					
Proteobacteria	11.116 (4.455-27.167)	12.011 (5.399-24.770)	0.531	9.881 (6.384-28.543)	0.742
Family					
Enterobacteriaceae	9.327 (2.619-21.985)	9.894(4.501-22.871)	0.316	8.284 (2.348-27.252)	0.705
Genus					
Citrobacter	0.023 (0.000-0.086)	0.023 (0.000-0.128)	0.847	0.008 (0.000-0.099)	0.221
Enterobacter_unclassified	9.311 (2.311-21.978)	9.870 (4.353-22.530)	0.305	8.062 (2.297-27.050)	0.632

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.6b
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births without intrapartum antibiotics prophylaxis (IAP) with exclusive breastfeeding, according to the duration of active first stage of labour (n=257)

Bacterial Taxa	1st Stage of labour ≤ 6 hours [Reference: Group 1]	1st Stage of labour > 6 to ≤ 13 hours [Group 2]	p-value	1st Stage of labour > 13 hours [Group 3]	p-value
	(n=143; 55.6%)	(n= 88; 34.2%)		(n=26; 10.1%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	10.042 (3.130-22.446)	5.732 (1.316-14.948)	**0.035**	8.364 (0.223-20.283)	0.180
Family					
Actinomycetaceae	0.015 (0.000-0.054)	0.016 (0.000-0.078)	0.471	0.008 (0.000-0.062)	0.922
Bifidobacteriaceae	9.871 (2.575-21.615)	5.613 (1.281-14.520)	**0.048**	5.516 (0.123-19.703)	0.171
Coriobacteriaceae	0.039 (0.008-0.141)	0.015 (0.000-0.062)	**0.008**	0.008 (0.000-0.054)	**0.029**
Genus					
Bifidobacterium	9.871 (2.542-21.615)	5.613 (1.281-14.520)	0.047	5.516 (0.123-19.703)	0.173
Actinomyces	0.008 (0.000-0.046)	0.008 (0.000-0.053)	0.479	0.008 (0.000-0.062)	0.848
Phylum					
Bacteroidetes	36.607 (0.396-63.376)	20.267 (0.180-58.939)	0.245	37.323(0.426-64.504)	0.937
Family					
Bacteroidaceae	31.846 (0.186-61.559)	19.703 (0.142-54.563)	0.284	37.323(0.159-61.827)	0.927
Genus					
Bacteroides	31.846 (0.186-61.559)	19.703 (0.142-54.563)	0.284	37.323(0.159-61.827)	0.927
Phylum					
Firmicutes	13.521 (6.746-28.984)	22.674 (6.172-44.995)	0.161	15.514 4.073-45.007)	0.841
Family					
Enterococcaceae	0.015 (0.000-0.062)	0.008 (0.000-0.060)	0.546	0.008 (0.000-0.025)	0.117
Lactobacillaceae	0.000 (0.000-0.070)	0.008 (0.000-0.052)	0.982	0.000 (0.000-0.010)	0.127
Streptoccocaceae	0.571 (0.194 -2.067)	0.441 (0.147-1.373)	0.264	0.531 (0.219-2.107)	0.939
Clostridiaceae	0.085 (0.008-1.899)	0.199 (0.017-2.389)	0.115	1.253 (0.084-6.247)	0.014
Lachnospiraceae	0.982 (0.023-4.803)	0.803 (0.039-6.638)	0.287	0.492 (0.021-6.470)	0.886
Ruminococcaceae	0.015 (0.000-0.357)	0.015 (0.000-0.178)	0.717	0.035 (0.000-1.164)	0.227
Veillionellaceae	2.308 (0.463-9.139)	3.381 (0.232-16.950)	0.409	2.543 (0.592-13.788)	0.879

Genus					
Enterococcus	0.015 (0.000-0.062)	0.008 (0.000-0.060)	0.695	0.008 (0.000-0.025)	0.162
Lactobacillus	0.000 (0.000-0.070)	0.008 (0.000-0.052)	0.982	0.000 (0.000-0.010)	0.127
Streptococcus	0.541 (0.194-2.067)	0.441 (0.147-1.373)	0.278	0.529 (0.219-2.107)	0.960
Clostridium	0.008 (0.000-0.660)	0.016 (0.000-1.120)	0.423	0.236 (0.000-5.854)	0.094
Ruminococcus	0.016 (0.000-1.819)	0.023 (0.000-1.987)	0.629	0.035 (0.000-0.359)	0.817
Veillionella	1.199 (0.147-7.517)	1.677 (0.157-14.553)	0.294	2.543 (0.176-12.573)	0.353
Phylum					
Proteobacteria	17.283 (8.110-39.129)	21.763 (11.961-43.020)	0.051	25.810(8.503-39.986)	0.701
Family					
Enterobacteriaceae	15.003 (6.114-36.604)	20.373 (9.980-42.871)	**0.040**	24.758(7.585-36.185)	0.568
Genus					
Citrobacter	0.008 (0.000-0.140)	0.062 (0.000-0.495)	**0.001**	0.016 (0.000-0.107)	0.814
Enterobacter_ unclassified	14.994 (6.106-36.596)	20.132 (9.599-42.648)	**0.044**	24.630(7.487-35.941)	0.562

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.7
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births with intrapartum antibiotics prophylaxis (IAP), according to the duration of active first stage of labour (n=189)

Bacterial Taxa	1st Stage of labour ≤ 6 hours [Reference: Group1]	1st Stage of labour > 6 to ≤ 13 hours [Group 2]	p-value	1st Stage of labour > 13 hours [Group 3]	p-value
	(n=79; 41.8%)	(n=80; 42.3%)		(n=30; 15.9%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	5.107 (1.528-15.118)	2.352 (0.352-7.313)	**0.003**	3.879 (0.937-19.328)	0.797
Family					
Actinomycetaceae	0.015 (0.000-0.117)	0.016 (0.000-0.077)	0.763	0.031 (0.000-0.105)	0.786
Bifidobacteriaceae	4.604 (1.450-14.629)	2.267 (0.058-6.657)	**0.003**	3.670 (0.717-19.039)	0.717
Coriobacteriaceae	0.024 (0.000-0.124)	0.023 (0.008-0.134)	0.469	0.039 (0.008-0.227)	0.442
Genus					
Bifidobacterium	4.604 (1.450-14.629)	2.267 (0.058-6.640)	**0.008**	3.670 (0.717-19.039)	0.717
Actinomyces	0.015 (0.000-0.117)	0.016 (0.000-0.077)	0.825	0.031 (0.000-0.105)	0.860
Phylum					
Bacteroidetes	21.330 0.062-69.675)	18.478 (0.046-68.225)	0.532	22.487 (0.095-58.094)	0.957
Family					
Bacteroidaceae	13.850(0.047-65.477)	11.017 (0.041-66.723)	0.989	15.197 (0.046-58.079)	0.973
Genus					
Bacteroides	13.854(0.047-65.477)	11.017 (0.041-66.723)	0.989	15.197 (0.046-58.079)	0.973
Parabacteroides	0.000 (0.000-0.023)	0.008 (0.000-0.037)	0.490	0.000 (0.000-0.095)	0.939
Phylum					
Firmicutes	20.715(8.564-43.791)	24.005 (8.122-55.611)	0.487	23.951 (8.751-50.744)	0.724
Family					
Enterococcaceae	0.023 (0.000-0.147)	0.023 (0.000-0.118)	0.598	0.035 (0.000-0.254)	0.607
Lactobacillaceae	0.008(0.000-0.016)	0.000 (0.000-0.015)	0.643	0.000 (0.000-0.008)	0.553
Streptoccocaceae	0.402 (0.109-1.565)	0.794 (0.272-2.394)	**0.032**	0.574 (0.335-1.381)	0.122
Clostridiaceae	0.201 (0.015-2.539)	0.519 (0.054-4.199)	0.144	0.267 (0.023-1.398)	0.892
Lachnospiraceae	2.905 (0.039-10.227)	1.441 (0.047-8.520)	0.808	1.622 (0.049-9.621)	0.847
Ruminococcaceae	0.287 (0.000-2.935)	0.023 (0.000-0.642)	**0.016**	0.299 (0.000-2.731)	0.739
Veillionellaceae	3.960 (0.464-11.792)	6.961 (0.862-17.075)	0.133	3.127 (0.813-24.455)	0.455

Genus					
Enterococcus	0.016 (0.000-0.147)	0.023 (0.000-0.093)	0.594	0.035 (0.000-0.254)	0.583
Lactobacillus	0.000 (0.000-0.016)	0.000 (0.000-0.015)	0.643	0.000 (0.000-0.008)	0.553
Streptococcus	0.402 (0.109-1.565)	0.794 (0.272-2.394)	0.033	0.574 (0.335-1.381)	0.117
Clostridium	0.016 (0.000-0.366)	0.051 (0.000-0.536)	0.266	0.035 (0.000-0.571)	0.815
Ruminococcus	0.008 (0.000-1.268)	0.016 (0.000-1.174)	0.917	0.023 (0.000-1.638)	0.664
Veillionella	3.300 (0.383-11.163)	6.766 (0.578-16.743)	0.205	2.276 (0.325-21.268)	0.786
Phylum					0.849
Proteobacteria	14.424 (7.665-37.562)	22.811 (9.399-40.682)	0.115	19.123 (4.626-40.580)	
Family					
Enterobacteriaceae	12.791 (4.196-36.208)	20.107 (7.902-39.165)	0.170	14.661 (4.166-34.912)	0.941
Genus					
Citrobacter	0.031 (0.000-0.201)	0.031 (0.000-0.391)	0.308	0.019 (0.000-0.165)	0.652
Enterobacter_ unclassified	12.781(4.141-35.743)	18.817 (7.854-38.702)	0.209	12.683 (3.778-34.840)	0.849

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.8a
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births with intrapartum antibiotic prophylaxis (IAP) without exclusive breastfeeding, according to the duration of active first stage of labour (n= 84)

Bacterial Taxa	1st Stage of labour ≤ 6 hours [Reference: Group 1]	1st Stage of labour > 6 to ≤ 13 hours [Group 2]	p-value	1st Stage of labour > 13 hours [Group 3]	p-value
	(n=35; 41.7%)	(n=34; 40.5%)		(n=15; 17.9%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	3.328 (1.277-10.535)	1.293 (0.381-3.472)	0.017	3.962 (0.719-18.759)	0.857
Family					
Actinomycetaceae	0.016 (0.000-0.100)	0.024 (0.006-0.075)	0.823	0.031 (0.008-0.132)	0.495
Bifidobacteriaceae	2.933 (1.270-10.223)	1.286 (0.072-3.443)	0.016	3.619 (0.696-18.417)	0.907
Coriobacteriaceae	0.031 (0.008-0.141)	0.031 (0.008-0.155)	0.847	0.062 (0.015-0.233)	0.355
Genus					
Bifidobacterium	2.933 (1.270-10.223)	1.286 (0.072-3.443)	**0.016**	3.619 (0.696-18.417)	0.907
Actinomyces	0.016 (0.000-0.100)	0.024 (0.006-0.072)	0.795	0.031 (0.000-0.132)	0.631
Phylum					
Bacteroidetes	65.477(0.155-76.495)	26.139(0.053-68.935)	0.140	13.817(0.047-78.530)	0.478
Family					
Bacteroidaceae	45.972(0.078-68.381)	12.171(0.045-67.062)	0.428	13.717(0.039-78.390)	0.634
Genus					
Bacteroides	45.972(0.078-68.381)	12.171(0.045-67.062)	0.428	13.717(0.039-78.390)	0.634
Phylum					
Firmicutes	22.022 (10.848-37.609)	26.305 8.018-72.220)	0.450	25.62 (7.379-51.024)	0.695
Family					
Enterococcaceae	0.016 (0.000-0.078)	0.019 (0.008-0.064)	0.923	0.023 (0.000-0.187)	0.855
Lactobacillaceae	0.000 (0.000-0.016)	0.000 (0.000-0.000)	0.230	0.000 (0.000-0.031)	0.784
Streptoccocaceae	0.218 (0.101-1.145)	0.643 (0.275-1.877)	0.034	1.005 (0.248-5.060)	0.053
Clostridiaceae	0.226 (0.062-2.295)	0.855 (0.227-6.640)	0.039	0.814 (0.070-1.309)	0.672
Lachnospiraceae	4.699 (0.868-10.227)	3.850 (0.507-19.614)	0.881	4.003 (0.25733.561)	0.992
Ruminococcaceae	1.353 (0.278-4.083)	0.422 (0.006-1.266)	0.027	1.691 (0.023-5.862)	0.824
Veillionellaceae	5.644 (1.683-14.343)	6.794 (1.604-18.808)	0.556	2.707 (0.820-11.484)	0.346

Genus					
Enterococcus	0.015 (0.000-0.078)	0.016 (0.008-0.062)	0.942	0.023 (0.000-0.187)	0.821
Lactobacillus	0.000 (0.000-0.016)	0.000 (0.000-0.000)	0.230	0.000 (0.000-0.031)	0.784
Streptococcus	0.218 (0.101-1.145)	0.643 (0.275-1.877)	**0.035**	1.005 (0.248-5.060)	0.053
Clostridium	0.023 (0.000-0.329)	0.148 (0.008-0.785)	0.157	0.156 (0.000-0.814)	0.771
Ruminococcus	0.320 (0.000-1.986)	0.410 (0.000-1.343)	0.654	0.101 (0.000-1.884)	0.781
Veillionella	4.860 (0.571-12.918)	6.718 (0.651-17.499)	0.320	2.338 (0.791-11.484)	0.575
Phylum					
Proteobacteria	8.679 (3.128-16.668)	17.450(7.289-25.253)	0.053	15.633 (4.475-35.676)	0.325
Family					
Enterobacteriaceae	8.419 (2.271-16.591)	15.576(5.275-23.820)	0.084	13.750 (4.460-22.815)	0.403
Genus					
Citrobacter	0.047 (0.008-0.179)	0.059 (0.008-0.368)	0.477	0.039 (0.008-0.170)	0.678
Enterobacter_ unclassified	8.302 (2.255-14.864)	15.541(5.105-23.443)	0.078	11.678(2.164-21.201)	0.546

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type

Table 2.8b
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births with intrapartum antibiotic prophylaxis (IAP) with exclusive breastfeeding, according to the duration of active first stage of labour (n= 102)

Bacterial Taxa	1st Stage of labour ≤ 6 hours [Reference: Group 1]	1st Stage of labour > 6 to ≤ 13 hours [Group 2]	p-value	1st Stage of labour > 13 hours [Group 3]	p-value
	(n=42; 41.2%)	(n=45; 44.1%)		(n=15; 14.9%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	6.474 (1.973-24.042)	2.854 (0.214-8.123)	**0.030**	3.797 (1.733-21.037)	0.638
Family					
Actinomycetaceae	0.015 (0.000-0.209)	0.008 (0.000-0.081)	0.488	0.031 (0.000-0.069)	0.684
Bifidobacteriaceae	6.392 (1.616-23.541)	2.800 (0.051-7.540)	**0.030**	3.720 (1.308-20.905)	0.704
Coriobacteriaceae	0.019 (0.000-0.089)	0.023 (0.008-0.128)	0.500	0.008 (0.008-0.047)	0.956
Genus					
Bifidobacterium	6.392 (1.616-23.539)	2.671 (0.051-7.540)	**0.027**	3.720 (1.308-20.905)	0.704
Actinomyces	0.012 (0.000-0.207)	0.008 (0.000-0.081)	0.550	0.031 (0.000-0.069)	0.738
Phylum					
Bacteroidetes	2.298 (0.050-53.067)	9.573 (0.046-67.231)	0.668	31.157 (0.101-50.280)	0.587
Family					
Bacteroidaceae	0.648 (0.031-51.510)	9.542 (0.039-65.964)	0.541	16.678 0.054-48.508)	0.618
Genus					
Bacteroides	0.648 (0.031-51.510)	9.542 (0.039-65.964)	0.541	16.678 (0.054-48.508)	0.618
Phylum					
Firmicutes	19.716 (7.665-44.149)	23.908 (7.523-46.184)	0.709	21.095 (9.208-45.998)	0.786
Family					
Enterococcaceae	0.023 (0.000-0.205)	0.031 (0.000-0.249)	0.428	0.109 (0.000-0.349)	0.356
Lactobacillaceae	0.000 (0.000-0.017)	0.000 (0.000-0.020)	0.678	0.000 (0.000-0.008)	0.394
Streptococcaceae	0.558 (0.180-2.026)	0.797 (0.256-3.033)	0.304	0.538 (0.395-0.907)	0.574
Clostridiaceae	0.155 (0.008-3.644)	0.248 (0.019-2.682)	0.676	0.047 (0.023-1.664)	0.957
Lachnospiraceae	0.170 (0.016-11.293)	0.426 (0.019-5.571)	0.829	1.006 (0.031-4.300)	0.793
Ruminococcaceae	0.031 (0.000-0.727)	0.008 (0.000-0.051)	0.252	0.000 (0.000-0.482)	0.435
Veillionellaceae	1.463 (0.206-9.585)	7.086 (0.484-16.583)	0.126	8.481 (0.343-27.929)	0.147

Genus					
Enterococcus	0.019 (0.000-0.205)	0.031 (0.000-0.249)	0.428	0.109 (0.000-0.349)	0.346
Lactobacillus	0.000 (0.000-0.017)	0.000 (0.000-0.020)	0.678	0.000 (0.000-0.008)	0.394
Streptococcus	0.554 (0.180-2.026)	0.797 (0.256-3.033)	0.300	0.538 (0.395-0.907)	0.574
Clostridium	0.016 (0.000-1.093)	0.039 (0.000-0.295)	0.614	0.031 (0.000-0.490)	0.869
Ruminococcus	0.008 (0.000-0.253)	0.008 (0.000-0.105)	0.694	0.008 (0.000-1.049)	0.564
Veillionella	1.460 (0.200-9.583)	6.794 (0.279-16.575)	0.221	1.822 (0.254-25.262)	0.538
Phylum					
Proteobacteria	22.064 (12.024-44.544)	34.908 (14.178-43.438)	0.391	29.832 (6.191-42.467)	0.856
Family					
Enterobacteriaceae	18.110 (9.327-40.705)	24.640 (8.930-43.152)	0.508	29.660 (3.946-39.935)	
Genus					
Citrobacter	0.008 (0.000-0.232)	0.031 (0.000-0.500)	0.300	0.000 (0.000-0.163)	0.701
Enterobacter_ unclassified	18.106 (9.132-40.534)	23.985 (8.802-41.707)	0.616	28.925 (3.792-39.912)	0.664

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type

Table 2.9
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among C-section with labour, according to the duration of active first stage of labour (n=121)

Bacterial Taxa	**1st Stage of labour ≤ 6 hours [Reference: Group 1]**	**1st Stage of labour > 6 hours [Group 2]**	p-value
	(n= 102; 84.3%)	(n=19; 15.7%)	
	Median (IQR)	Median (IQR)	
	N (%); IQR	N (%); IQR	
Phylum			
Actinobacteria	6.532 (0.653-15.717)	10.464 (0.309-23.755)	0.787
Family			
Actinomycetaceae	0.039 (0.008-0.178)	0.031 (0.000-0.055)	0.310
Bifidobacteriaceae	6.066 (0.457-14.212)	10.441 (0.054-23.632)	0.643
Coriobacteriaceae	0.047 (0.000-0.203)	0.023 (0.000-0.109)	0.552
Genus			
Bifidobacterium	0.035 (0.000-0.178)	0.031 (0.000-0.047)	0.332
Actinomyces	6.066 (0.457-14.212)	10.441 (0.047-23.632)	0.776
Phylum			
Bacteroidetes	0.118 (0.045-1.326)	0.124 (0.039-0.255)	0.538
Family			
Bacteroidaceae	0.100 (0.037-0.646)	0.070 (0.031-0.255)	0.415
Genus			
Bacteroides	0.100 (0.037-0.646)	0.070 (0.031-0.255)	0.415
Phylum			
Firmicutes	36.248 (20.155-61.111)	33.393 (14.577-57.941)	0.559
Family			
Enterococcaceae	0.047 (0.015-0.155)	0.101 (0.015-0.325)	0.246
Lactobacillaceae	0.000 (0.000-0.018)	0.000 (0.000-0.000)	0.130
Streptoccocaceae	1.018 (0.373-2.858)	0.765 (0.277-1.825)	0.598
Clostridiaceae	1.384 (0.166-7.013)	1.043 (0.541-1.972)	0.613
Lachnospiraceae	4.533 (0.039-14.365)	7.658 (0.062-13.761)	0.554
Ruminococcaceae	0.085 (0.008-2.401)	0.326 (0.008-6.305)	0.920
Veillionellaceae	10.590 (3.024-27.844)	9.393 (0.835-22.164)	0.512

Genus			
Enterococcus	0.043 (0.008-0.149)	0.094 (0.015-0.317)	0.256
Lactobacillus	0.000 (0.000-0.018)	0.000 (0.000-0.000)	0.130
Streptococcus	1.016 90.373-2.852)	0.765 (0.277-1.825)	0.603
Clostridium	0.256 (0.016-2.378)	0.195 (0.093-0.920)	0.721
Ruminococcus	0.008 (0.000-1.542)	0.023 (0.000-5.357)	0.461
Veillionella	8.916 (1.447-25.974)	9.370 (0.820-22.156)	0.732
Phylum			
Proteobacteria	27.509 (12.997-50.867)	17.026 (6.996-42.365)	0.312
Family			
Enterobacteriaceae	24.568 (11.027-49.294)	17.018 (6.996-42.202)	0.401
Genus			
Citrobacter	0.147 (0.014-0.904)	0.047 (0.008-0.287)	0.098
Enterobacter_ unclassified	22.463 (9.091-48.012)	16.431 (6.942-42.187)	0.508

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.10
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among C-section without labour versus C-section with labour

Bacterial Taxa	C-section without labour	C-section with labour	p-value
	(n= 116; 48.9%)	(n=121; 51.1%)	
	Median (IQR)	Median (IQR)	
	N (%); IQR	N (%); IQR	
Phylum Actinobacteria	5.666 (1.403-15.205)	6.753 (0.589-17.179)	0.931
g_Actinomyces	0.031 (0.000-0.127)	0.031 (0.000-0.113)	0.903
g_Bifidobacterium	5.260 (1.023-15.139)	6.184 (0.352-15.937)	0.933
Phylum Bacteroidetes	0.120 (0.046-2.279)	0.119 (0.043-1.080)	0.908
g_Bacteroides	0.082 (0.039-1.122)	0.100 (0.031-0.636)	0.937
Phylum Firmicutes	37.151 (13.559-54.210)	35.457 (19.434-60.879)	0.258
g_Enterococcus	0.039 (0.000-0.191)	0.048 (0.012-0.182)	0.471
g_Lactobacillus	0.000 (0.000-0.086)	0.000 (0.000-0.016)	0.010
g_Streptococcus	0.577 (0.271-1.918)	0.964 (0.368-2.693)	0.070
g_Clostridia	0.161 (0.008-1.797)	0.255 (0.019-2.116)	0.198
g_Ruminococcus_L	0.012 (0.000-1.747)	0.008 (0.000-2.134)	0.719
g_Veilloinella	7.441 (1.297-22.603)	9.096 (1.208-25.511)	0.706
Phylum Proteobacteria	29.925 (11.524-51.000)	25.946 (11.769-49.901)	0.968
g_Citrobacter	0.093 (0.008-0.824)	0.124 (0.008-0.818)	0.553

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.11

ACTINOBACTERIA

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of active 1st stage of labour

Microbiota Measure						
Ref. Group 1 = 1st Stage ≤ 6 Hours Group 2 = 1st Stage > 6 to ≤ 13 Hrs Group 3 = 1st Stage > 13 Hrs	**Infant's gut microbiota at 3 to 4 months of age**					
			Phylum Actinobacteria (below vs above median)	**Family Bifidobacteriaceae** (below vs above median)	**Family Coriobacteriaceae** (below vs above median)	**Genus Bifidobacterium** (below vs above median)
			OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 1st stage of labour	Group2		0.59 (0.44-0.80)*	0.63 (0.47-0.85)*	0.73 (0.54-0.98)*	0.63 (0.47-0.85)*
	Group3		0.66 (0.42-1.03)	0.57 (0.36-0.90)*	0.57 (0.36-0.90)*	0.57 (0.36-0.90)*
Adjusted for delivery MODE by IAP	Group2		0.55 (0.41-0.75)**	0.59 (0.44-0.81)**	0.68 (0.50-0.93)*	0.59 (0.44-0.81)**
	Group3		0.66 (0.41-1.05)	0.58 (0.36-0.92)*	0.56 (0.35-0.90)*	0.58 (0.36-0.92)*
Adjusted for gestational age	Group2		0.57 (0.42-0.77)**	0.61 (0.45-0.82)**	0.71 (0.53-0.95)*	0.61 (0.45-0.82)**
	Group3		0.63 (0.40-0.99)*	0.55 (0.35-0.87)*	0.55 (0.35-0.87)*	0.55 (0.35-0.87)*
Adjusted for infant diet at 3 months	Group2		0.58 (0.43-0.78)**	0.61 (0.46-0.83)**	0.72 (0.53-0.96)*	0.61 (0.46-0.83)**
	Group3		0.66 (0.42-1.05)	0.57 (0.36-0.91)*	0.55 (0.35-0.88)*	0.57 (0.36-0.91)*
Adjusted for parity	Group2		0.61 (0.45-0.82)	0.64 (0.48-0.86)*	0.77 (0.58-1.04)	0.64 (0.48-0.86)*
	Group3		0.69 (0.44-1.09)	0.59 (0.37-0.93)*	0.62 (0.39-0.99)*	0.59 (0.37-0.93)*
Adjusted for ROM >18 hours	Group2		0.60 (0.44-0.80)**	0.64 (0.48-0.86)*	0.74 (0.55-0.99)*	0.64 (0.48-0.86)*
	Group3		0.62 (0.39-0.99)*	0.55 (0.34-0.88)*	0.62 (0.39-0.99)*	0.55 (0.34-0.88)*
Adjusted for baby's length of hospital stay	Group2		0.57 (0.42-0.78)**	0.61 (0.45-0.82)**	0.71 (0.53-0.96)*	0.61 (0.45-0.82)**
	Group3		0.67 (0.42-1.07)	0.58 (0.36-0.92)*	0.59 (0.37-0.94)*	0.58 (0.36-0.92)*
Adjusted for infant's age at the time of	Group2		0.58 (0.39-0.86)*	0.61 (0.41-0.91)*	0.82 (0.55-1.22)	0.61 (0.41-0.91)*
	Group3		0.58 (0.31-1.10)	0.48 (0.25-0.91)*	0.54 (0.28-1.03)	0.48 (0.25-0.91)*

stool collection					
Adjusted for maternal pre-pregnancy weight	Group2	0.58 (0.43-0.79)*	0.62 (0.46-0.84)*	0.73 (0.54-0.98)*	0.62 (0.46-0.84)*
	Group3	0.62 (0.39-0.79)*	0.56 (0.35-0.89)*	0.52 (0.33-0.83)*	0.56 (0.35-0.89)*
MODEL 1	Group2	0.53 (0.38-0.74)**	0.57 (0.41-0.81)**	0.68 (0.48-0.95)*	0.57 (0.41-0.81)**
	Group3	0.63 (0.38-1.06)	0.56 (0.34-0.95)*	0.64 (0.34-1.08)	0.56 (0.34 - 0.95)*

MODEL1: Adjusted for delivery mode by IAP, gestational age, infant diet at 3 months, parity, ROM > 18 hours, length of hospital stay, age of stool collection, maternal pre-pregnancy weight

* p <0.05; ** p<0.005;
OR = odds ratio; CI = confidence interval
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes
Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Table 2.12

BACTEROIDETES

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of active 1st stage of labour

Microbiota Measure				
Ref. Group 1 = 1st Stage ≤ 6 Hours Group 2 = 1st Stage > 6 to ≤ 13 Hrs Group 3 = 1st Stage > 13 Hrs	**Infant's gut microbiota at 3 to 4 months of age**			
	Phylum Bacteroidetes (below vs above median)		**Family Bacteroidaceae** (below vs above median)	**Genus *Bacteroides*** (below vs above median)
	OR (95% CI)		OR (95% CI)	OR (95% CI)
Crude OR for 1st stage of labour	Group2	1.50 (1.12-2.01)*	1.49 (1.11-1.99)*	1.49 (1.11-1.99)*
	Group3	1.60 (1.02-2.53)*	1.86 (1.17-2.95)*	1.86 (1.17-2.95)*
Adjusted for delivery MODE by IAP	Group2	0.99 (0.72-1.37)	0.95 (0.69-1.31)	0.95 (0.69-1.31)
	Group3	1.08 (0.67-1.76)	1.24 (0.76-2.03)	1.24 (0.76-2.03)
Adjusted for gestational age	Group2	1.47 (1.09-1.98)*	1.46 (1.09-1.96)*	1.46 (1.09-1.96)*
	Group3	1.57 (0.99-2.49)	1.83 (1.15-2.90)*	1.83 (1.15-2.90)*
Adjusted for infant diet at 3 months	Group2	1.50 (1.12-2.03)*	1.48 (1.10-1.98)*	1.48 (1.10-1.98)*
	Group3	1.61 (1.02-2.55)*	1.86 (1.17-2.96)*	1.86 (1.17-2.96)*
Adjusted for parity	Group2	1.55 (1.15-2.09)*	1.57 (1.16-2.11)*	1.57 (1.16-2.11)*
	Group3	1.70 (1.07-2.70)*	2.04 (1.27-3.26)*	2.04 (1.27-3.26)*
Adjusted for ROM > 18 hours	Group2	1.46 (1.09-1.97)*	1.45 (1.08-1.94)*	1.45 (1.08-1.94)*
	Group3	1.59 (1.00-2.54)	1.86 (1.16-2.98)*	1.86 (1.16-2.98)*
Adjusted for baby's length of hospital stay	Group2	1.37 (1.01-1.85)*	1.38 (1.02-1.86)*	1.38 (1.02-1.86)*
	Group3	1.43 (0.89-2.29)	1.67 (1.04-2.68)*	1.67 (1.04-2.68)*
Adjusted for infant's age at the time of stool collection	Group2	1.81 (0.21-2.70)*	1.93 (1.30-2.88)*	1.93 (1.30-2.88)*
	Group3	1.47 (0.78-2.77)	2.16 (1.13-2.88)*	2.16 (1.13-2.14)*
Adjusted for maternal pre-pregnancy weight	Group2	1.52 (1.13-2.05)*	1.50 (1.11-2.02)*	1.50 (1.11-2.02)*
	Group3	1.65 (1.04-2.62)*	1.91 (1.19-3.05)*	1.91 (1.19-3.05)*

MODEL 1	Group2	0.93 (0.66-1.33)	0.92 (0.65-1.31)	0.92 (0.65-1.31)
	Group3	1.06 (0.62-1.80)	1.26 (0.73-2.17)	1.26 (0.73-2.17)

MODEL1: Adjusted for delivery mode by IAP, gestational age, infant diet at 3 months, parity, ROM > 18 hours, length of hospital stay, age of stool collection, maternal pre-pregnancy weight

* p <0.05; ** p<0.005;
OR = odds ratio; CI = confidence interval
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes
Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Table 2.13a **FIRMICUTES**

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of active 1st stage of labour

Microbiota Measure		Infant's gut microbiota at 3 to 4 months of age					
Ref. Group 1: 1st Stage ≤ 6 Hours Group 2 :1st Stage >6 to ≤13 Hrs Group 3: 1st Stage > 13 Hrs		**PHYLUM**	**FAMILY**				
		FIRMICUTES (below vs above median) OR (95% CI)	**Streptococcaceae** (below vs above median) OR (95% CI)	**Clostridiaceae** (below vs above median) OR (95% CI)	**Lactobacillaceae** (below vs above median) OR (95% CI)	**Ruminococcaceae** (below vs above median) OR (95% CI)	**Veillonellaceae** (below vs above median) OR (95% CI)
Crude OR for 1st stage of labour	Group2	0.92 (0.68-1.23)	0.84 (0.63-1.13)	0.90 (0.67-1.21)	0.83 (0.61-1.12)	0.73 (0.55-0.98)*	0.98 (0.73-1.31)
	Group3	0.80 (0.51 -1.25)	0.57 (0.36-0.90)*	1.18 (0.75-1.86)	0.54 (0.32-0.89)*	1.04 (0.66-1.63)	0.68 (0.43-1.07)
Adjusted for delivery MODE by IAP	Group2	1.24 (0.91-1.70)	0.88 (0.65-1.19)	1.14 (0.83-1.55)	0.80 (0.59-1.10)	0.73 (0.53-0.99)*	1.29 (0.94-1.75)
	Group3	1.05 (0.66-1.69)	0.64 (0.40-1.02)	1.47 (0.92-2.35)	0.56 (0.33-0.93)*	1.01 (0.64-1.62)	0.85 (0.53-1.36)
Adjusted for gestational age	Group2	0.93 (0.69-1.25)	0.81 (0.61-1.09)	0.92 (0.69-1.24)	0.84 (0.62-1.14)	0.73 (0.55-0.98)*	0.98 (0.73-1.31)
	Group3	0.81 (0.51-1.28)	0.55 (0.34-0.87)*	1.21 (0.77-1.91)	0.55 (0.33-0.91)*	1.04 (0.66-1.63)	0.67 (0.43-1.07)
Adjusted for infant diet at 3 months	Group2	0.92 (0.68-1.23)	0.83 (0.62-1.11)	0.91 (0.68-1.22)	0.82 (0.60-1.12)	0.72 (0.52-0.99)*	0.98 (0.73-1.32)
	Group3	0.79 (0.50-1.24)	0.57 (0.36-0.90)*	1.19 (0.75-1.87)	0.53 (0.32-0.89)*	1.03 (0.63-1.68)	0.67 (0.42-1.06)
Adjusted for parity	Group2	0.89 (0.66-1.19)	0.82 (0.61-1.10)	0.82 (0.61-1.11)	0.83 (0.61-1.12)	0.75 (0.56-1.01)	0.95 (0.71-1.28)
	Group3	0.75 (0.48-1.19)	0.54 (0.34-0.87)*	1.03 (0.65-1.63)	0.54 (0.32-0.90)*	1.09 (0.69-1.72)	0.64 (0.41-1.02)
Adjusted for ROM >18 hours	Group2	0.92 (0.68-1.23)	0.87 (0.65-1.17)	0.89 (0.66-1.19)	0.83 (0.61-1.12)	0.74 (0.55-0.99)*	0.96 (0.71-1.28)
	Group3	0.76 (0.48-1.21)	0.64 (0.40-1.02)	1.08 (0.68-1.71)	0.54 (0.32-0.91)*	0.99 (0.63-1.58)	0.63 (0.39-1.00)
Adjusted for baby's length of hospital stay	Group2	1.02 (0.76-1.38)	0.78 (0.58-1.06)	0.95 (0.70-1.28)	0.75 (0.55-1.03)	0.68 (0.51-0.92)*	1.06 (0.78-1.42)
	Group3	0.89 (0.56-1.42)	0.53 (0.33-0.86)*	1.34 (0.84-2.13)	0.52 (0.31-0.87)*	0.95 (0.60-1.52)	0.69 (0.43-1.10)
Adjusted for infant's age at the time of stool collection	Group2	1.06 (0.71-1.57)	0.97 (0.65-1.44)	0.92 (0.62-1.37)	1.17 (0.78-1.74)	0.88 (0.58-1.32)	1.08 (0.73-1.61)
	Group3	0.88 (0.46-1.65)	0.59 (0.31-1.14)	1.23 (0.65-2.33)	0.38 (0.18-0.82)*	0.89 (0.46-1.71)	0.59 (0.31-1.14)
Adjusted for maternal pre-pregnancy weight	Group2	0.94 (0.70-1.27)	0.84 (0.63-1.14)	0.90 (0.67-1.21)	0.82 (0.60-1.12)	0.74 (0.55-1.01)	0.96 (0.71-1.29)
	Group3	0.83 (0.53-1.32)	0.57 (0.36-0.91)*	1.16 (0.74-1.84)	0.51 (0.30-0.85)*	1.02 (0.64-1.62)	0.69 (0.44-1.10)
MODEL 1	Group2	1.35 (0.96-1.90)	0.78 (0.56-1.10)	1.00 (0.72-1.42)	0.78 (0.55-1.10)	**0.66 (0.45-0.95)***	1.31 (0.93-1.84)
	Group3	1.10 (0.66-1.85)	0.60 (0.36-1.00)	1.23 (0.73-2.06)	**0.53 (0.30-0.95)***	0.90 (0.51-1.59)	0.79 (0.47-1.33)

MODEL1: Adjusted for delivery mode by IAP, gestational age, infant diet at 3 months, parity, ROM > 18 hours, length of hospital stay, age of stool collection, maternal pre-pregnancy weight

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval; IAP = Intrapartum antibiotic prophylaxis; GA = gestational age; ROM = rupture of membranes

Table 2.13b

FIRMICUTES

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of active 1st stage of labour

Ref. Group 1 = 1st Stage ≤ 6 Hours Group 2 = 1st Stage > 6 to ≤ 13 Hrs Group 3 = 1st Stage > 13 Hrs		**Infant's gut microbiota at 3 to 4 months of age**				
		GENUS				
		Lactobacillus (below vs above median)	***Streptococcus*** (below vs above median)	***Clostridium*** (below vs above median)	***Ruminococcus*** (below vs above median)	***Veillionella*** (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 1st stage of labour	Group2	0.83 (0.61-1.12)	0.82 (0.62-1.10)	0.91 (0.68-1.22)	1.13 (0.84-1.51)	0.83 (0.62-1.12)
	Group3	0.54 (0.32-0.89)*	0.57 (0.36-0.90)*	1.24 (0.78-1.95)	1.06 (0.68-1.67)	0.75 (0.47-1.18)
Adjusted for delivery MODE by IAP	Group2	0.80 (0.59-1.10)	0.87 (0.64-1.18)	1.27 (0.93-1.74)	0.98 (0.72-1.33)	1.11 (0.82-1.52)
	Group3	0.56 (0.33-0.93)*	0.64 (0.40-1.03)	1.64 (1.02-2.65)*	0.90 (0.57-1.44)	0.97 (0.61-1.56)
Adjusted for gestational age	Group2	0.84 (0.62-1.14)	0.79 (0.59-1.07)	0.93 (0.70-1.25)	1.12 (0.84-1.51)	0.83 (0.62-1.11)
	Group3	0.55 (0.33-0.91)*	0.54 (0.34-0.86)*	1.27 (0.80-2.01)	1.06 (0.67-1.66)	0.74 (0.47-1.17)
Adjusted for infant diet at 3 months	Group2	0.82 (0.60-1.12)	0.81 (0.61-1.09)	0.92 (0.69-1.23)	1.17 (0.87-1.58)	0.84 (0.63-1.13)
	Group3	0.53 (0.32-0.89)*	0.57 (0.36-0.90)*	1.23 (0.78-1.94)	1.06 (0.67-1.70)	0.74 (0.47-1.17)
Adjusted for parity	Group2	0.83 (0.61-1.12)	0.80 (0.59-1.07)	0.84 (0.63-1.13)	1.18 (0.88-1.59)	0.81 (0.60-1.09)
	Group3	0.54 (0.32-0.90)*	0.53 (0.34-0.85)*	1.09 (0.69-1.74)	1.15 (0.73-1.82)	0.71 (0.45-1.12)
Adjusted for ROM >18 hours	Group2	0.83 (0.61-1.12)	0.86 (0.64-1.15)	0.89 (0.66-1.20)	1.18 (0.88-1.58)	0.81 (0.61-1.09)
	Group3	0.54 (0.32-0.91)*	0.63 (0.39-1.01)	1.15 (0.74-1.84)	1.15 (0.73-1.83)	0.70 (0.44-1.11)
Adjusted for baby's length of hospital stay	Group2	0.75 (0.55-1.03)	0.77 (0.57-1.04)	0.95 (0.70-1.29)	1.09 (0.81-1.47)	0.89 (0.66-1.20)
	Group3	0.52 (0.31-0.87)*	0.53 (0.3-0.85)*	1.45 (0.90-2.32)	1.03 (0.65-1.64)	0.78 (0.49-1.24)
Adjusted for infant's age at the time of stool collection	Group2	1.17 (0.78-1.74)	0.91 (0.61-1.35)	1.03 (0.70-1.53)	1.33 (0.89-2.00)	0.91 (0.61-1.35)
	Group3	0.38 (0.18-0.82)*	0.57 (0.30-1.10)	1.19 (0.63-2.25)	1.10 (0.58-2.10)	0.69 (0.36-1.32)
Adjusted for maternal pre-pregnancy weight	Group2	0.82 (0.60-1.12)	0.82 (0.61-1.11)	0.93 (0.69-1.25)	1.16 (0.86-1.56)	0.83 (0.62-1.12)
	Group3	0.51 (0.30-0.85)*	0.56 (0.35-0.90)	1.26 (0.79-2.00)	1.07 (0.68-1.69)	0.75 (0.47-1.19)
MODEL 1	Group2	0.78 (0.55-1.10)	0.78 (0.55-1.07)	1.14 (0.81-1.61)	1.12 (0.79-1.60)	1.12 (0.80-1.58)
	Group3	**0.53 (0.30-0.95)***	0.59 (0.36-1.00)	1.50 (0.89-2.53)	1.10 (0.64-1.88)	0.91 (0.54-1.54)

MODEL1: Adjusted for delivery mode by IAP, gestational age, infant diet at 3 months, parity, ROM > 18 hours, length of hospital stay, age of stool collection, maternal pre-pregnancy weight

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval; IAP = Intrapartum antibiotic prophylaxis; GA = gestational age; ROM = rupture of membranes

Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Table 2.14

PROTEOBACTERIA

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of active 1st stage of labour

Microbiota Measure					
Ref. Group 1 = 1st Stage ≤ 6 Hours Group 2 = 1st Stage > 6 to ≤ 13 Hrs Group 3 = 1st Stage > 13 Hrs	**Infant's gut microbiota at 3 to 4 months of age**				
		Phylum Proteobacteria (below vs above median)	**Family Enterobacteriaceae** (below vs above median)	**Genus Citrobacter** (below vs above median)	**Genus Enterobacter** (unclassified) (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 1st stage of labour	Group2	1.10 (0.82-1.47)	1.02 (0.76-1.36)	1.06 (0.79-1.42)	1.03 (0.77-1.38)
	Group3	0.90 (0.58-1.42)	093 (0.59-1.46)	0.76 (0.48-1.20)	0.93 (0.59-1.46)
Adjusted for delivery MODE by IAP	Group2	1.34 (0.98-1.82)	1.22 (0.90-1.66)	1.30 (0.96-1.77)	1.21 (0.90-1.65)
	Group3	1.11 (0.69-1.77)	1.13 (0.71-1.81)	0.86 (0.53-1.38)	1.11 (0.69-1.77)
Adjusted for gestational age	Group2	1.10 (0.82-1.48)	1.01 (0.76-1.36)	1.08 (0.81-1.46)	1.03 (0.77-1.38)
	Group3	0.90 (0.57-1.42)	0.93 (0.59-1.46)	0.78 (0.49-1.22)	0.93 (0.59-1.46)
Adjusted for infant diet at 3 months	Group2	1.10 (0.82-1.49)	1.01 (0.75-1.37)	1.07 (0.80-1.44)	1.03 (0.76-1.39)
	Group3	0.91 (0.57-1.44)	0.93 (0.58-1.48)	0.76 (0.48-1.20)	0.93 (0.58-1.48)
Adjusted for parity	Group2	1.08 (0.81-1.45)	0.99 (0.74-1.32)	1.02 (0.76-1.37)	1.01 (0.75-1.36)
	Group3	0.88 (0.56-1.39)	0.88 (0.56-1.39)	0.71 (0.45-1.12)	0.90 (0.57-1.42)
Adjusted for ROM >18 hours	Group2	1.12 (0.84-1.51)	1.02 (0.76-1.37)	1.04 (0.77-1.39)	1.04 (0.77-1.39)
	Group3	0.92 (0.58-1.46)	0.92 (0.58-1.47)	0.75 (0.47-1.20)	0.93 (0.58-1.47)
Adjusted for baby's length of hospital stay	Group2	1.12 (0.3-1.52)	1.04 (0.77-1.40)	1.04 (0.77-1.40)	1.05 (0.78-1.42)
	Group3	0.89 (0.56-1.42)	0.92 (0.58-1.46)	0.74 (0.47-1.19)	0.92 (0.58-1.46)
Adjusted for infant's age at the time of stool collection	Group2	0.90 (0.60-1.33)	0.84 (0.57-1.25)	0.88 (0.59-1.31)	0.89 (0.60-1.32)
	Group3	1.42 (0.74-2.71)	1.47 (0.76-2.83)	0.41 (0.21-0.80)*	1.50 (0.78-2.89)
Adjusted for maternal pre-pregnancy weight	Group2	1.07 (0.80-1.45)	0.99 (0.74-1.34)	1.06 (0.79-1.43)	1.01 (0.75-1.35)
	Group3	0.88 (0.56-1.39)	0.91 (0.57-1.43)	0.77 (0.48-1.22)	0.91 (0.57-1.43)
MODEL 1	Group2	1.41 (1.00-1.99)	1.24 (0.88-1.76)	1.29 (0.92-1.81)	1.26 (0.89-1.77)
	Group3	1.05 (0.62-1.79)	1.04 (0.62-1.77)	0.82 (0.49-1.38)	1.04 (0.62 -1.77)

MODEL1: Adjusted for delivery mode by IAP, gestational age, infant diet at 3 months, parity, ROM > 18 hours, length of hospital stay, age of stool collection, maternal pre-pregnancy weight

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes
Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Table 2.15

Crude and adjusted likelihood ratio of gut microbiota richness and diversity measures at 3-4 months according to duration of active 1st stage of labour

Ref. Group 1 = 1st Stage ≤ 6 Hours Group 2 = 1st Stage > 6 to ≤ 13 Hrs Group 3 = 1st Stage > 13 Hrs		**Chao1 richness** (below vs above median) OR (95% CI)	**Shannon diversity** (below vs above median) OR (95% CI)
Crude OR for 1st stage of labour	Group2	1.05 (0.79-1.41)	0.69 (0.51-0.93)*
	Group3	0.99 (0.63-1.56)	0.74 (0.47-1.17)
Adjusted for delivery MODE by IAP	Group2	0.99 (0.73-1.34)	0.68 (0.50-0.92)*
	Group3	0.86 (0.54-1.36)	0.73 (0.46-1.17)
Adjusted for gestational age	Group2	1.03 (0.77-1.38)	0.66 (0.49-0.89)*
	Group3	0.97 (0.62-1.52)	0.71 (0.45-1.11)
Adjusted for infant diet at 3 months	Group2	1.06 (0.78-1.43)	0.69 (0.51-0.93)
	Group3	0.98 (0.61-1.56)	0.74 (0.46-1.17)
Adjusted for parity	Group2	1.04 (0.78-1.40)	0.70 (0.52-0.94)*
	Group3	0.97 (0.62-1.53)	0.75 (0.48-1.19)
Adjusted for ROM >18 hours	Group2	1.05 (0.78-1.41)	0.70 (0.52-0.94)
	Group3	1.00 (0.63-1.59)	0.76 (0.48-1.21)
Adjusted for baby's length of hospital stay	Group2	1.02 (0.76-1.37)	0.66 (0.48-0.89)*
	Group3	0.92 (0.58-1.47)	0.68 (0.42-1.08)
Adjusted for infant's age at the time of stool collection	Group2	1.07 (0.80-1.43)	0.70 (0.52-0.94)*
	Group3	1.04 (0.66-1.65)	0.79 (0.50-1.24)
Adjusted for maternal pre-pregnancy weight	Group2	1.05 (0.78-1.41)	0.70 (0.52-0.94)*
	Group3	0.99 (0.63-1.58)	0.79 (0.50-1.24)
MODEL 1	Group2	0.86 (0.61-1.22)	**0.64 (0.45-0.90)***
	Group3	0.74 (0.43-1.26)	0.74 (0.44-1.26)

MODEL1: Adjusted for delivery mode by IAP, gestational age, infant diet at 3 months, parity, ROM > 18 hours, length of hospital stay, age of stool collection, maternal pre-pregnancy weight

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes
Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Table 2.16 Summary table showing **significant** ($p<0.05$) changes in median relative abundance of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the **duration of second stage of labour**, and following different levels of stratifications:

ALL MODES OF BIRTHS (n=955)		
Reference group: 2nd Stage of labour <= 1 hour	2nd Stage of labour > 1 to ≤2 hours	2nd Stage of labour > 2 hours
Phylum Actinobacteria	--	↓
Bifidobacteriaceae	--	↓
Coriobacteriaceae	--	↓
g_*Bifidobacterium*	--	↓
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Lactobacillaceae	--	↓
Ruminococcaceae	--	↓
Clostridiaceae	--	↑
g_*Lactobacillus*	--	↓
Phylum Proteobacteria	--	--

VAGINAL BIRTHS WITHOUT IAP (n=503)		
Reference group: 2nd Stage of labour <= 1 hour	2nd Stage > 1 to ≤ 2hrs	2d Stage >2 hrs
Phylum Actinobacteria	--	↓
Coriobacteriaceae	↓	↓
Bifidobacteriaceae	--	↓
genus_Bifidobacterium	--	↓
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Clostridiaceae	--	↑
genus_Clostridium	↑	↑
genus_Veillionella	--	↑
Phylum Proteobacteria	--	--
genus_Citrobacter	--	↑

VAGINAL BIRTHS WITH IAP (n=198)		
Reference group: 2nd Stage of labour <= 1 hour	2nd Stage > 1 to ≤2 hrs	2nd Stage >2 hrs
Phylum Actinobacteria	–	--
Bifidobacteriaceae	–	--
genus_Bifidobacterium	–	--
Phylum Bacteroidetes	–	--
Phylum Firmicutes	↑	--
Clostridiaceae	↑	--
genus_Lactobacillus	–	--
Phylum Proteobacteria	–	↑
Enterobacteriaceae	–	↑
genus_Enterobacter	–	↑

VAGINAL BIRTHS WITHOUT IAP WITH EXCLUSIVEL BREASTFEEDING (n=269)		
Reference group: 2nd Stage of labour <= 1 hour	2nd Stage >1 to ≤2 hrs	2nd Stage >2 hrs
Phylum Actinobacteria	–	↓
Bifidobacteriaceae	–	↓
genus_Bifidobacterium	–	↓
Clostridiaceae	–	↑

VAGINAL BIRTHS WITH IAP WITH EXCLUSIVEL BREASTFEEDING (n=106)		
Reference group: 2nd Stage of labour <= 1 hour	2nd Stage >1 to ≤2 hrs	2nd Stage >2 hrs
Phylum Actinobacteria	–	–
Bifidobacteriaceae	–	–
genus_Bifidobacterium	–	–
Phylum Proteobacteria	–	↑

VAGINAL BIRTHS WITHOUT IAP WITHOUT EXCLUSIVELY BREASTFEEDING (n=230)		
Reference group: 2nd Stage of labour <= 1 hour	2nd Stage >2 to ≤2 hrs	2nd Stage > 2 hrs
Phylum Actinobacteria	–	--
Bifidobacteriaceae	–	↓
genus_Bifidobacterium	–	↓
Bacteroidaceae	↓	--

VAGINAL BIRTHS WITH IAP WITHOUT EXCLUSIVELY BREASTFEEDING (n=89)		
Reference group: 2nd Stage of labour <= 1 hour	2nd Stage >2 to ≤2 hrs	2nd Stage > 2 hrs
Phylum Actinobacteria	–	--
Bifidobacteriaceae	–	--
genus_Bifidobacterium	–	--
Clostridiaceae	↑	↑

Table 2.17
Median relative abundance of dominant bacterial taxa at the phylum and family level in infant gut microbiota at 3-4 months among *all modes* of delivery, according to the duration of second stage of labour (n=955)

Bacterial Taxa	**2nd Stage of labour ≤ 1 hour [Reference: Group 1]**	**2nd Stage of labour > 1 to ≤ 2 hours [Group 2]**	p-value	**2nd Stage of labour > 2 hours [Group 3]**	p-value
	(n=667; 69.8%)	(n=125; 13.1%)		(n=163; 17.1%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	6.163 (1.821-16.291)	5.104 (0.754-13.993)	0.087	3.559 (0.00-13.693)	**0.010**
Family					
Actinomycetaceae	0.023 (0.000-0.108)	0.016 (0.000-0.077)	0.526	0.031 (0.000-0.100)	0.919
Bifidobacteriaceae	5.402 (1.450-15.374)	4.524 (0.512-12.978)	0.103	3.164 (0.263-13.024)	**0.006**
Coriobacteriaceae	0.046 (0.008-0.187)	0.047 (0.000-0.147)	0.332	0.023 (0.000-0.117)	**0.005**
Genus					
Bifidobacterium	5.402 (1.450-15.374)	4.524 (0.492-12.978)	0.096	3.164(0.263-13.024)	**0.005**
Actinomyces	0.023 (0.000-0.093)	0.016 (0.000-0.074)	0.644	0.023 (0.000-0.086)	0.547
Phylum					
Bacteroidetes	18.252 (0.109-60.674)	26.577 (0.081-61.234)	0.872	14.775 (0.085-66.620)	0.852
Family					
Bacteroidaceae	12.658 (0.077-54.501)	21.228 (0.066-58.826)	0.855	9.542 (0.062-62.317)	0.920
Genus					
Bacteroides	12.658 (0.077-54.501)	21.228 (0.066-58.826)	0.855	9.542 (0.062-62.317)	0.920
Phylum					
Firmicutes	22.709 (8.529-44.531)	21.624 (9.348-46.198)	0.916	22.681 (8.564-45.256)	0.998
Family					
Enterococcaceae	0.023 (0.000-0.108)	0.016 (0.000-0.097)	0.264	0.031 (0.000-0.101)	0.664
Lactobacillaceae	0.000 (0.000-0.024)	0.000 (0.000-0.023)	0.474	0.000 (0.000-0.008)	**0.008**
Streptoccocaceae	0.591 (0.217-1.914)	0.642(0.205-1.894)	0.936	0.564 (0.201-1.568)	0.392
Clostridiaceae	0.322 (0.031-1.994)	0.542 (0.023-4.051)	0.464	0.805 (0.063-4.040)	**0.003**
Lachnospiraceae	2.800 (0.054-9.853)	1.773 (0.043-9.554)	0.455	1.999 (0.046-8.393)	0.471
Ruminococcaceae	0.132 (0.000-2.012)	0.031 (0.000-1.433)	0.151	0.046 (0.000-1.028)	**0.051**
Veillionellaceae	4.459 (0.819-16.416)	3.847 (0.951-13.797)	0.415	5.284 (0.658-17.214)	0.929

Genus					
Enterococcus	0.016 (0.000-0.101)	0.015 (0.000-0.093)	0.352	0.031 (0.000-0.094)	0.696
Lactobacillus	0.000 (0.000-0.024)	0.000 (0.000-0.023)	0.474	0.000 (0.000-0.008)	**0.008**
Streptococcus	0.591 (0.217-1.914)	0.642(0.203-1.894)	0.926	0.557 (0.201-1.568)	0.370
Clostridium	0.016 (0.000-0.450)	0.031 (0.000-0.977)	0.431	0.085 (0.008-1.262)	0.009
Ruminococcus	0.031 (0.000-1.827)	0.023 (0.000-2.307)	0.300	0.016 (0.000-1.575)	0.016
Veillionella	3.079 (0.403-14.374)	2.540 (0.533-11.941)	0.526	4.314 (0.364-16.856)	0.526
Phylum					
Proteobacteria	17.937 (7.563-39.072)	22.224 (7.835-41.245)	0.430	18.982 (9.131-42.053)	0.361
Family					
Enterobacteriaceae	15.995 (5.468-36.360)	21.181 (4.795-40.816)	0.299	18.051(7.845-40.974)	0.172
Genus					
Citrobacter	0.031 (0.000-0.232)	0.039 (0.000-0.464)	0.546	0.047(0.000-0.248)	0.450
Enterobacter_unclss	15.275 (5.106-35.681)	16.926 (4.730-39.522)	0.312	17.812 (7.798-39.751)	0.145

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.18
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among VAGINAL births without intrapartum antibiotic prophylaxis (IAP), according to duration of second stage of labour (n= 503)

Bacterial Taxa	2nd Stage of labour ≤ 1 hour [Reference: Group 1]	2nd Stage of labour > 1 to ≤ 2 hours [Group 2]	p-value	2nd Stage of labour > 2 hours [Group 3]	p-value
	(n=338; 67.2%)	(n=83; 16.5%)		(n=82; 16.3%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	6.938 (2.492-17.318)	6.194 (1.192-14.554)	0.179	3.511 (0.883-13.465)	**0.012**
Family					
Actinomycetaceae	0.023 (0.000-0.080)	0.015 (0.000-0.070)	0.532	0.039 (0.008-0.127)	0.096
Bifidobacteriaceae	6.478 (2.085-16.298)	6.033 (1.036-14.204)	0.253	3.193 (0.515-13.108)	**0.005**
Coriobacteriaceae	0.050 (0.008-0.221)	0.046 (0.000-0.101)	**0.020**	0.023 (0.000-0.117)	**0.050**
Genus					
Bifidobacterium	6.478 (2.034-16.257)	6.033 (1.036-14.204)	0.249	3.193 (0.515-13.108)	**0.005**
Actinomyces	0.016 (0.000-0.064)	0.008 (0.000-0.062)	0.568	0.031 (0.008-0.117)	**0.032**
Phylum					
Bacteroidetes	42.140 (5.581-66.116)	29.644(0.140-61.839)	0.072	27.599(0.393-68.904)	0.353
Family					
Bacteroidaceae	34.402 (2.394-59.950)	26.424(0.085-60.701)	0.067	23.310(0.122-66.685)	0.346
Genus					
Bacteroides	34.402 (2.394-59.950)	26.424 (0.085-60.701)	0.067	23.310 (0.122-66.685)	0.346
Phylum					
Firmicutes	15.850 (6.963-33.700)	18.637(8.130-36.415)	0.418	19.276(7.090-41.920)	0.311
Family					
Enterococcaceae	0.016 (0.000-0.057)	0.016 (0.000-0.079)	0.814	0.012 (0.000-0.063)	0.916
Lactobacillaceae	0.000 (0.000-0.023)	0.000 (0.000-0.015)	0.713	0.000 (0.000-0.008)	0.072
Streptoccocaceae	0.564 (0.155-1.808)	0.642 (0.170-1.784)	0.816	0.400 (0.174-1.225)	0.417
Clostridiaceae	0.151 (0.016-0.956)	0.396 (0.016-3.981)	0.103	0.636 (0.068-5.046)	**0.000**
Lachnospiraceae	2.131 (0.066-8.420)	1.389 (0.039-9.463)	0.760	2.188 (0.052-6.703)	0.872
Ruminococcaceae	0.120 (0.008-1.784)	0.023 (0.000-1.545)	0.143	0.082 (0.000-1.103)	0.289
Veillionellaceae	2.940 (0.493-13.574)	3.261 (0.952-10.972)	0.912	6.704 (0.556-15.422)	0.219

Genus					
Enterococcus	0.015 (0.000-0.055)	0.015 (0.000-0.070)	0.817	0.008 (0.000-0.057)	0.719
Lactobacillus	0.000 (0.000-0.023)	0.000 (0.000-0.015)	0.713	0.000 (0.000-0.008)	0.072
Streptococcus	0.562 (0.155-1.808)	0.642 (0.170-1.784)	0.809	0.400 (0.174-1.176)	0.378
Clostridium	0.008 (0.000-0.140)	0.016 (0.000-1.200)	**0.040**	0.031 (0.000-0.810)	**0.006**
Ruminococcus	0.120 (0.000-2.086)	0.023 (0.000-2.310)	0.417	0.031 (0.006-1.830)	0.470
Veillionella	1.698 (0.202-10.212)	2.465 (0.599-8.001)	0.309	4.540 (0.283-15.416)	**0.053**
Phylum					
Proteobacteria	14.767 (6.411-32.198)	16.434 (7.665-41.473)	0.069	16.685 (9.292-35.209)	0.147
Family					
Enterobacteriaceae	12.067 (4.144-29.471)	15.824 (4.914-41.372)	0.041	14.278 (7.613-33.707)	0.093
Genus					
Citrobacter	0.016 (0.000-0.101)	0.031 (0.000-0.435)	0.117	0.062 (0.000-0.320)	**0.016**
Enterobacter_unclss	11.938 (4.037-29.087)	14.741 (4.852-40.859)	**0.040**	14.068 (7.578-33.660)	0.103

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.19a
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births without intrapartum antibiotics prophylaxis (IAP) without exclusive breastfeeding, according to the duration of second stage of labour (n=230)

Bacterial Taxa	2nd Stage of labour ≤ 1 hour [Reference: Group 1]	2nd Stage of labour > 1 to ≤ 2 hours [Group 2]	p-value	2nd Stage of labour > 2 hours [Group 3]	p-value
	(n=157; 68.3%)	(n= 34; 14.8%)		(n=39; 17.0%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	5.773 (2.158-14.534)	3.451 (1.466-14.268)	0.331	3.388 (1.094-8.715)	0.072
Family *Actinomycetaceae*	0.031 (0.000-0.105)	0.039 (0.008-0.209)	0.274	0.047 (0.016-0.132)	**0.040**
Bifidobacteriaceae	5.043 (1.749-13.877)	2.880 (1.426-12.838)	0.513	2.620 (0.630-5.833)	**0.025**
Coriobacteriaceae	0.086 (0.015-0.509)	0.047 (0.000-0.107)	0.075	0.070 (0.008-0.291)	0.756
Genus *Bifidobacterium*	5.043 (1.749-13.877)	2.880 (1.426-12.838)	0.498	2.620 (0.630-5.833)	**0.025**
Actinomyces	0.023 (0.000-0.101)	0.023 (0.000-0.185)	0.493	0.046 (0.016-0.118)	**0.031**
Phylum Bacteroidetes	49.751 (16.782-72.109)	29.788 (0.307-61.651)	0.096	35.400 (9.936-71.631)	0.548
Family *Bacteroidaceae*	40.016 (12.301-62.046)	26.501 (0.084-52.984)	**0.048**	25.601 (7.159-68.632)	0.383
Genus *Bacteroides*	40.016 (12.301-62.046)	26.501 (0.084-52.984)	**0.048**	25.601 (7.159-68.632)	0.383
Phylum Firmicutes	10.203 (4.704-24.216)	11.663 (5.359-33.612)	0.415	12.367 (7.308-26.301)	0.529
Family *Enterococcaceae*	0.023 (0.000-0.058)	0.035 (0.008-0.118)	0.193	0.023 (0.008-0.062)	0.621
Lactobacillaceae	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.553	0.000 (0.000-0.000)	**0.018**
Streptoccocaceae	0.644 (0.179-1.698)	0.703 (0.201-1.816)	0.632	0.307 (0.108-1.164)	0.139
Clostridiaceae	0.202 (0.047-0.950)	0.600 (0.070-3.263)	0.070	0.342 (0.071-2.209)	0.112
Lachnospiraceae	2.929 (0.572-8.834)	5.407 (1.169-16.699)	0.066	5.522 (1.793-9.684)	0.217
Ruminococcaceae	0.922 (0.023-3.017)	0.794 (0.035-6.816)	0.506	0.624 (0.016-2.326)	0.371
Veillionellaceae	3.258 (0.934-14.133)	5.765 (1.399-14.611)	0.443	7.329 (1.984-15.060)	0.122

Genus					
Enterococcus	0.016 (0.000-0.055)	0.035 (0.008-0.118)	0.152	0.023 (0.000-0.055)	0.788
Lactobacillus	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.553	0.000 (0.000-0.000)	**0.018**
Streptococcus	0.644 (0.175-1.698)	0.703 (0.195-1.816)	0.637	0.307 (0.108-1.164)	0.141
Clostridium	0.008 (0.000-0.074)	0.085 (0.000-0.710)	0.079	0.016 (0.000-0.189)	0.607
Ruminococcus	0.313 (0.000-2.296)	0.627 (0.008-3.186)	0.440	0.209 (0.008-3.486)	0.996
Veillionella	1.974 (0.326-9.851)	3.189 (1.273-11.476)	0.113	5.999 (0.825-14.722)	0.079
Phylum					
Proteobacteria	10.203 (4.704-24.216)	11.663 (5.359-33.612)	0.415	12.367 (7.308-26.301)	0.529
Family					
Enterobacteriaceae	7.455 (2.869-20.620)	11.543 (3.926-32.217)	0.290	11.116 (4.161-24.350)	0.456
Genus					
Citrobacter	0.016 (0.000-0.078)	0.019 (0.000-0.113)	0.903	0.063 (0.008-0.201)	**0.027**
Enterobacter_unclss	7.366 (2.752-20.320)	11.531 (3.876-30.455)	0.277	11.109 (3.983-23.613)	0.473

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.19b
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births without intrapartum antibiotics prophylaxis (IAP) with exclusive breastfeeding, according to the duration of second stage of labour (n= 269)

Bacterial Taxa	2nd Stage of labour ≤ 1 hour [Reference: Group 1]	2nd Stage of labour > 1 to ≤ 2 hours [Group 2]	p-value	2nd Stage of labour > 2 hours [Group 3]	p-value
	(n=178; 66.2%)	(n= 49; 18.2%)		(n= 42; 15.6%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	8.932 (2.903-20.355)	6.632 (0.579-20.869)	0.279	3.736 (0.310-16.986)	**0.050**
Family					
Actinomycetaceae	0.015 (0.000-0.077)	0.008 (0.000-0.039)	0.15	0.012 (0.000-0.093)	0.957
Bifidobacteriaceae	8.622 (2.567-19.370)	6.282 (0.524-18.795)	0.31	3.376 (0.074-16.589)	0.047
Coriobacteriaceae	0.031 (0.006-0.139)	0.023 (0.000-0.078)	0.17	0.008 (0.000-0.043)	**0.015**
Genus					
Bifidobacterium	8.622 (2.539-19.353)	6.282 (0.505-18.783)	0.310	3.345 (0.068-16.589)	**0.046**
Actinomyces	0.008 (0.000-0.047)	0.008 (0.000-0.039)	0.31	0.012 (0.000-0.068)	0.574
Phylum					
Bacteroidetes	33.105 (0.654-62.249)	29.644 (0.097-62.674)	0.429	21.152 (0.130-63.566)	0.428
Family					
Bacteroidaceae	27.034(0.269-57.930)	23.154 (0.081-61.116)	0.586	21.148 (0.112-63.465)	0.694
Genus					
Bacteroides	27.034(0.269-57.930)	23.154 (0.081-61.116)	0.586	21.148 (0.112-63.465)	0.694
Phylum					
Firmicutes	15.440 (6.733-35.125)	14.460 (5.060-28.531)	0.482	23.460 (5.098-44.775)	0.559
Family					
Enterococcaceae	0.015 (0.000-0.056)	0.015 (0.000-0.046)	0.51	0.008 (0.000-0.060)	0.385
Lactobacillaceae	0.000 (0.000-0.062)	0.000 (0.000-0.031)	0.29	0.000 (0.000-0.041)	0.784
Streptoccocaceae	0.519 (0.149-1.993)	0.456 (0.139-1.796)	0.90	0.427 (0.292-1.580)	0.827
Clostridiaceae	0.086 (0.008-1.110)	0.170 (0.008-4.841)	0.36	1.499 (0.031-15.102)	0.001
Lachnospiraceae	1.478(0.029-7.265)	0.201 (0.023-3.802)	0.14	0.151(0.029-3.771)	0.161
Ruminococcaceae	0.027 (0.000-0.385)	0.008 (0.000-0.070)	0.03	0.008 (0.000-0.238)	0.390
Veillionellaceae	2.550 (0.370-13.039)	2.548 (0.807-6.473)	0.73	4.248 (0.184-17.577)	0.829

Genus					
Enterococcus	0.012 (0.000-0.056)	0.008 (0.000-0.043)	0.453	0.008 (0.000-0.056)	0.337
Lactobacillus	0.000 (0.000-0.0626)	0.000 (0.000-0.031)	0.289	0.000 (0.000-0.041)	0.784
Streptococcus	0.519 (0.149-1.993)	0.456 (0.139-1.796)	0.896	0.427 (0.292-1.580)	0.897
Clostridium	0.008 (0.000-0.367)	0.008 (0.000-1.721)	0.233	0.078 (0.008-5.850)	**0.003**
Ruminococcus	0.039 (0.000-1.980)	0.008 (0.000-0.639)	0.124	0.012 (0.000-0.151)	0.355
Veillionella	1.437 (0.151-10.346)	1.562 (0.198-6.406)	0.929	3.271 (0.166-17.573)	0.364
Phylum					
Proteobacteria	17.865 (8.664-38.800)	30.422 (10.316-52.459)	0.133	21.763 (12.763-42.349)	0.122
Family					
Enterobacteriaceae	16.498(6.933-35.552)	30.083 (7.864-50.593)	0.11	20.744 (11.125-42.148)	0.088
Genus					
Citrobacter	0015 (0.000-0.187)	0.055 (0.000-0.477)	**0.049**	0.054 (0.000-0.673)	0.164
Enterobacter_unclss	16.294 (6.768-35.521)	28.782 (7.667-50.555)	0.110	20.132 (9.989-41.500)	0.096

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.20

Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births with intrapartum antibiotics prophylaxis (IAP), according to the duration of second stage of labour (n= 198)

Bacterial Taxa	**2nd Stage of labour ≤ 1 hour [Reference: Group 1]**	**2nd Stage of labour > 1 to ≤ 2 hours [Group 2]**	p-value	**2nd Stage of labour > 2 hours [Group 3]**	p-value
	(n= 105; 53.0%)	(n= 38; 19.2%)		(n= 55; 27.8%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	3.389 (1.128-16.016)	3.261 (0.353-12.096)	0.398	2.173 (0.326-10.133)	0.123
Family					
Actinomycetaceae	0.015 (0.000-0.124)	0.035 (0.006-0.111)	0.260	0.016 (0.000-0.069)	0.742
Bifidobacteriaceae	3.328 (1.004-15.596)	2.825 (0.078-9.084)	0.253	2.142 (0.085-9.231)	0.120
Coriobacteriaceae	0.031 (0.008-0.104)	0.077 (0.008-0.353)	0.072	0.015 (0.008-0.118)	0.379
Genus					
Bifidobacterium	3.328 (1.004-15.596)	2.825 (0.078-9.073)	0.248	2.142 (0.085-9.231)	0.114
Actinomyces	0.015 (0.000-0.117)	0.035 (0.006-0.111)	0.216	0.016 (0.000-0.069)	0.749
Phylum					
Bacteroidetes	28.692 (0.086-69.635)	1.118 (0.047-55.877)	0.086	4.061 (0.046 -66.620)	0.333
Family					
Bacteroidaceae	23.083 (0058-66.432)	1.107 (0.039-48.172)	0.115	0.667 (0.039-65.791)	0.420
Genus					
Bacteroides	23.083 (0058-66.432)	1.107 (0.039-48.172)	0.115	0.667 (0.039-65.791)	0.420
Phylum					
Firmicutes	19.488 (6.593-39.532)	28.594 (15.495-54.824)	**0.009**	24.486 (8.103-49.189)	0.480
Family					
Enterococcaceae	0.023 (0.000-0.203)	0.015 (0.000-0.157)	0.475	0.024 (0.000-0.119)	0.867
Lactobacillaceae	0.000 (0.000-0.016)	0.000 (0.000-0.023)	0.904	0.000 (0.000-0.008)	0.502
Streptoccocaceae	0.503 (0.200-1.892)	0.628 (0.333-2.845)	0.235	0.710 (0.193-1.608)	0.873
Clostridiaceae	0.201 (0.024-1.491)	0.832 (0.047-5.271)	**0.045**	0.725 (0.039-5.712)	0.132
Lachnospiraceae	1.813 (0.051-8.744)	2.073 (0.060-7.899)	0.873	1.408 (0.031-9.646)	0.734
Ruminococcaceae	0.239 (0.000-1.845)	0.085 (0.000-1.044)	0.811	0.008 (0.000-0.685)	0.120
Veillionellaceae	4.041 (0.476-11.689)	8.887 (0.929-24.915)	0.098	4.212 (0.785-20.373)	0.430

Genus					
Enterococcus	0.023 (0.000-0.203)	0.015 (0.000-0.157)	0.537	0.023 (0.000-0.119)	0.919
Lactobacillus	0.000 (0.000-0.016)	0.000 (0.000-0.023)	0.904	0.000 (0.000-0.008)	0.502
Streptococcus	0.503 (0.200-1.892)	0.628 (0.333-2.845)	0.229	0.710 (0.193-1.608)	0.859
Clostridium	0.016 (0.000-0.339)	0.077 (0.000-0.464)	0.335	0.062 (0.000-1.215)	0.177
Ruminococcus	0.016 (0.000-1.140)	0.023 (0.008-1.861)	0.424	0.008 (0.000-1.222)	0.238
Veillionella	3.312 (0.312-11.525)	7.231 (0.401-21.840)	0.291	2.214 (0.544-19.495)	0.420
Phylum					
Proteobacteria	15.664 (7.547-39.341)	22.993 (11.131-40.951)	0.210	22.815 (9.420-46.768)	**0.039**
Family					
Enterobacteriaceae	12.791 (5.501-34.823)	22.529 (4.623-39.988)	0.168	21.832 (8.714-45.482)	**0.014**
Genus					
Citrobacter	0.023 (0.000-0.178)	0.070 (0.000-0.517)	0.075	0.047 (0.000-0.364)	0.098
Enterobacter_unclss	12.737 (5.226-34.815)	21.188 (4.474-37.516)	0.240	21.514 (8.632-43.868)	**0.016**

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.21a
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births with intrapartum antibiotics prophylaxis (IAP) without exclusive breastfeeding, according to the duration of second stage of labour (n= 89)

Bacterial Taxa	**2nd Stage of labour ≤ 1 hour [Reference: Group 1]**	**2nd Stage of labour > 1 to ≤ 2 hours [Group 2]**	p-value	**2nd Stage of labour > 2 hours [Group 3]**	p-value
	(n=47; 52.8%)	(n= 14; 15.7%)		(n= 28; 31.5%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	2.626 (0.806-8.691)	2.594 (0.353-5.494)	0.372	1.955 (0.308-6.931)	0.128
Family *Actinomycetaceae*	0.015 (0.000-0.132)	0.035 (0.014-0.128)	0.347	0.023 (0.002-0.079)	0.686
Bifidobacteriaceae	2.306 (0.555-7.811)	2.370 (0.112-4.407)	0.295	1.881 (0.37-5.651)	0.154
Coriobacteriaceae	0.023 (0.008-0.124)	0.097 (0.026-0.284)	0.050	0.031 (0.008-0.145)	0.575
Genus *Bifidobacterium*	0.015 (0.000-0.132)	0.035 (0.014-0.128)	0.330	0.019 (0.000-0.074)	0.587
Actinomyces	2.306 (0.555-7.811)	2.370 (0.112-4.407)	0.295	1.881 (0.037-4.407)	0.154
Phylum Bacteroidetes	52.750 (0.155-70.349)	23.732 (0.045-60.780)	0.251	17.570 (0.054-71.266)	0.470
Family *Bacteroidaceae*	43.965 (0.078-66.757)	23.447 (0.029-59.138)	0.337	0.656 (0.042-70.072)	0.518
Genus *Bacteroides*	43.965 (0.078-66.757)	23.447 (0.029-59.138)	0.337	0.656 (0.042-70.072)	0.518
Phylum Firmicutes	15.278 (6.676-46.683)	33.358 (13.198-72.866)	0.069	26.173 (11.607-64.813)	0.363
Family *Enterococcaceae*	0.016 (0.008-0.077)	0.008 (0.000-0.056)	0.186	0.023 (0.000-0.074)	0.847
Lactobacillaceae	0.000 (0.000-0.000)	0.000 (0.000-0.014)	0.785	0.000 (0.000-0.006)	0.804
Streptoccocaceae	0.402 (0.109-1.565)	0.628 (0.238-2.219)	0.515	0.575 (0.152-1.707)	0.827
Clostridiaceae	0.244 (0.062-1.347)	2.493 (0.275-5.674)	0.029	1.206 (0.517-6.021)	0.033
Lachnospiraceae	4.512 (0.536-14.395)	3.902 (1.937-15.280)	0.745	3.824 (0.461-19.859)	0.921
Ruminococcaceae	0.886 (0.047-3.833)	0.740 (0.068-7.980)	0.770	0.625 (0.000-2.944)	0.368
Veillionellaceae	4.419 (0.921-11.272)	11.152 (0.540-26.754)	0.223	4.803 (0.883-22.030)	0.540

Genus					
Enterococcus	0.015 (0.008-0.077)	0.008 (0.000-0.056)	0.216	0.023 (0.000-0.062)	0.847
Lactobacillus	0.000 (0.000-0.000)	0.000 (0.000-0.014)	0.785	0.000(0.000-0.006)	0.804
Streptococcus	0.402 (0.109-1.565)	0.628 (0.238-2.219)	0.504	0.575 (0.152-1.707)	0.801
Clostridium	0.016 (0.000-0.319)	0.082 (0.006-1.182)	0.377	0.277 (0.000-0.285)	**0.047**
Ruminococcus	0.124 (0.000-1.565)	1.007 (0.014-2.621)	0.333	0.043 (0.000-1.512)	0.491
Veillionella	3.926 (0.797-10.268)	8.062 (0.206-26.754)	0.548	3.499 (0.691-18.722)	0.638
Phylum					
Proteobacteria	12.401 (3.833-24.835)	15.836 (6.320-27.117)	0.482	17.280 (3.406-33.552)	0.393
Family					
Enterobacteriaceae	9.595 (3.613-19.858)	12.777 (4.245-27.030)	0.758	17.208 (3.366-32.261)	0.212
Genus					
Citrobacter	0.031 (0.008-0.178)	0.074 (0.012-0.622)	0.199	0.067 (0.008-0.207)	0.267
Enterobacter_unclss	9.186 (3.598-19.347)	12.412 (3.257-26.086)	0.797	17.065 (3.282-32.103)	0.224

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.21b

Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among vaginal births with intrapartum antibiotics prophylaxis (IAP) with exclusive breastfeeding, according to the duration of second stage of labour (n= 106)

Bacterial Taxa	**2nd Stage of labour ≤ 1 hour [Reference: Group 1]**	**2nd Stage of labour > 1 to ≤ 2 hours [Group 2]**	p-value	**2nd Stage of labour > 2 hours [Group 3]**	p-value
	(n= 57; 53.8%)	(n= 22; 20.8%)		(n= 27; 25.5%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	4.807 (1.444-21.717)	4.194 (0.126-15.447)	0.431	4.492 0.811-13.703)	0.506
Family *Actinomycetaceae*	0.015 (0.000-0.093)	0.039 (0.000-0.225)	0.392	0.008(0.000-0.069)	0.844
Bifidobacteriaceae	4.366 (1.406-20.986)	3.734 (0.041-11.498)	0.274	4.476 (0.788-10.543)	0.487
Coriobacteriaceae	0.039 (0.004-0.081)	0.054 (0.008-0.548)	0.248	0.008 (0.008-0.023)	0.077
Genus *Bifidobacterium*	4.366 (1.406-20.963)	3.734 (0.041-11.498)	0.260	4.476 (0.551-10.543)	0.464
Actinomyces	0.008 (0.000-0.093)	0.039 (0.000-0.225)	0.340	0.008 (0.000-0.069)	0.988
Phylum Bacteroidetes	12.515 (0.062-69.415)	1.078 (0.044-56.256)	0.353	2.686 (0.046-50.280)	0.524
Family *Bacteroidaceae*	9.529 (0.047-65.720)	1.047 (0.039-49.073)	0.320	2.686 (0.039-48.967)	0.635
Genus *Bacteroides*	9.529 (0.047-65.720)	1.047 (0.039-49.073)	0.320	2.686 (0.039-48.967)	0.635
Phylum Firmicutes	21.619 (5.931-38.200)	24.135 (15.495-53.194)	0.101	18.919 (4.960-45.998)	0.989
Family *Enterococcaceae*	0.031 (0.000-0.286)	0.027 (0.000-0.212)	0.598	0.062 (0.000-0.241)	0.803
Lactobacillaceae	0.000 (0.000-0.063)	0.000 (0.000-0.023)	0.589	0.000 (0.000-0.008)	0.325
Streptoccocaceae	0.518 (0.241-2.495)	0.541 (0.352-4.068)	0.562	0.710 (0.201-1.608)	0.844
Clostridiaceae	0.132 (0.008-1.621)	0.414 (0.021-8.092)	0.259	0.194 (0.008-3.602)	0.992
Lachnospiraceae	0.467 (0.023-7.465)	0.444 (0.021-6.496)	0.870	0.108 (0.015-4.887)	0.470
Ruminococcaceae	0.008 (0.000-0.825)	0.016 (0.000-0.462)	0.837	0.008 (0.000-0.031)	0.196
Veillionellaceae	3.399 (0.241-14.391)	8.075 (1.405-21.972)	0.238	1.501 (0.317-20.373)	0.595

Genus					
Enterococcus	0.023 (0.000-0.286)	0.027 (0.000-0.212)	0.638	0.062 (0.000-0.241)	0.744
Lactobacillus	0.000 (0.000-0.063)	0.000 (0.000-0.023)	0.589	0.000 (0.000-0.008)	0.325
Streptococcus	0.518 (0.241-2.495)	0.541 (0.352-4.068)	0.562	0.710 (0.201-1.608)	0.844
Clostridium	0.016 (0.000-0.700)	0.101 (0.000-2.704)	0.601	0.031 (0.000-0.575)	0.973
Ruminococcus	0.008 (0.000-0.174)	0.008 (0.000-1.861)	0.773	0.000 (0.000-0.062)	0.167
Veillionella	3.208 (0.190-14.391)	7.231 (0.824-17.013)	0.394	1.149 (0.295-20.373)	0.518
Phylum					
Proteobacteria	18.951 (9.584-40.815)	29.370 (11.267-43.596)	0.526	40.721 (18.982-63.415)	**0.023**
Family					
Enterobacteriaceae	16.970 (8.930-38.258)	29.308 (7.116-43.489)	0.325	36.933 (18.799-53.122)	**0.019**
Genus					
Citrobacter	0.008 (0.000-0.178)	0.051 (0.000-0.446)	0.325	0.046 (0.000-1.307)	0.281
Enterobacter_unclss	16.628 (8.802-38.196)	26.334 (7.034-40.035)	0.457	36.825 (18.799-51.103)	**0.021**

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.22
Median relative abundance of dominant bacterial taxa at the genus level in infant gut microbiota at 3-4 months among C-section with labour, according to the duration of second stage of labour (n =121)

Bacterial Taxa	**2nd Stage of labour ≤ 1 hour [Reference: Group 1]**	**2nd Stage of labour > 1 hour [Group 2]**	p-value
	(n= 95; 78.5%)	(n=26; 21.5%)	
	Median (IQR)	Median (IQR)	
	N (%); IQR	N (%); IQR	
Phylum Actinobacteria	6.300 (0.699-14.836)	12.843 (0.344-26.763)	0.316
Family *Actinomycetaceae*	0.039 (0.008-0.177)	0.031 (0.006-0.089)	0.603
Bifidobacteriaceae	5.539 (0.478-13.926)	12.576 (0.052-26.612)	0.286
Coriobacteriaceae	0.047 (0.000-0.201)	0.031 (0.006-0.128)	0.803
Genus *Bifidobacterium*	0.031 (0.000-0.177)	0.031 (0.006-0.065)	0.665
Actinomyces	5.539 (0.478-13.773)	12.553 (0.043-26.591)	0.357
Phylum Bacteroidetes	0.116 (0.046-1.225)	0.139 (0.037-0.892)	0.736
Family *Bacteroidaceae*	0.095 (0.039-0.644)	0.116 (0.029-0.861)	0.684
Genus *Bacteroides*	0.095 (0.039-0.644)	0.116 (0.029-0.861)	0.684
Phylum Firmicutes	36.620 (22.709-61.669)	29.880 (16.229-48.427)	0.298
Family *Enterococcaceae*	0.046 (0.015-0.143)	0.183 (0.037-0.424)	0.009
Lactobacillaceae	0.000 (0.000-0.016)	0.000 (0.000-0.015)	0.420
Streptoccocaceae	1.052 (0.379-2.826)	0.826 (0.275-2.775)	0.840
Clostridiaceae	1.223 (0.170-7.011)	1.253 (0.460-3.532)	0.955
Lachnospiraceae	4.812 (0.039-14.438)	6.361 (0.056-13.827)	0.820
Ruminococcaceae	0.077 (0.008-2.616)	0.210 (0.008-1.471)	0.919
Veillionellaceae	11.666 (3.246-29.785)	4.997 (0.553-19.722)	0.068

Genus			
Enterococcus	0.031 (0.008-0.143)	0.151 (0.037-0.424)	**0.008**
Lactobacillus	0.000 (0.000-0.016)	0.000 (0.000-0.015)	0.420
Streptococcus	1.048 (0.378-2.810)	0.826 (0.275-2.775)	0.845
Clostridium	0.209 (0.016-2.207)	0.321 (0.091-2.131)	0.429
Ruminococcus	0.015 (0.000-1.608)	0.008 (0.000-4.509)	0.765
Veillionella	9.652 (2.189-27.317)	4.923 (0.553-19.685)	0.148
Phylum			
Proteobacteria	27.777 (13.426-51.000)	18.473 (6.916-42.204)	0.187
Family			
Enterobacteriaceae	25.264 (11.272-50.253)	18.312 (6.881-38.661)	0.286
Genus			
Citrobacter	0.151 (0.016-0.953)	0.023 (0.000-0.160)	**0.007**
Enterobacter_unclss	22.900 (10.167-48.247)	17.726 (6.839-38.634)	0.427

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

Table 2.23

ACTINOBACTERIA

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of 2nd stage of labour

Microbiota Measure					
Ref. Group 1: 2nd Stage ≤ 1 Hour Group 2: 2nd Stage > 1 to ≤2 Hrs Group 3: 2nd Stage > 2 Hrs	**Infant's gut microbiota at 3 to 4 months of age**				
	Phylum Actinobacteria (below vs above median)		**Family Bifidobacteriaceae** (below vs above median)	**Family Coriobacteriaceae** (below vs above median)	**Genus Bifidobacterium** (below vs above median)
	OR (95% CI)		OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 2nd stage of labour	Group2	0.82 (0.56-1.20)	0.87 (0.59-1.28)	1.04 (0.71-1.52)	0.87 (0.59-1.28)
	Group3	0.61 (0.43-0.86)*	0.57 (0.40-0.81)**	0.56 (0.39-0.79)**	0.57 (0.40-0.81)**
Adjusted for delivery MODE by IAP	Group2	0.81 (0.55-1.20)	0.86 (0.58-1.28)	1.02 (0.69-1.51)	0.86 (0.58-1.28)
	Group3	0.61 (0.43-0.86)*	0.57 (0.40-0.82)**	0.57 (0.40-0.81)**	0.57 (0.40-0.82)**
Adjusted for gestational age	Group2	0.78 (0.53-1.15)	0.83 (0.57-1.22)	1.01 (0.69-1.48)	0.83 (0.57-1.22)
	Group3	0.58 (0.41-0.82)*	0.55 (0.39-0.78)*	0.54 (0.38-0.77)**	0.55 (0.39-0.78)**
Adjusted for infant diet at 3 months	Group2	0.76 (0.51-1.12)	0.80 (0.54-1.18)	1.15 (0.78-1.70)	0.80 (0.54-1.18)
	Group3	0.59 (0.42-0.84)*	0.56 (0.39-0.79)**	0.57 (0.40-0.81)**	0.56 (0.39-0.79)**
Adjusted for parity	Group2	0.87 (0.59-1.29)	0.89 (0.60-1.32)	1.19 (0.80-1.76)	0.89 (0.60-1.32)
	Group3	0.65 (0.45-0.94)*	0.59 (0.41-0.85)**	0.65 (0.45-0.95)*	0.59 (0.41-0.85)**
Adjusted for ROM >18 hours	Group2	0.80 (0.54-1.18)	0.85 (0.58-1.26)	1.05 (0.72-1.55)	0.85 (0.58-1.26)
	Group3	0.54 (0.38-0.78)**	0.52 (0.36-0.75)**	0.59 (0.41-0.85)**	0.52 (0.36-0.75)**
Adjusted for baby's length of hospital stay	Group2	0.79 (0.54-1.17)	0.84 (0.57-1.24)	1.05 (0.71-1.54)	0.84 (0.57-1.24)
	Group3	0.60 (0.42-086)*	0.57 (0.40-0.81)**	0.56 (0.39-0.80)**	0.57 (0.40-0.81)**
Adjusted for infant's age at time of stool collection	Group2	0.82 (0.56-1.20)	0.86 (0.59-1.27)	1.05 (0.72-1.54)	0.86 (0.59-1.27)
	Group3	0.60 (0.42-0.85)**	0.57 (0.40-0.80)**	0.57 (0.40-0.80)**	0.57 (0.40-0.80)**

Adjusted for maternal pre-pregnancy weight	Group2	0.80 (0.54-1.19)	0.85 (0.57-1.26)	1.13 (0.76-1.67)	0.85 (0.57-1.26)
	Group3	0.55 (0.38-0.79)**	0.53 (0.37-0.76)**	0.56 (0.39-0.81)**	0.53 (0.37-0.76)**
MODEL 1	Group2	0.74 (0.48-1.15)	0.78 (0.51-1.21)	1.36 (0.88-2.11)	0.78 (0.51-1.21)
	Group3	0.51 (0.34-0.77)**	0.48 (0.32-0.73)**	0.70 (0.46-1.06)	0.48 (0.32-0.73)**

MODEL 1: Adjusted for delivery mode by IAP, gestational age, infant diet, parity, ROM > 18 hours, length of hospital stay, stool collection age, maternal pre-pregnancy weight

* p <0.05; ** p<0.005;
OR = odds ratio; CI = confidence interval
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes
Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Table 2.24

BACTEROIDETES

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of 2nd stage of labour

Microbiota Measure				
Ref. Group 1: 2nd Stage ≤ 1 Hour Group 2: 2nd Stage > 1 to ≤2 Hrs Group 3: 2nd Stage > 2 Hrs	**Infant's gut microbiota at 3 to 4 months of age**			
		Phylum Bacteroidetes (below vs above median)	**Family Bacteroidaceae** (below vs above median)	**Genus *Bacteroides*** (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 2nd stage of labour	Group2	1.12 (0.77-1.65)	1.06 (0.72-1.56)	1.06 (0.72-1.56)
	Group3	0.97 (0.69-1.36)	0.88 (0.62-1.24)	0.88 (0.62-1.24)
Adjusted for delivery MODE by IAP	Group2	0.70 (0.46-1.04)	0.63 (0.42-0.95)*	0.63 (0.42-0.95)*
	Group3	0.84 (0.58-1.21)	0.74 (0.51-1.06)	0.74 (0.51-1.06)
Adjusted for gestational age	Group2	1.09 (0.74-1.60)	1.02 (0.70-1.51)	1.02 (0.70-1.51)
	Group3	0.94 (0.67-1.33)	0.85 (0.60-1.20)	0.85 (0.60-1.20)
Adjusted for infant diet at 3 months	Group2	1.22 (0.83-1.80)	1.15 (0.78-1.69)	1.15 (0.78-1.69)
	Group3	0.97 (0.69-1.37)	0.90 (0.64-1.27)	0.90 (0.64-1.27)
Adjusted for parity	Group2	1.19 (0.81-1.77)	1.15 (0.78-1.71)	1.15 (0.78-1.71)
	Group3	1.04 (0.73-1.50)	0.98 (0.68-1.40)	0.98 (0.68-1.40)
Adjusted for ROM > 18 hours	Group2	1.08 (0.73-1.59)	1.02 (0.69-1.49)	1.02 (0.69-1.49)
	Group3	0.98 (0.69-1.40)	0.88 (0.62-1.26)	0.88 (0.62-1.26)
Adjusted for baby's length of hospital stay	Group2	1.10 (0.74-1.62)	1.04 (0.70-1.53)	1.04 (0.70-1.53)
	Group3	0.94 (0.66-1.33)	0.87 (0.61-1.24)	0.87 (0.61-1.24)
Adjusted for infant's age at the time of stool collection	Group2	1.13 (0.77-1.66)	1.07 (0.73-1.57)	1.07 (0.73-1.57)
	Group3	0.98 (0.69-1.38)	0.89 (0.63-1.25)	0.89 (0.63-1.25)
Adjusted for maternal pre-pregnancy weight	Group2	1.10 (0.74-1.62)	1.03 (0.70-1.53)	1.03 (0.70-1.53)
	Group3	0.95 (0.67-1.35)	0.88 (0.62-1.25)	0.88 (0.62-1.25)

MODEL 1	Group2	0.68 (0.44-1.07)	0.63 (0.40-0.98)	0.63 (0.40-0.98)
	Group3	0.78 (0.51-1.19)	0.73 (0.47-1.12)	0.73 (0.47-1.12)

MODEL 1: Adjusted for delivery mode by IAP, gestational age, infant diet, parity, ROM > 18 hours, length of hospital stay, stool collection age, maternal pre-pregnancy weight

* p <0.05; ** p<0.005;
OR = odds ratio; CI = confidence interval
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes
Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Table 2.25a

FIRMICUTES

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of 2nd stage of labour

Ref. Group 1: 2nd Stage ≤ 1 Hour Group 2: 2nd Stage > 1 to ≤2 Hrs Group 3: 2nd Stage > 2 Hrs		**Infant's gut microbiota at 3 to 4 months of age**					
		PHYLUM	**FAMILY**				
		FIRMICUTES (below vs above median)	**Streptococcaceae** (below vs above median)	**Clostridiaceae** (below vs above median)	**Lactobacillaceae** (below vs above median)	**Ruminococcaceae** (below vs above median)	**Veillonellaceae** (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 2nd stage of labour	Group2	0.94 (0.64-1.37)	1.03 (0.70-1.51)	1.23 (0.84-1.81)	0.85 (0.57-1.27)	0.75 (0.51-1.11)	0.87 (0.59-1.27)
	Group3	1.00 (0.71-1.41)	0.97 (0.69-1.36)	1.67 (1.18-2.37)**	0.62 (0.42-0.89)*	0.75 (0.53-1.06)	1.18 (0.84-1.66)
Adjusted for delivery MODE by IAP	Group2	1.28 (0.86-1.90)	1.14 (0.77-1.69)	1.61 (1.08-2.40)*	0.82 (0.54-1.23)	0.77 (0.52-1.15)	1.11 (0.75-1.65)
	Group3	1.09 (0.77-1.55)	1.00 (0.71-1.42)	1.80 (1.26-2.58)**	0.61 (0.42-0.89)*	0.76 (0.55-1.10)	1.26 (0.89-1.79)
Adjusted for gestational age	Group2	0.96 (0.65-1.41)	0.99 (0.67-1.46)	1.28 (0.87-1.89)	0.86 (0.58-1.28)	0.75 (0.51-1.11)	0.86 (0.59-1.27)
	Group3	1.02 (0.72-1.44)	0.94 (0.67-1.33)	1.73 (1.22-2.46)**	0.62 (0.43-0.90)*	0.75 (0.53-1.06)	1.17 (0.83-1.65)
Adjusted for infant diet at 3 months	Group2	0.96 (0.65-1.42)	1.00 (0.68-1.47)	1.29 (0.87-1.89)	0.77 (0.51-1.16)	0.85 (0.56-1.29)	0.90 (0.61-1.33)
	Group3	0.99 (0.70-1.40)	0.96 (0.68-1.35)	1.67 (1.18-2.38)**	0.60 (0.41-0.88)**	0.72 (0.50-1.05)	1.20 (0.85-1.70)
Adjusted for parity	Group2	0.88 (0.59-1.30)	0.98 (0.66-1.45)	1.03 (0.70-1.54)	0.85 (0.5701.28)	0.80 (0.54-1.18)	0.84 (0.57-1.25)
	Group3	0.92 (0.64-1.33)	0.91 (0.64-1.31)	1.34 (0.93-1.94)	0.61 (0.41-0.90)	0.81 (0.56-1.16)	1.13 (0.79-1.62)
Adjusted for ROM >18 hours	Group2	0.96 (0.65-1.41)	1.07 (0.72-1.57)	1.23 (0.84-1.81)	0.85 (0.57-1.27)	0.75 (0.51-.11)	0.88 (0.60-1.29)
	Group3	1.00 (0.65-1.42)	1.05 (0.74-1.50)	1.46 (1.02-2.10)*	0.66 (0.45-0.97)*	0.77 (0.54-1.11)	1.14 (0.79-1.62)
Adjusted for baby's length of hospital stay	Group2	0.98 (0.66-1.45)	1.10 (0.69-1.49)	1.30 (0.88-1.92)	0.84 (0.56-1.25)	0.77 (0.52-1.14)	0.88 (0.60-1.29)
	Group3	1.00 (0.70-1.43)	0.91 (0.64-1.29)	1.59 (1.12-2.28)*	0.58 (0.40-0.86)*	0.76 (0.53-1.08)	1.22 (0.86-1.74)
Adjusted for infant's age at	Group2	0.96 (0.65-1.40)	1.01 (0.69-1.48)	1.24 (0.85-1.82)	0.85 (0.57-1.26)	0.77 (0.52-1.14)	0.89 (0.60-1.31)

the time of stool collection	Group3	1.03 (0.73-1.45)	0.95 (0.67-1.33)	1.69 (1.19-2.39)*	0.61 (0.42-0.88)*	0.78 (0.55-1.11)	1.22 (0.86-1.73)
Adjusted for maternal pre-pregnancy weight	Group2	1.00 (0.68-1.48)	1.07 (0.73-1.59)	1.17 (0.79-1.73)	0.84 (0.56-1.26)	0.77 (0.52-1.14)	0.85 (0.57-1.26)
	Group3	1.00 (0.70-1.42)	0.92 (0.65-1.31)	1.60 (1.12-2.27)*	0.60 (0.41-0.88)*	0.75 (0.53-1.08)	1.17 (0.82-1.66)
MODEL 1	Group2	1.41 (0.91-2.18)	1.14 (0.74-1.76)	1.36 (0.88-2.11)	0.75 (0.48-1.19)	0.92 (0.57-1.47)	1.16 (0.75-1.79)
	Group3	1.05 (0.70-1.59)	0.88 (0.59-1.32)	1.16 (0.77-1.75)	**0.63 (0.41-0.98)***	0.86 (0.55-1.36)	1.31 (0.87-1.99)

MODEL 1: Adjusted for delivery mode by IAP, gestational age, infant diet, parity, ROM > 18 hours, length of hospital stay, stool collection age, maternal pre-pregnancy weight

* p <0.05; ** p<0.005;
OR = odds ratio; CI = confidence interval
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes
Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Table 2.25b

FIRMICUTES

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of 2nd stage of labour

Infant's gut microbiota at 3 to 4 months of age						
Ref. Group 1: 2nd Stage ≤ 1 Hour Group 2: 2nd Stage > 1 to ≤2 Hrs Group 3: 2nd Stage > 2 Hrs		GENUS				
		Lactobacillus (below vs above median)	***Streptococcus*** (below vs above median)	***Clostridium*** (below vs above median)	***Ruminococcus*** (below vs above median)	***Veillionella*** (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 2nd stage of labour	Group2	0.85 (0.57-1.27)	1.02 (0.70-1.50)	1.10 (0.75-1.62)	0.89 (0.61-1.31)	0.83 (0.56-1.21)
	Group3	0.62 (0.42-0.89)*	0.94 (0.67-1.32)	1.68 (1.18-2.38)**	0.83 (0.59-1.16)	1.10 (0.78-1.55)
Adjusted for delivery MODE by IAP	Group2	0.82 (0.54-1.23)	1.14 (0.77-1.70)	1.56 (1.05-2.33)*	0.76 (0.51-1.12)	1.11 (0.75-1.66)
	Group3	0.61 (0.42-0.89)*	0.98 (0.69-1.38)	1.84 (1.28-2.65)**	0.78 (0.55-1.10)	1.20 (0.84-1.71)
Adjusted for gestational age	Group2	0.86 (0.58-1.28)	0.98 (0.67-1.45)	1.15 (0.78-1.70)	0.88 (0.60-1.30)	0.82 (0.56-1.20)
	Group3	0.62 (0.43-0.90)*	0.91 (0.64-1.29)	1.74 (1.23-2.48)**	0.82 (0.58-1.15)	1.09 (0.77-1.54)
Adjusted for infant diet at 3 months	Group2	0.77 (0.51-1.16)	0.99 (0.68-1.46)	1.10 (0.74-1.61)	0.98 (0.66-1.46)	0.85 (0.58-1.26)
	Group3	0.60 (0.41-0.88)*	0.93 (0.66-1.31)	1.64 (1.16-2.33)**	0.82 (0.58-1.17)	1.13 (0.80-1.59)
Adjusted for parity	Group2	0.85 (0.57-1.28)	0.96 (0.65-1.42)	0.95 (0.64-1.42)	1.00 (0.67-1.48)	0.79 (0.54-1.18)
	Group3	0.61 (0.41-0.90)*	0.87 (0.61-1.25)	1.40 (0.97-2.02)	0.95 (0.66-1.37)	1.05 (0.73-1.50)
Adjusted for ROM >18 hours	Group2	0.85 (0.57-1.27)	1.06 (0.72-1.56)	1.12 (0.76-1.65)	0.93 (0.63-1.37)	0.84 (0.57-1.23)
	Group3	0.66 (0.45-0.97)*	1.02 (0.71-1.45)	1.56 (1.08-2.24)*	0.84 (0.59-1.20)	1.06 (0.74-1.52)
Adjusted for baby's length of hospital stay	Group2	0.84 (0.56-1.25)	1.00 (0.68-1.48)	1.14 (0.77-1.69)	0.89 (0.60-1.31)	0.84 (0.57-1.24)

	Group3	0.58 (0.40-0.86)*	0.88 (0.62-1.25)	1.66 (1.16-2.39)*	0.85 (0.60-1.21)	1.11 (0.78-1.59)
Adjusted for infant's age at the time of stool collection	Group2	0.85 (0.57-1.26)	1.00 (0.68-1.47)	1.10 (0.75-1.61)	0.92 (0.62-1.36)	0.84 (0.57-1.24)
	Group3	0.61 (0.42-0.88)*	0.92 (0.65-1.29)	1.67 (1.18-2.37)	0.86 (0.60-1.21)	1.13 (0.80-1.60)
Adjusted for maternal pre-pregnancy weight	Group2	0.84 (0.56-1.26)	1.07 (0.72-1.58)	1.06 (0.72-1.57)	1.00 (0.68-1.48)	0.82 (0.55-1.22)
	Group3	0.60 (0.41-0.88)*	0.89 (0.63-1.27)	1.66 (1.16-2.38)*	0.84 (0.59-1.20)	1.08 (0.76-1.53)
MODEL 1	Group2	0.75 (0.48-1.19)	1.14 (0.74-1.75)	1.35 (0.88-2.09)	0.99 (0.63-1.55)	1.16 (0.75-1.79)
	Group3	**0.63 (0.41-0.98)***	0.84 (0.56-1.25)	1.44 (0.58-1.37)	0.90 (0.58-1.37)	1.20 (0.79-1.81)

MODEL 1: Adjusted for delivery mode by IAP, gestational age, infant diet, parity, ROM > 18 hours, length of hospital stay, stool collection age, maternal pre-pregnancy weight

* p <0.05; ** p<0.005;
OR = odds ratio; CI = confidence interval
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes
Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Table 2.26

PROTEOBACTERIA

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of 2nd stage of labour

Microbiota Measure					
Ref. Group 1: 2nd Stage ≤1Hr Group 2: 2nd Stage > 1 to ≤2 Hrs Group 3: 2nd Stage > 2 Hrs	**Infant's gut microbiota at 3 to 4 months of age**				
		Phylum Proteobacteria (below vs above median)	**Family Enterobacteriaceae** (below vs above median)	**Genus Citrobacter** (below vs above median)	**Genus Enterobacter** (unclassified) (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 2nd stage of labour	Group2	1.11 (0.76-1.63)	1.12 (0.76-1.64)	1.23 (0.84-1.81)	1.26 (0.86-1.85)
	Group3	1.12 (0.80-1.58)	1.13(0.80-1.59)	1.24 (0.88-1.75)	1.07 (0.76-1.51)
Adjusted for delivery MODE by IAP	Group2	1.37 (0.92-2.03)	1.38 (0.93-2.05)	1.53 (1.03-2.27)	1.63 (1.09-2.43)
	Group3	1.19 (0.84-1.69)	1.120 (0.84-1.70)	1.33 (0.94-1.89)	1.17 (0.82-1.66)
Adjusted for gestational age	Group2	1.12 (0.76-1.65)	1.12 (0.77-1.65)	1.27 (0.86-1.87)	1.30 (0.88-1.91)
	Group3	1.13 (0.80-1.59)	1.13 (0.80-1.60)	1.27 (0.90-1.80)	1.10 (0.78-1.55)
Adjusted for infant diet at 3 months	Group2	0.98 (0.66-1.46)	0.99 (0.67-1.46)	1.21 (0.82-1.78)	1.19 (0.81-1.75)
	Group3	1.11 (0.78-1.58)	.12 (0.79-1.59)	1.23 (0.87-1.73)	1.08 (0.76-0.52)
Adjusted for parity	Group2	1.06 (0.72-1.58)	1.05 (0.70-1.54)	1.15 (0.78-1.71)	1.12 (0.76-1.67)
	Group3	1.06 (0.74-1.52)	1.03 (0.72-1.47)	1.14 (0.79-1.63)	0.92 (0.64-1.32)
Adjusted for ROM >18 hours	Group2	1.14 (0.78-1.68)	1.14 (0.78-1.67)	1.19 (0.81-1.75)	1.26 (0.85-1.85)
	Group3	1.07 (0.75-1.53)	1.05 (0.74-1.50)	1.29 (0.90-1.84)	1.03 (0.72-1.47)
Adjusted for length of hospital stay	Group2	1.10 (0.75-1.62)	1.11 (0.76-1.64)	1.18 (0.80-1.73)	1.22 (0.83-1.80)
	Group3	1.13 (0.80-1.61)	1.14 (0.81-1.63)	1.14 (0.80-1.62)	0.93 (0.66-1.33)
Adjusted for infant's age at the time of stool collection	Group2	1.09 (0.74-1.60)	1.10 (0.75-1.61)	1.22 (0.83-1.79)	1.06 (0.72-1.57)
	Group3	1.09 (0.77-1.54)	1.10 (0.78-1.55)	1.22 (0.87-1.73)	1.12 (0.80-1.59)
Adjusted for maternal pre-pregnancy weight	Group2	1.10 (0.74-1.63)	1.11 (0.75-1.64)	1.22 (0.82-1.80)	1.24 (0.83-1.83)
	Group3	1.13 (0.79-1.61)	1.14 (0.80-1.62)	1.23 (0.87-1.75)	1.12 (0.79-1.60)

MODEL 1	Group2	1.28 (0.82-2.00)	1.27 (0.82-1.97)	1.43 (0.92-2.20)	1.21 (0.78-1.89)
	Group3	1.14 (0.75-1.73)	1.10 (0.72-1.67)	1.26 (0.83-1.89)	1.16 (0.76-1.76)

MODEL 1: Adjusted for delivery mode by IAP, gestational age, infant diet, parity, ROM > 18 hours, length of hospital stay, stool collection age, maternal pre-pregnancy weight

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes
Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Table 2.27

Crude and adjusted likelihood ratio of gut microbiota richness and diversity measures at 3-4 months according to duration of 2nd stage of labour

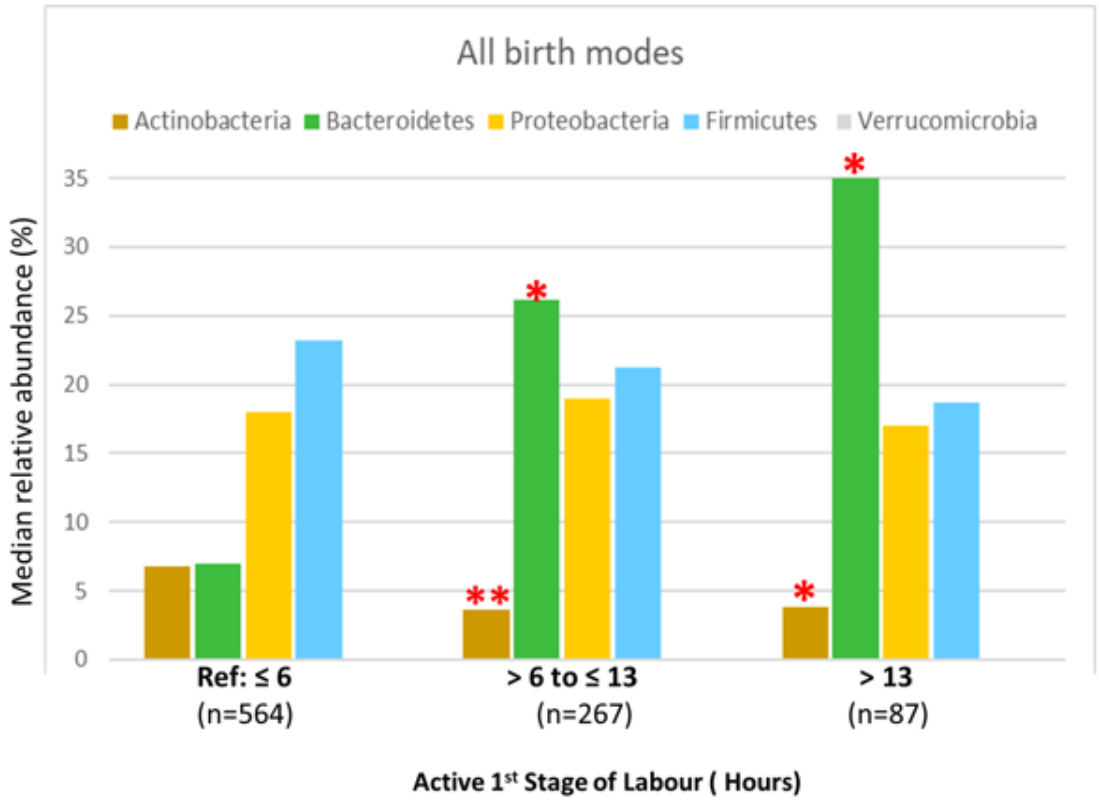
Ref. Group 1: 2nd Stage ≤1Hr Group 2: 2nd Stage > 1 to ≤2 Hrs Group 3: 2nd Stage > 2 Hrs		**Chao1 richness** (below vs above median) OR (95% CI)	**Shannon diversity** (below vs above median) OR (95% CI)
Crude OR for 2nd stage of labour	Group2	0.75 (0.51-1.10)	0.78 (0.54-1.15)
	Group3	0.94 (0.67-1.32)	0.68 (0.48-0.96)*
Adjusted for delivery MODE by IAP	Group2	0.73 (0.49-1.08)	0.81 (0.55-1.20)
	Group3	0.92 (0.65-1.29)	0.69 (0.49-0.97)*
Adjusted for gestational age	Group2	0.72 (0.49-1.06)	0.75 (0.51-1.10)
	Group3	0.91 (0.65-1.29)	0.65 (0.46-0.93)*
Adjusted for infant diet at 3 months	Group2	0.81 (0.54-1.21)	0.83 (0.56-1.23)
	Group3	0.97 (0.68-1.38)	0.67 (0.47-0.96)*
Adjusted for parity	Group2	0.71 (0.48-1.05)	0.79 (0.53-1.17)
	Group3	0.88 (0.61-1.26)	0.69 (0.48-0.99)*
Adjusted for ROM >18 hours	Group2	0.74 (0.50-1.09)	0.80 (0.54-1.18)
	Group3	0.90 (0.63-1.28)	0.66 (0.46-0.95)*
Adjusted for baby's length of hospital stay	Group2	0.71 (0.48-1.05)	0.73 (0.49-1.07)
	Group3	0.93 (0.65-1.32)	0.63 (0.45-0.91)*
Adjusted for infant's age at the time of stool collection	Group2	0.76 (0.52-1.12)	080 (0.54-1.18)
	Group3	0.96 (0.68-1.36)	0.70 (0.49-0.99)*
Adjusted for maternal pre-pregnancy weight	Group2	0.74 (0.50-1.10)	0.78 (0.53-1.15)
	Group3	0.93 (0.66-1.33)	0.66 (0.46-0.94)*
MODEL 1	Group2	0.64 (0.41-1.00)	0.80 (0.51-1.24)
	Group3	0.076 (0.50-1.16)	0.60 (0.39-0.91)* ; p=0.016

MODEL 1: Adjusted for delivery mode by IAP, gestational age, infant diet, parity, ROM > 18 hours, length of hospital stay, stool collection age, maternal pre-pregnancy weight

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes
Delivery mode by IAP categories: Vaginal and no IAP, Vaginal with IAP, Elective C-section and C-section with labour

Figure 2.2

Median relative abundance of dominant bacterial taxa at the **phylum level** in infant gut microbiota at 3-4 months ***among all birth modes*** (vaginal plus C-section), according to the duration of active first stage of labour **(n=918)**



Group 1: Active 1st Stage duration ≤ 6 hour (Reference group)

Group 2: Active 1st Stage duration > 6 to ≤ 13 hours

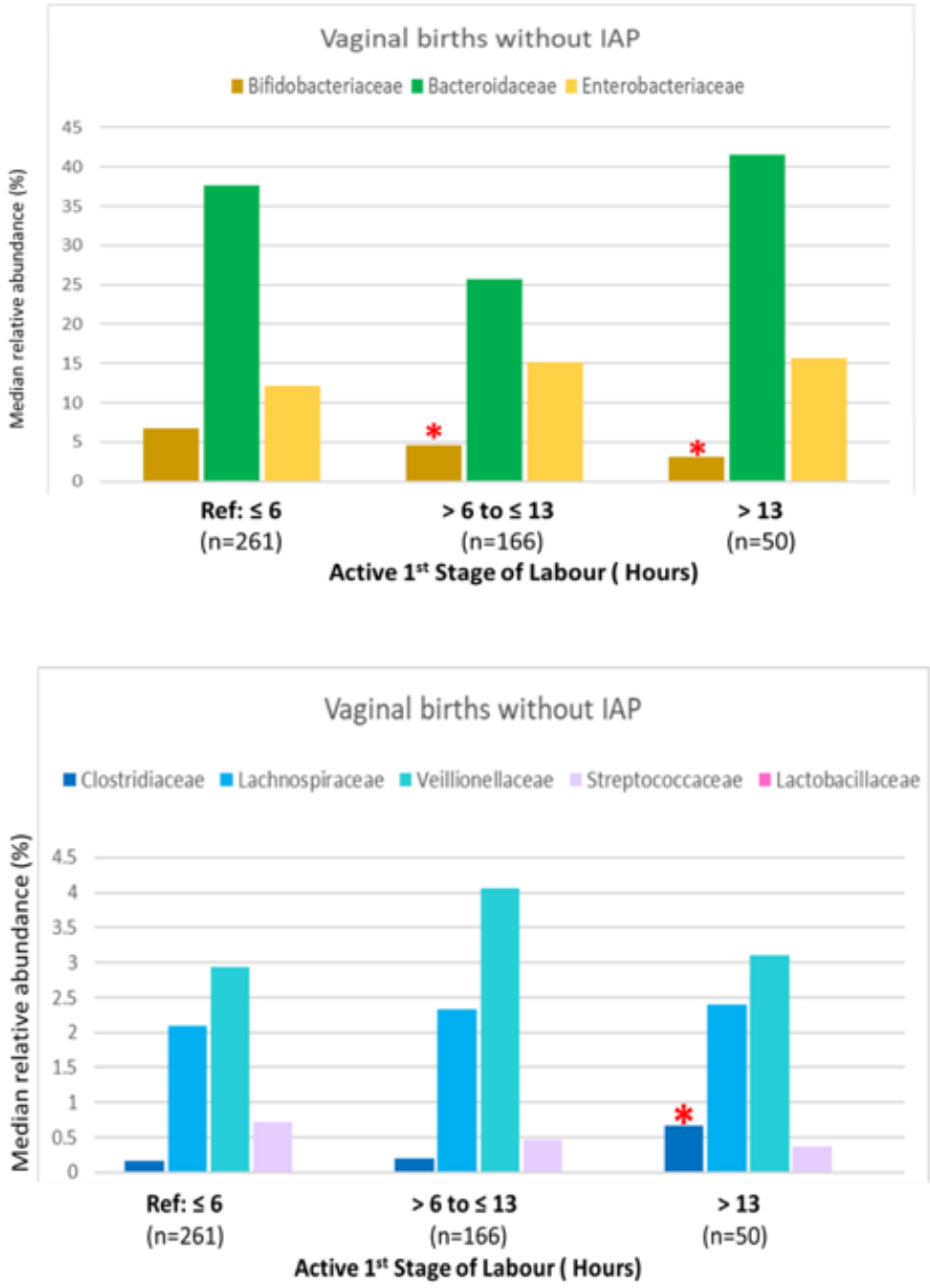
Group 3: Active 1st Stage duration > 13 hours

* indicates p <0.05; ** indicates p<0.005

Graph generated based on comparisons made by Mann-Whitney U test

Figure 2.3

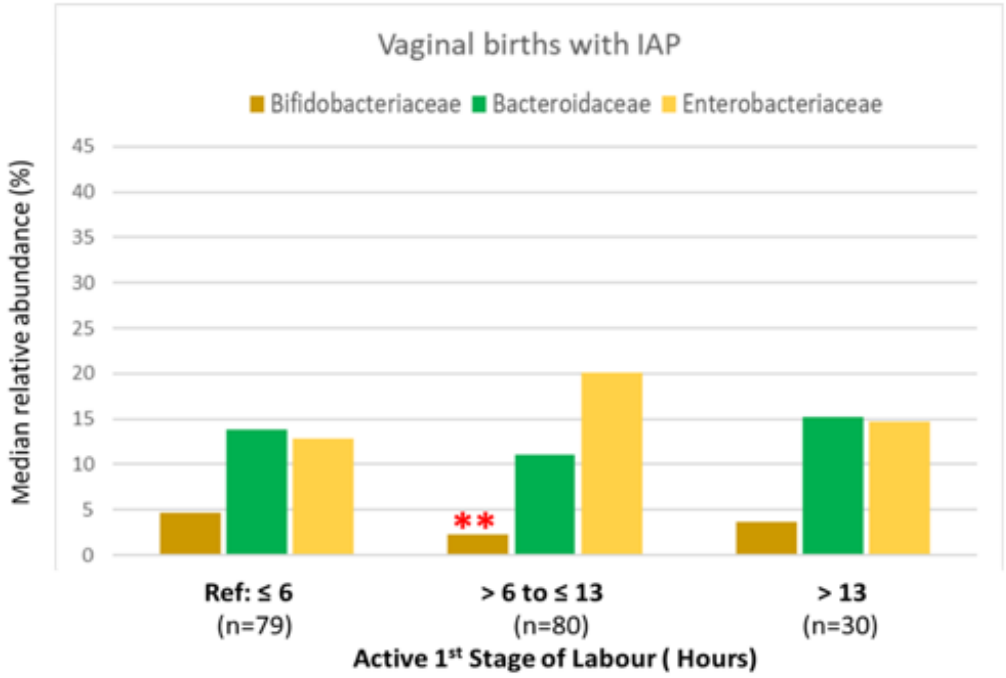
Median relative abundance of dominant bacterial taxa at the **family level** in infant gut microbiota at 3-4 months among **VAGINAL births without intrapartum antibiotic prophylaxis (IAP),** according to the duration of active first stage of labour (n=477)

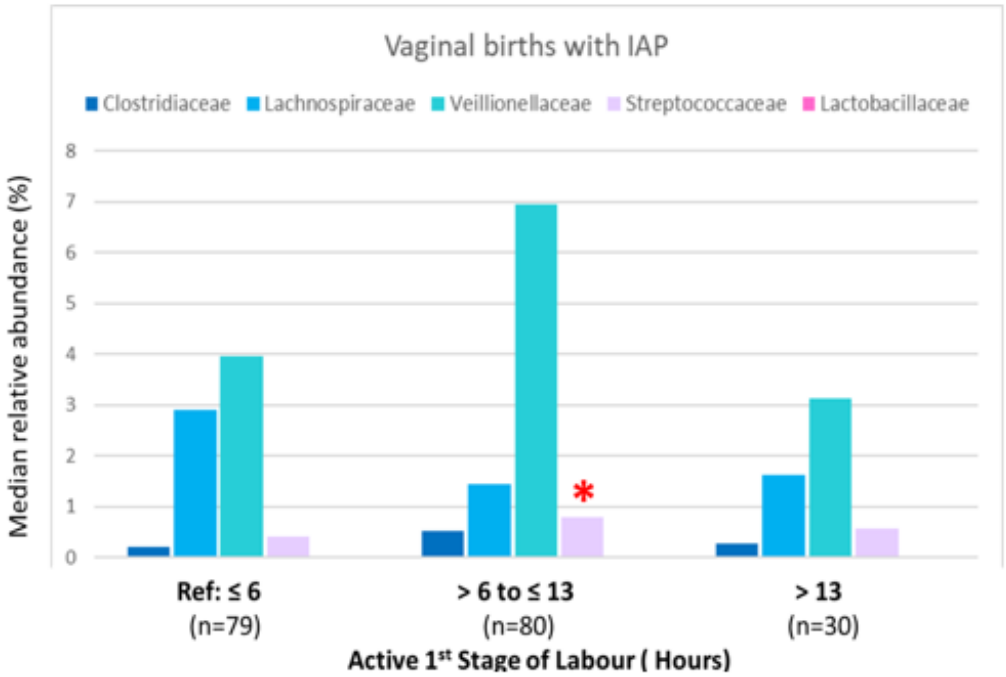


* indicates p <0.05; ** indicates p<0.005; IAP = Intrapartum Antibiotic Prophylaxis

Figure 2.4

Median relative abundance of dominant bacterial taxa at the **family level** in infant gut microbiota at 3-4 months among **VAGINAL births with intrapartum antibiotics prophylaxis (IAP),** according to the duration of active first stage of labour (n=189)

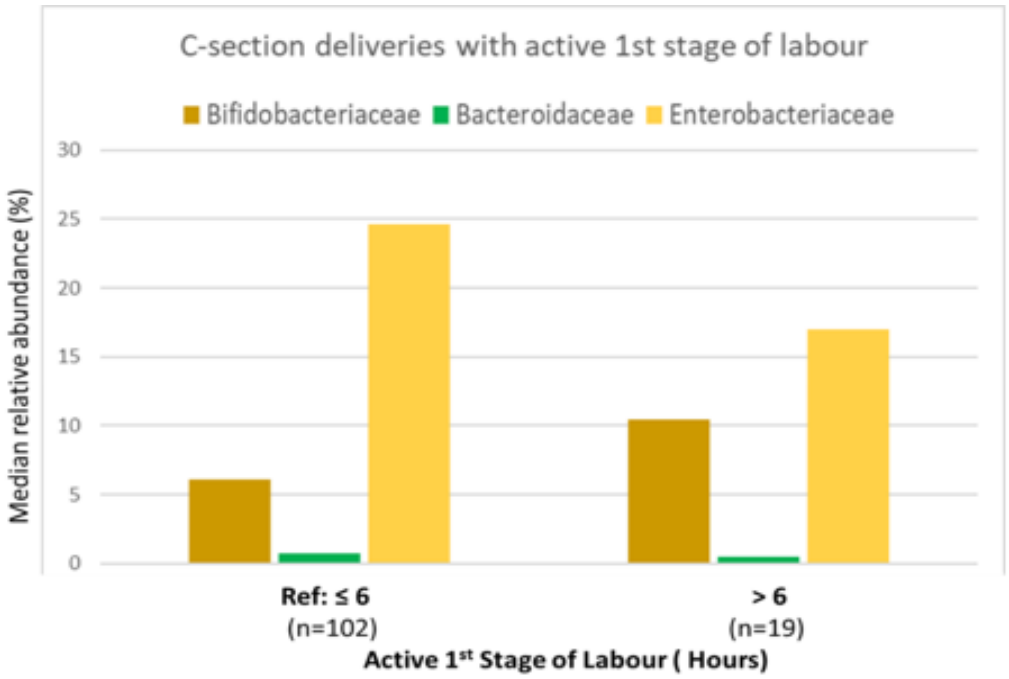




* indicates p <0.05; ** indicates p<0.005; IAP = Intrapartum Antibiotic Prophylaxis

Figure 2.5

Median relative abundance of dominant bacterial taxa at the **family level** in infant gut microbiota at 3-4 months among infants born by **C-section with active 1st stage of labour**, according to the duration of active first stage of labour



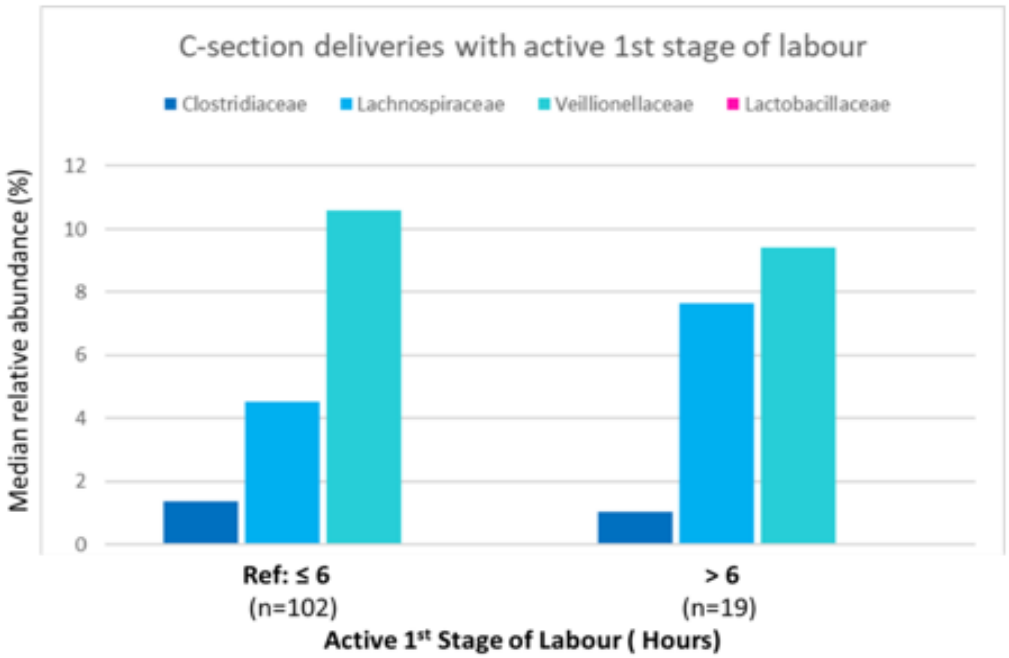
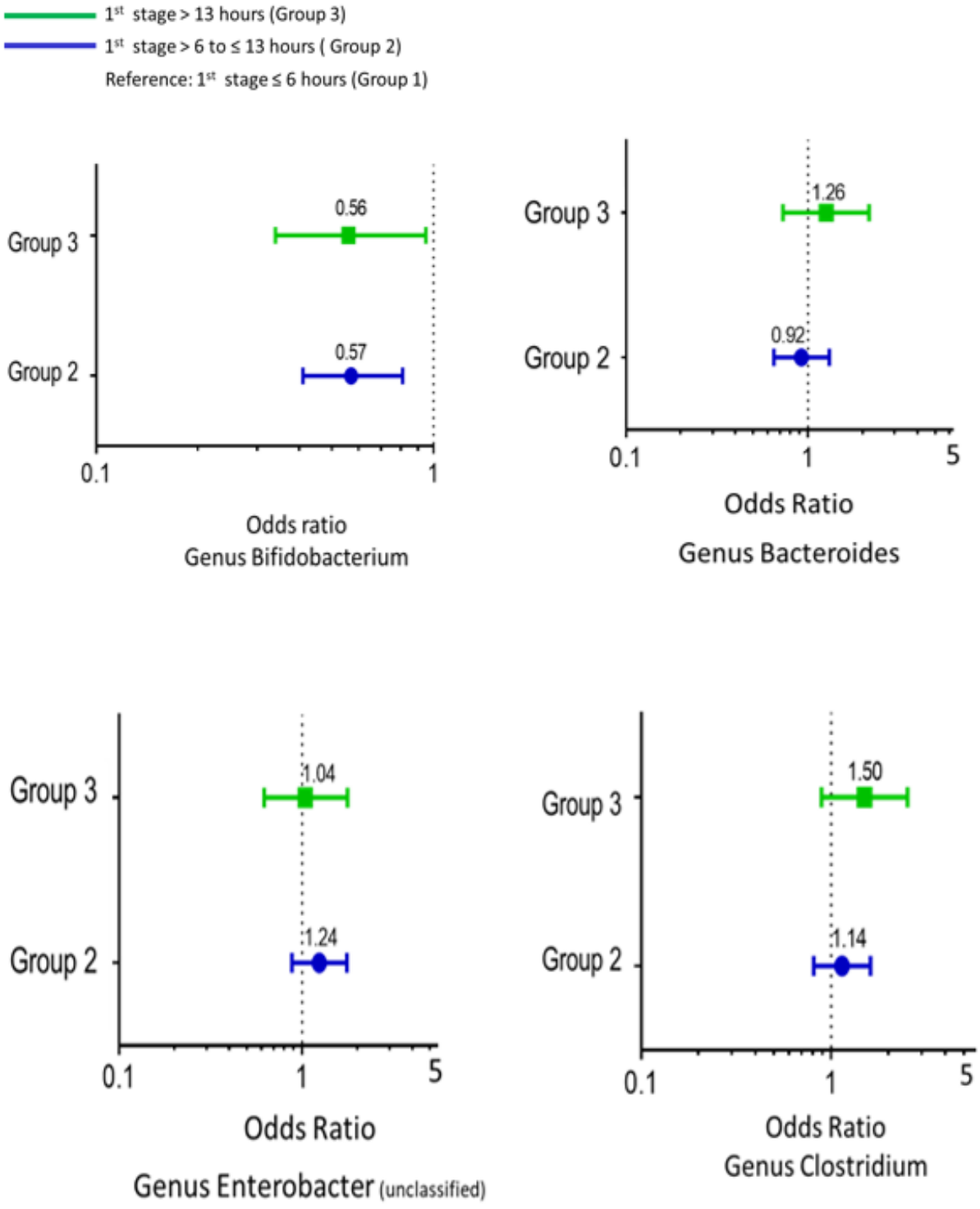
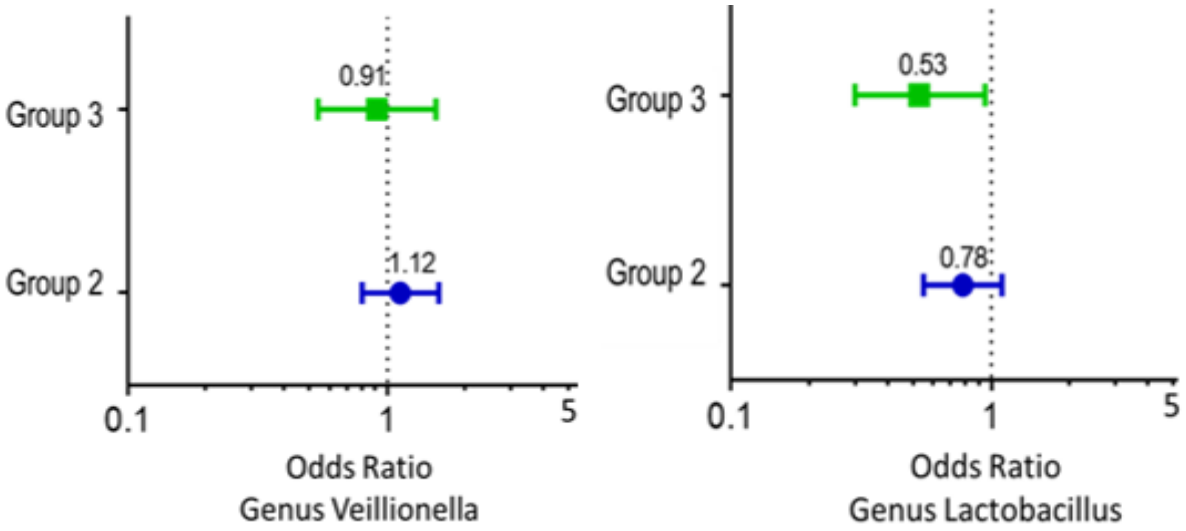


Figure 2.6 *Adjusted likelihood ratio of abundance of key gut microbiota measures at **genus level** (below and above median) at 3-4 months according to **duration of 1st stage of labour**

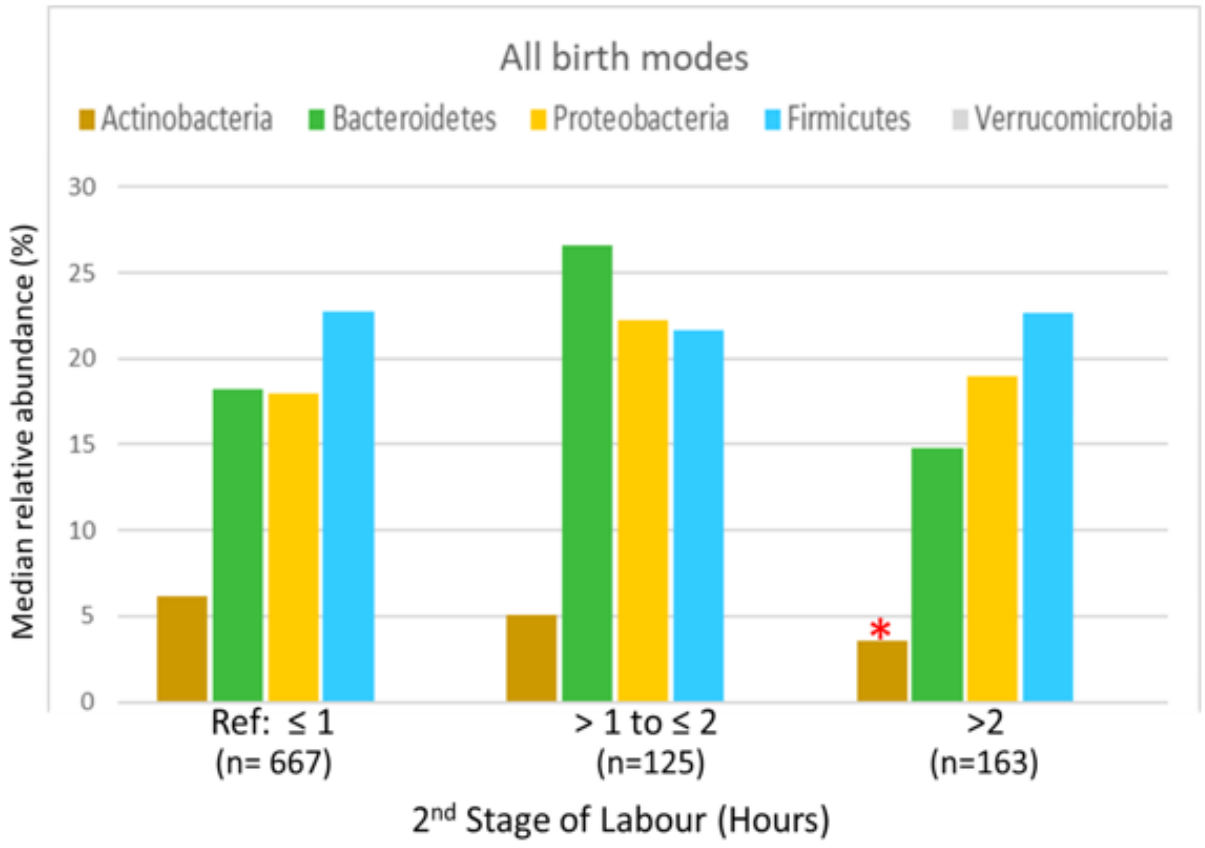




*Odds ratio adjusted for delivery mode by IAP, gestational age, infant diet, parity, membrane rupture >18 hours, length of baby's hospital stay, age at stool collection and maternal pre-pregnancy weight

Figure 2.7

Median relative abundance of dominant bacterial taxa at the **phylum level** in infant gut microbiota at 3-4 months among ***all birth modes*** (vaginal plus C-section), according to the duration of **second stage of labour**



Group 1: 2nd Stage duration ≤ 1 hour (Reference group)

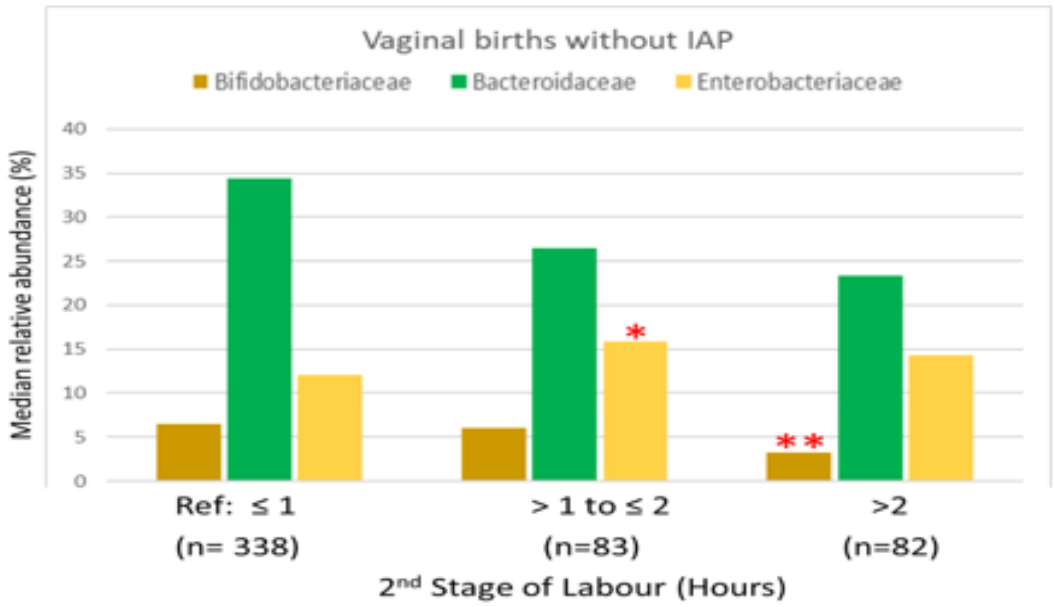
Group 2: 2nd Stage duration >1 to ≤ 2 hours

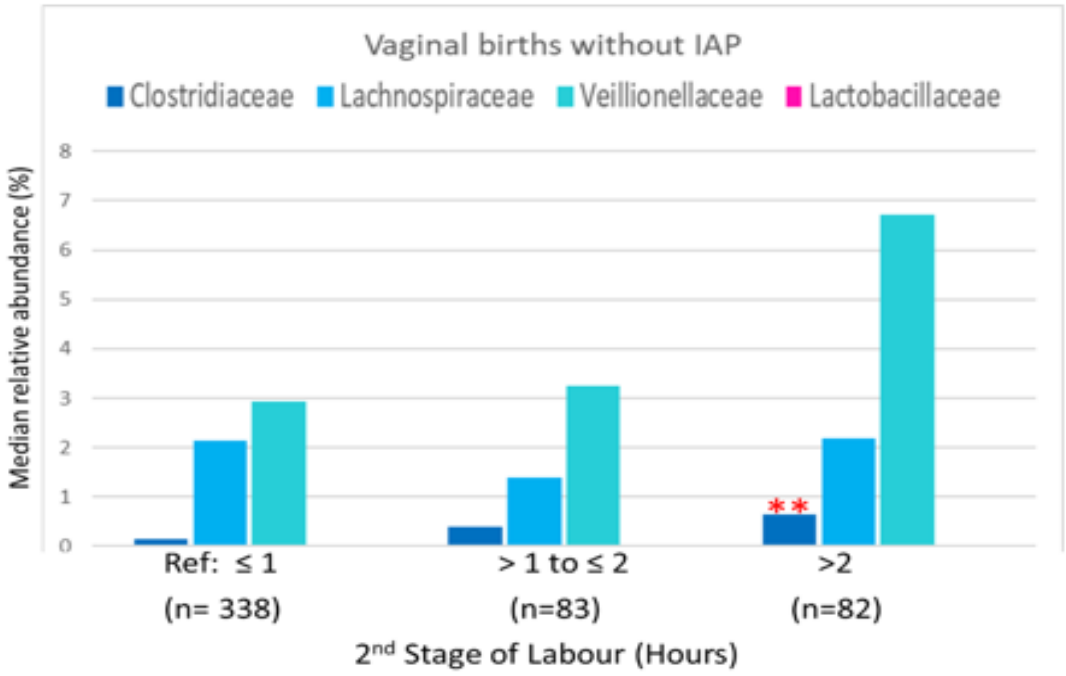
Group 3: 2nd Stage duration >2 hours

** indicates p<0.005; * indicates p<0.05

Graphs based on comparisons made by Mann-Whitney U test

Figure 2.8 Median relative abundance of dominant bacterial taxa at the **family level** in infant gut microbiota at 3-4 months among **VAGINAL births without intrapartum antibiotic prophylaxis (IAP),** according to duration of second stage of labour (n= 503)



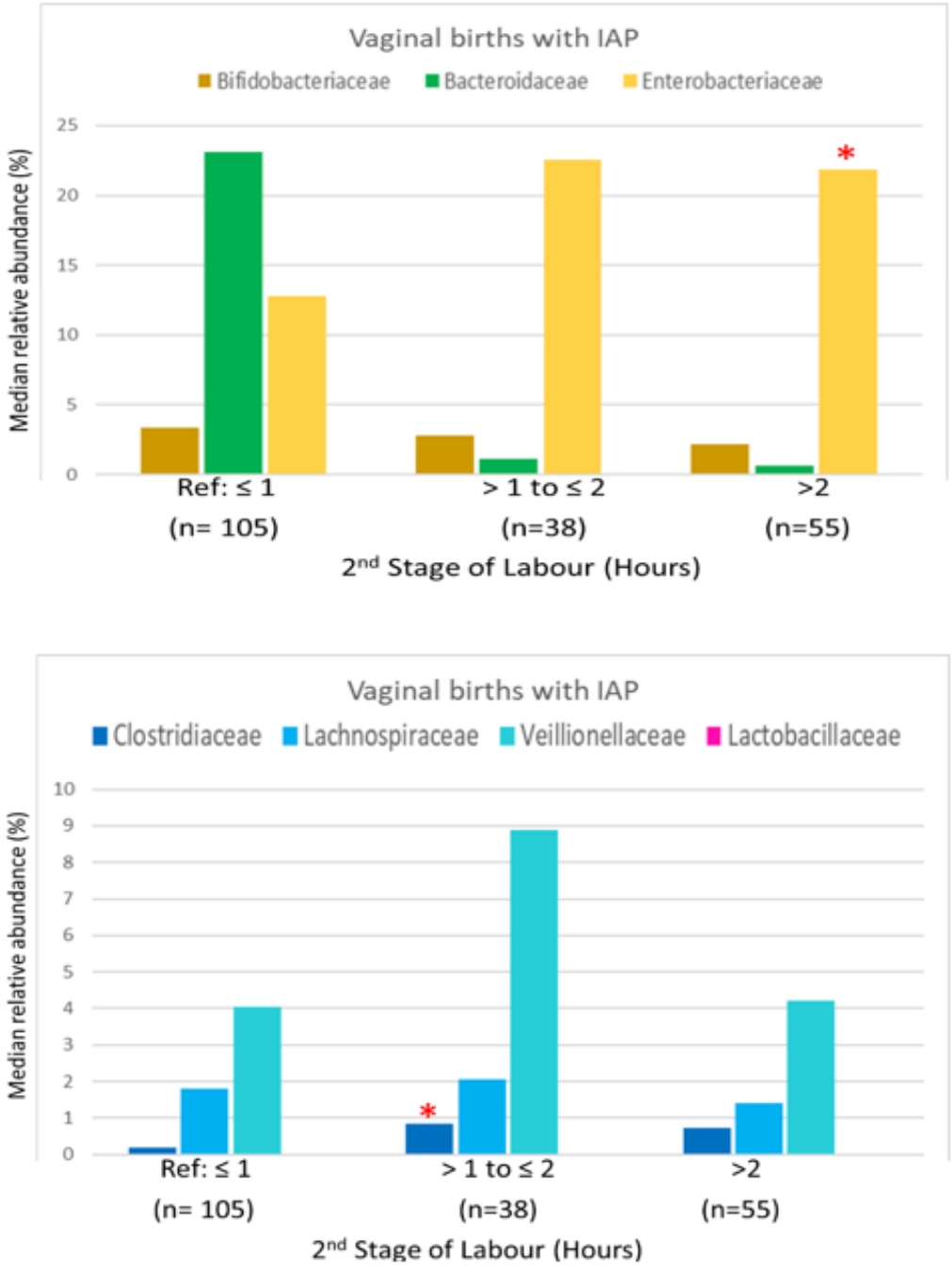


** indicates p<0.005; * indicates p<0.05

IAP = Intrapartum Antibiotic Prophylaxis

Figure 2. 9

Median relative abundance of dominant bacterial taxa at the **family level** in infant gut microbiota at 3-4 months among **VAGINAL births with intrapartum antibiotics prophylaxis (IAP),** according to the duration of second stage of labour (n= 198)

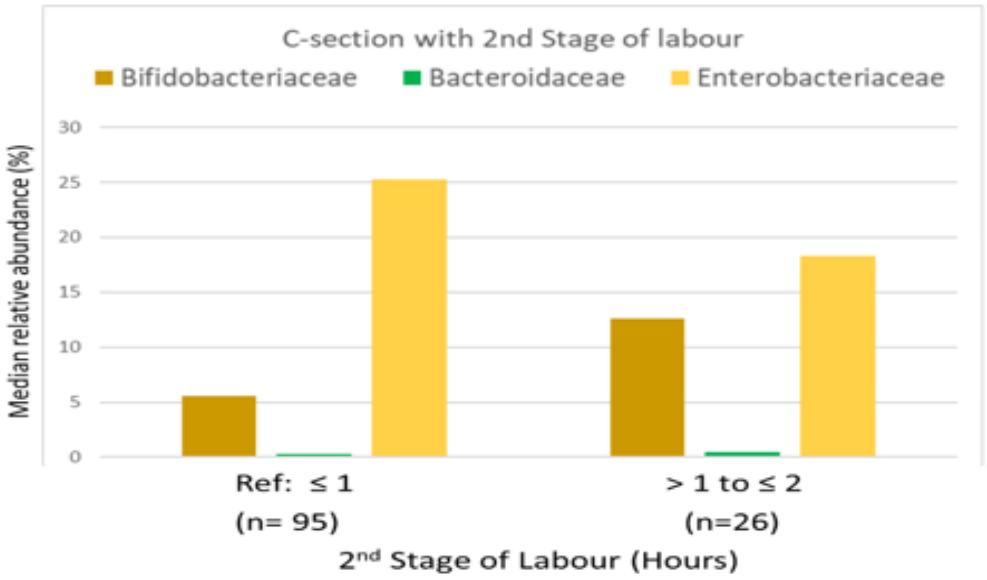


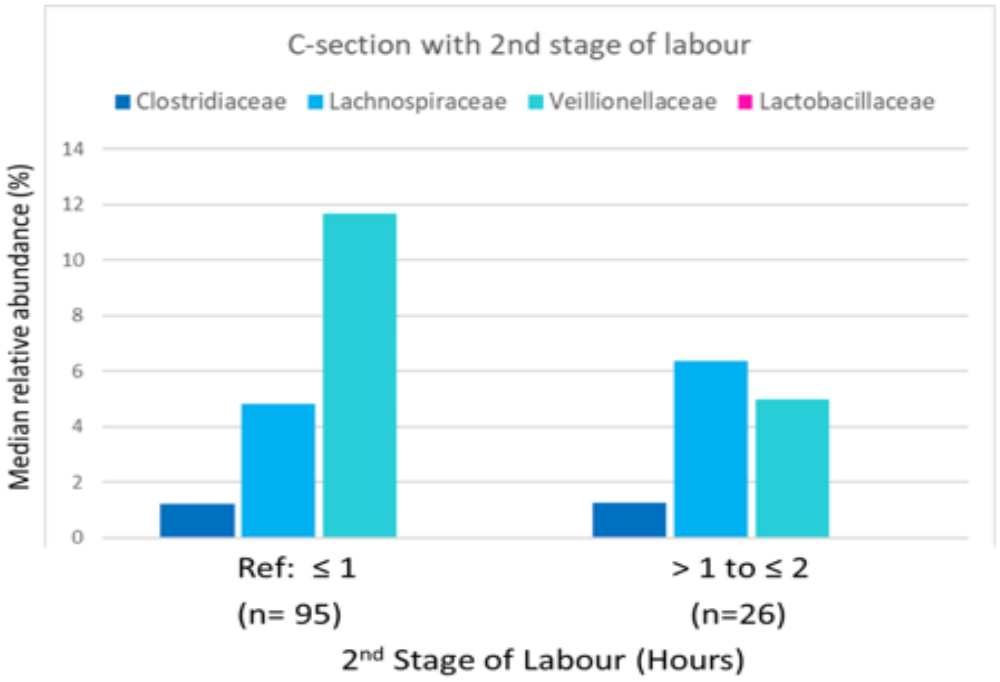
** indicates p<0.005; * indicates p<0.05

IAP = Intrapartum Antibiotic Prophylaxis

Figure 2.10

Median relative abundance of dominant bacterial taxa at the **family level** in infant gut microbiota at 3-4 months among infants born by **C-section with labour,** according to the duration of second stage of labour **(n =121)**

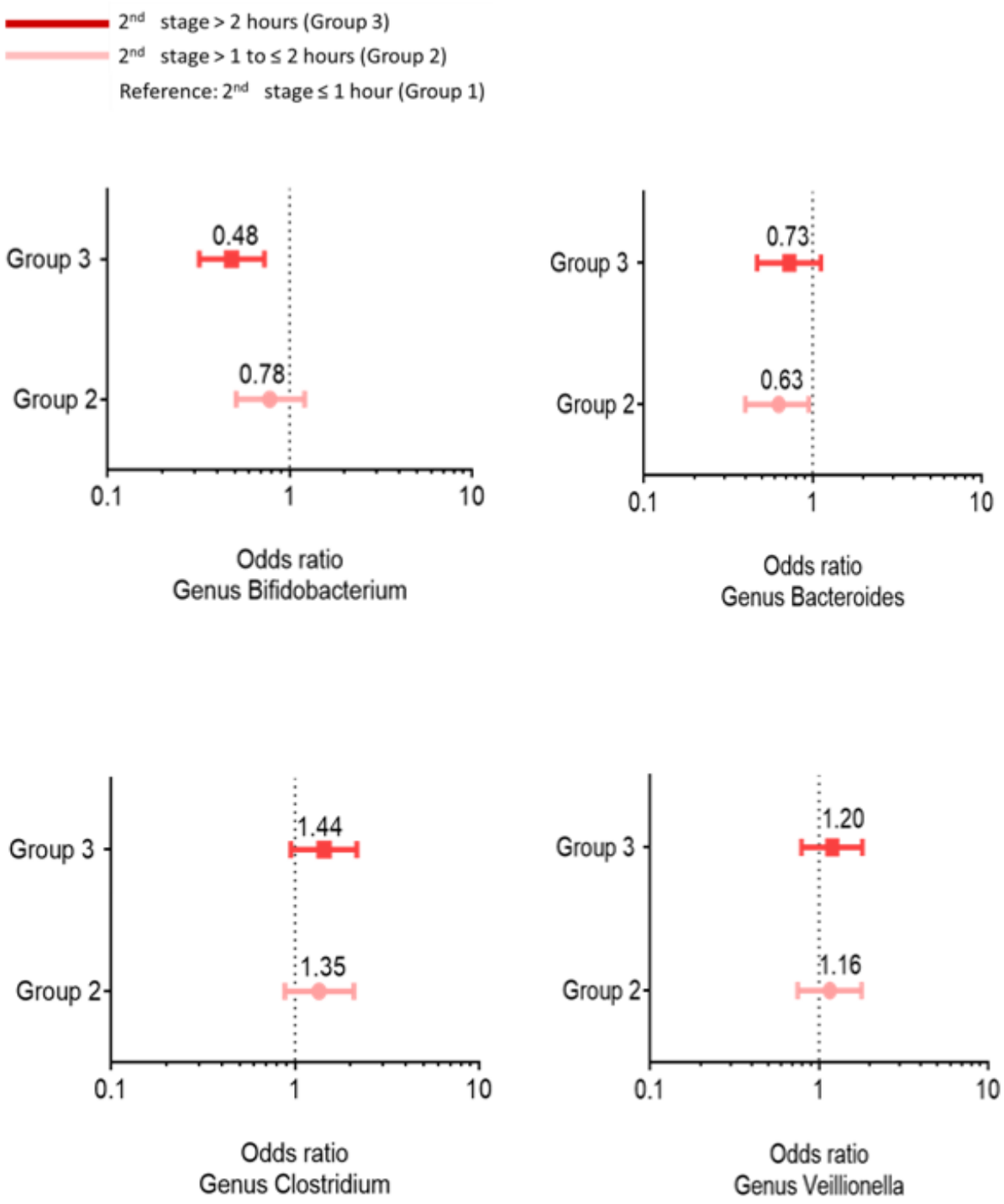


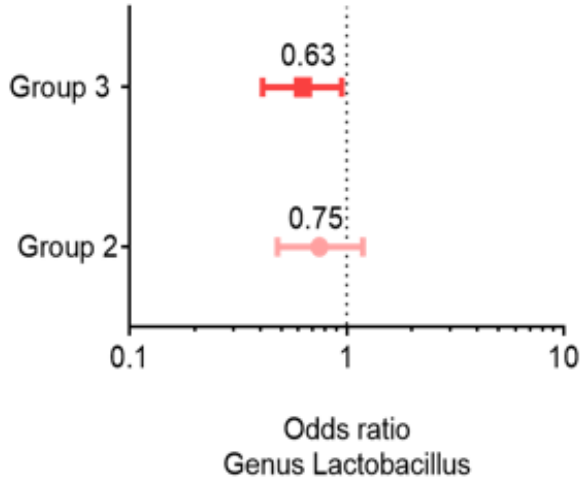


** indicates p<0.005; * indicates p<0.05; IAP = Intrapartum Antibiotic Prophylaxis

Figure 2.11

*Adjusted likelihood ratio of abundance of key gut microbiota measures at **genus level** (below and above median) at 3-4 months according to duration of 2nd stage of labour





*Odds ratios adjusted for delivery mode by IAP, gestational age, infant diet, parity, membrane rupture >18 hours, length of baby's hospital stay, age at stool collection and maternal pre-pregnancy weight

CHAPTER 3

Labour Duration and Maternal Pre-Pregnancy Weight: Influence on Infant Gut Microbiota Composition at 3-4 months of age

3.1 Background

Pediatric obesity is a global epidemic, and Canadian children are among the most affected. Despite its decrease in prevalence from 30.7% to 27.0% between 2004 and 2013 (1), childhood obesity remains a serious public health challenge in Canada. To add to the alarming burden, increasing number of children are at risk of overweight at an earlier age. Biro et al found that 6.7% of Canadian toddlers aged less than 2 years are already overweight or obese, and one in four (18.0%) of toddlers are at risk of overweight (2). Obese children are not only at risk of growing up into obese adolescents and obese adults, but also have higher risk of metabolic syndrome and Type 2 Diabetes Mellitus, and higher morbidity and mortality (3). Thus, many research efforts have focused on delineating the roots of childhood obesity.

Childhood obesity has multi-factorial etiology. While increased caloric intake and sedentary life-style are obvious causes, genetics and perinatal influences also play important roles in development of childhood obesity (4). Interestingly, increased maternal weight gain prior to conception and increased gestational weight gain can also influence the progeny's weight gain. Both maternal pre-pregnancy overweight (5) (6), and excessive gestational weight gain (7) (8) are potent predictors of weight gain in the offspring, and recent evidence suggests that study of gut microbiome might elucidate the unique connection between maternal overweight and offspring adiposity.

The gut microbiota help maintain energy balance by breaking down the short-chain fatty acids of dietary fiber and facilitating host energy extraction. The role of gut microbiota in energy homeostasis has been demonstrated by several studies (9) (10) (11) (12). Imbalances in the gut microbial composition i.e. gut dysbiosis, has been linked to aberrant energy storage and adiposity in murine and adult human studies. Turnbaugh et al compared the gut microbiota of lean mice and

mice with diet-induced obesity, and observed higher abundance of Firmicutes in the latter group (10). Upon investigation of obese human subjects, perturbations of two dominant gastrointestinal phyla, Bacteroidetes and Firmicutes, has been found to be obesogenic. Some studies have observed a dysbiotic adult gut microbial profile comprising of higher abundance of Firmicutes and lower abundance of Bacteroidetes is associated with increased adiposity (13) (14) (15) whereas other studies have documented lower abundance of gut Firmicutes and higher abundance of Bacteroidetes (16) (17). Recent evidence suggest that pediatric obesity is also associated with imbalances in gut microbiota. Riva et al observed alterations in the gut microbiota in obese children characterized by divergent colonization of Firmicutes and Bacteroidetes compared to normal weight counterparts (18). Besides, based on limited evidence available for infant studies, obese-host phenotype in children may be influenced by more complex disruption in the gut microbial composition than simple alterations of just the Bacteroidetes and Firmicutes phyla. In a study of age-matched children from a prospective follow-up cohort, Kalliomäki et al found lower abundance of *Bifidobacteria* (of phylum Actinobacteria) and higher abundance of *Staphylococcus* (of phylum Firmicutes) in fecal samples during infancy was associated with higher body mass index (BMI) at a later age (19). Another study also observed that infants with reduced gut *Bifidobacteria* counts at age of 3 months went on to develop higher BMI at age 10 years (20). Compiling evidence from available studies, Kozyrskyj et al observed that a higher *Lactobacillus* yet a lower *Bacteroides* spp. colonization in infants aged less than 3 months may predict risk for childhood overweight, with male infants being disproportionately affected (21). In addition, concentrations of the main metabolites produced by gut bacteria, i.e. short chain fatty acids (SCFAs) such as acetate, propionate and butyrate were found to be significantly increased in fecal samples of obese children compared to normal-weight controls (18), suggesting that elevated substrate utilization and energy harvesting capacity facilitated by the dysbiotic gut microbiota may be the mechanism for increased adiposity in obese children. Interestingly, overweight or obesity associated compositional changes in the gut microbiota are also evident in expectant women with elevated BMI.

There is inter-individual variation in the composition of gut microbes in humans, and pregnancy promotes changes in the maternal gut microbiota. While the composition of gut microbiota in the first trimester are comparable to the healthy non-pregnant counterparts, expectant women in third trimester show substantial inter-individual variation in gut microbial diversity,

along with an increased abundance of phyla Proteobacteria and Actinobacteria, and reduced microbial richness (22). To add, the changes in maternal gut microbiota are also influenced by pre-pregnancy maternal body weight and weight gain during pregnancy. Compared to women with normal BMI, Collado et al found distinct composition of gut microbiota in overweight pregnant women characterized by significantly higher presence of *Clostridium* and *Staphylococcus* (both of phylum Firmicutes) and *Bacteroides* (of phylum Bacteroidetes) (23). In another study, Santacruz et al found increased numbers of *Staphylococcus* (of phylum Firmicutes), *Enterobacteriaceae* and *E. coli* (both of phylum Proteobacteria) but reduced numbers of *Bacteroides* and *Bifidobacterium* in overweight pregnant women as compared to the normal weight controls (24). In a more recent study, Stanislawski et al observed that maternal overweight or obesity is associated with lower maternal alpha diversity (gut microbial diversity), and distinct differences in the family *Christensenellaceae*, the genera *Lachnospira, Parabacteroides, Bifidobacterium*, and *Blautia* as compared to normal weight women (25). These studies suggest the presence of distinctly atypical gut microbiota in pregnant women with increased BMI.

Interestingly, maternal overweight during pregnancy can influence the composition of gut microbes in the offspring. Mueller et al compared the fecal microbiota of neonates based on maternal pre-pregnancy body mass index. Compared to neonates delivered vaginally to normal weight mothers, neonates born to overweight or obese mothers had a distinct gut microbial composition, enriched in *Bacteroides* and depleted in *Enterococcus, Acinetobacter, Pseudomonas*, and *Hydrogenophilus* (26). Since maternal microbiome is the first source of gut microbiota in the neonates, atypical maternal fecal microbiota could be implicated divergent microbial seeding for the newborn gut. In a prospective follow-up study, Collado et al examined the infant gut microbiota at 1 and 6 months of age based on maternal pre-pregnancy BMI and gestational weight gain. Compared to infants of normal weight mothers, infants of overweight mothers showed higher abundance of fecal *Bacteroides*, Clostridium and *Staphylococcus*, and lower concentrations of the *Bifidobacterium* group (27). In contrast, Stanislawski et al observed that although the specific gut microbial profile observed in overweight and obese parturients significantly increased the presence of those specific OTUs in their neonates at age of 4-10 days, these changes did not impact the overall differences in the infant gut microbiota over the first 2 years of life (25). These findings suggest that changes in early infant gut microbial composition may be influenced by increased maternal pre-pregnancy weight.

Overweight and obese parturients are at higher risk of labour complications such as dysfunctional labour, abnormally slow progression of labour (28) (29) (30) (31). Based on currently available evidence, altered labour progression associated with maternal obesity is hypothesized to occur due to altered metabolic regulation of uterine contractility associated with hyperlipidemia and leptin resistance. Hypercholesterolemia in obese women, secondary to increased insulin resistance and increased lipolysis, has been proposed to alter the effectiveness of myometrial contractions by affecting intracellular [Ca^{2+}] flux (32). In addition, obesity-associated leptin resistance and increased levels of circulating leptin may also contribute to protracted labour in obese women since leptin inhibits the onset of spontaneous and oxytocin induced myometrial activity (33). The *active* phase of first stage of labour, i.e. cervical dilation from 4 to 10 cm, is prolonged in parturients with elevated BMI. Kominiarek et al investigated 118,978 gravidas with a singleton term pregnancy and found that increasing maternal BMI was associated with increasing duration of active first stage of labour in both nulliparas and multiparas (28). Similarly, Carlhäll et al studied 63,829 nulliparous women with a singleton pregnancy and spontaneous onset of labour comparing overweight and obese women, and found that the risk of labour lasting more than 12 hours increased with increasing BMI (31). Once the second stage was reached, duration of labour shortened in obese women as compared to normal weight women (31). Since the fetus's first major contact to maternal vaginal and fecal microbes is during its passage through vaginal canal, it is arguable that the prolonged labour duration may affect the degree of gut microbial seeding in the offspring of overweight or obese mothers. Further, slow abnormal progression of labour with protracted first stage, often culminating into unplanned C-section is common obstetric course for many overweight nulliparas. Hillard et al compared pregnant women with based on their BMI [normal ($\leq$24), overweight (25 to 29.9), or obese ($\geq$30 kg/m^2)], and found different Cesarean delivery rates among the three groups (p=0.0001), with highest CS rate in the obese category (29). In a systematic review and meta-analysis of 11 papers, Poobalan et al found that the risk of unplanned CS delivery is increased by 50% in overweight women [pooled OR =1.64, 95% CI = 1.55-1.73] , and is more than double for obese women [pooled OR = 2.23, 95%CI=2.07-2.42], as compared to women with normal BMI (34). C-section born infants possess gut dysbiosis (35) (36), and this may add additional concern for infants of overweight and obese mothers born after prolonged labour.

Balanced development of the gut microbiota in infant is essential for its future health. Evidence links infant gut dysbiosis to higher risk of childhood obesity (19). Increased pre-pregnancy overweight or obesity, which have been associated separately with maternal gut dysbiosis (22) (23) (25), infant gut dysbiosis (26) (27), and increased weight gain in their offspring (5) (6) (8), are also risk factors for slow, dysfunctional labour (28) (29) (31) and other labour complications. Since mode of birth is a strong determinant for microbial seeding of the newborn gut and balanced development of the infant gut microbiota, the prolonged labour duration and higher propensity for emergency CS in overweight/obese mothers (who have atypical gut microbiota) might alter the gut microbial seeding and gut microbiota development in their infants. At present, no study has investigated the role of labour duration in overweight/ obese parturients on infant gut microbial composition of their infants.

Our study aims to investigate how labour duration is associated with infant gut microbiota composition at 3-4 months of age in infants born to mothers of different pre-pregnancy BMI categories.

3.2 Materials and Methods

3.2.1 Study design

This study involved a subsample of 999 infants from three study sites (Edmonton, Vancouver and Winnipeg) of the CHILD cohort (www.childstudy.ca) whose mothers were enrolled during pregnancy between 2009 and 2012. Information on labour duration and birth characteristics, mode of delivery and some covariates were obtained for hospital charts. Complete information on a*ctive* first stage of labour (cervical dilation from 4 cm to 10 cm) was obtained for 884 mothers. Complete information on second stage of labour (fully dilated cervix to expulsion of the fetus) was obtained for 921 mothers.

For vaginally delivered infants, a labour length variable denoting three mutually exclusive categories was created for active first stage with following cut-offs. These cut-offs were based on a recent metanalysis on duration of active first stage of labour conducted by Neal et al (37):

(1) Duration of active 1st stage of labour ≤ 6 hours [Reference category: Group 1]

(2) Duration of active 1st stage of labour > 6 to ≤ 13 hours [Group 2]

(3) Duration of active 1st stage of labour > 13 hours [Group 3].

For second stage of labour, a labour length variable denoting three mutually exclusive categories was created (38) as follows:

(1) Duration of 2nd stage of labour ≤ 1 hours (Reference category: Group 1]

(2) Duration of 2nd stage of labour > 1 to ≤ 2 hours [Group 2]

(3) Duration of 2nd stage of labour > 2 hours [Group3].

For infants delivered by C-section after onset of labour, a labour length variable for active 1st stage of labour denoting two mutually exclusive categories was created as follows:

(1) C-section with duration of active 1st stage of labour ≤ 6 hours [Reference category: Group 1]

(2) C-section with duration of active 1st stage of labour > 6 hours [Group 2].

For 2nd stage of labour for C-section births, the categories were:

(1) C-section with duration of 2nd stage of labour ≤ 1 hour [Reference category: Group 1] and

(2) C-section with duration of 2nd stage of labour > 1 hour [Group 2].

'Elective C-section births' and 'Emergency C-section without labour' were excluded from the analyses.

Information on maternal pre-pregnancy weight was derived from maternal body mass index (BMI) obtained from hospital records, and three maternal pre-pregnancy weight categories were identified:

1) Pre-pregnancy BMI < 25 = Normal weight pregnant women

2) Pre-pregnancy BMI ≥ 25 to < 30 = Overweight pregnant women

3) Pre-pregnancy BMI ≥ 30 = Obese pregnant women

Data on covariates that could potentially impact either the exposure or outcome, or both, were obtained from hospital records (mode of delivery, intrapartum antibiotic prophylaxis (IAP), parity, duration after rupture of membranes, infant gender, length of infant's hospital stay etc.) or from standardized questionnaires completed by mothers (breastfeeding, maternal ethnicity, maternal smoking, maternal asthma), and were considered in the study. Written informed consent was obtained from parents at enrollment. This study was approved by the ethics board at the University of Alberta.

3.2.2 Fecal sample collection, DNA extraction and PCR amplification

Faecal samples of infants were collected at 3-4 months of age using a standard protocol during a scheduled home visit. Samples were refrigerated immediately after collection and during transport, and stored at -80 °C until analysis. Genomic DNA was isolated with QIAamp DNA stool Mini Kit (Qiagen, Venlo, the Netherlands), and the hypervariable V4 region of the bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal bacterial primers. For sample multiplexing, reverse primers were barcoded uniquely for each sample (barcoded sequence was denoted in the primer sequence by Xs). PCR amplification consisted of an initial denaturation step for 3 min at 94 °C, followed by 20 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 50 °C and an extension step for 30 s at 72 °C. PCR reactions for each sample were performed in triplicate with a negative control in each run. One hundred nanograms of pooled PCR product from each sample was concentrated using an Amicon Ultra-4 30K centrifugal filter.

3.2.3 Sequencing and taxonomic nomenclature

Pooled PCR amplicons were sequenced using the MiSeq Illumina Sequencing at the University of Toronto Centre for the Analysis of Genome Evolution & Function (CAGEF). Using a QIIME pipeline, forward and reverse reads were assembled for a final length of 144 bp demultiplexed and filtered against the GREENGENES reference database (v13.8) to discard all

sequences with <60% similarity. Remaining sequences were clustered at 97% sequence similarity against the GREENGENES database (using closed picking algorithm in QIIME), and taxonomic assignment was achieved using the RDP classifier. After taxonomic assignment, operational taxonomic units (OTUs) representing bacterial origin were selected, and bacterial OTUs with overall relative abundance below 0.0001 were excluded from subsequence for downstream analyses. Microbiota diversity within samples (α diversity) was calculated using two standard metrics: the Chao1 estimator of OTU richness (which estimates the number of different OTUs present) and the Shannon diversity index (which evaluates both the number of OTUs and the evenness of their distribution). Those metrics were calculated at OTU and family levels.

3.2.4 Statistical analyses

Statistical analyses were performed in SPPS version 22.0 (SPSS, Inc., Chicago, IL, USA). Chi-square test was used to examine the distribution of potential confounders according to exposure to differential duration of labour. Within the three maternal pre-pregnancy categories, i.e. normal weight mothers, overweight mothers and obese mothers, gut microbial profile of infants with duration of active first stage of labour ≤ 6 hours (reference group) was compared to the gut microbiota profile of infants with first stage of labour > 6 to ≤ 13 hours and > 13 hours. Similarly, the gut microbial profile of infants with duration of second stage of labour ≤ 1 hours (reference group) was compared to the gut microbiota profile of infants with first stage of labour > 1 to <= 2 hours and > 2 hours. Median relative abundance, richness and diversity of dominant taxa were compared based on duration of active 1st stage and 2nd stage in each maternal BMI group. Non-parametric Mann-Whitney U-test was used for comparing the microbial abundance. A p-value of <0.05 was defined as statistically significant, and 95% confidence intervals (CIs) were calculated.

Univariate analysis and multivariate logistic regression were used to identify variables independently associated with the outcome variables. Variables with a p-value of <0.25 in univariate analyses and clinically significant covariates were included in multivariable analyses. Microbiota measures were classified in two groups (below vs. above median). The following variables were included in the multivariable models as potential confounders: mode of delivery,

maternal intrapartum antibiotic exposure, infant diet, parity and duration after rupture of membrane.

3.3 Results

3.3.1 Study population

In this study population, 921 infants had complete information on maternal pre-pregnancy body mass index (BMI). Of these, 556 infants (60.4%) were born to mothers with normal pre-pregnancy weight (BMI <25), 208 (22.6%) were born to mothers with pre-pregnancy overweight (BMI ≥25 to <30), and 157 (17.0%) infants were born to obese mothers (BMI ≥30).

In all three maternal BMI categories, majority of the women were Caucasians (73.4% in normal weight, 77.1% in overweight and 83.6% in obese category respectively), followed by those of Asian ethnicity (14.5% in normal weight, 10.4% in overweight and 2.0% in obese category respectively) and the rest belonged to other racial profiles.

Among the infants born to **normal weight mothers**, the mean duration of active 1st stage was 353.9 minutes (SD=364.4 minutes). 531 infants had complete information on active first stage of labour, were found to be distributed as follows [Fig. 3.1]:

322 (60.6%) were born after active 1st stage duration ≤6 hours [Reference group = Group 1 infants],
156 (29.4%) were born after active 1st stage duration > 6 to ≤ 13 hours [Group 2 infants], and
53 (10.0%) of infants were born after active 1st stage duration >13 hours [Group 3 infants].

Table 3.1a shows the demographic characteristics of the studied infants with differential duration of active 1st stage of labour (n=531) exposure status. There were significant differences between the three groups with respect to mode of delivery by intrapartum antibiotic prophylaxis (p<0.001), term gestation (p=0.031) and parity (p<0.001). No significant differences were detected

in other co-variates of interest such as infant gender (p=0.999), infant diet (p=0.972), maternal ethnicity (p=0.891) etc.

For the second stage, infants (n=556) of normal weight mothers were distributed as follows:

379 (68.2%) were born after 2nd stage duration ≤ 1 hour [Group 1 infants = Reference group],
75 (13.5%) were born after 2nd stage duration greater than 1 hour and ≤ 2 hours [Group 2 infants],
102 (18.3%) of infants were born after 2nd stage duration greater than 2 hours [Group 3 infants].

Table 3.1b shows demographic characteristics of the studied infants with differential duration of second stage of labour (n=556) exposure status. There were significant differences between the three groups with respect to mode of delivery by intrapartum antibiotic prophylaxis status (p<0.001), parity (p<0.001) and duration after rupture of membranes (p< 0.001). No significant differences were detected in other co-variates of interest such as infant gender (p=0.685), infant diet (p=0.983), maternal ethnicity (p=0.955) etc.

Among the infants born to **overweight mothers**, the mean duration of active 1st stage was 338.4 minutes (SD=343.6 minutes). 201 infants had complete information on active first stage of labour, were distributed as follows [Fig. 3.2]:
120 (59.7%) were born after active 1st stage duration ≤6 hours [Group 1 infants = Reference group],
62 (30.8%) were born after active 1st stage duration > 6 to ≤ 13 hours [Group 2 infants], and
19 (9.5%) of infants were born after active 1st stage duration >13 hours [Group 3 infants].

Table 3.2a shows the demographic characteristics of the studied infants with differential duration of active 1st stage of labour (n=201) exposure status. There were significant differences between the three groups with respect to mode of delivery by intrapartum antibiotic prophylaxis (p<0.001), term gestation (p=0.031) and infant gender (p=0.019). No significant differences were detected in other co-variates of interest such as parity (p=0.114, infant diet (p=0.853), maternal ethnicity (p=0.900) etc.

For the second stage, infants (n=208) of overweight mothers were distributed as follows:

150 (72.1%) were born after 2nd stage duration ≤ 1 hour [Group 1 infants = Reference group],
26 (12.5%) were born after 2nd stage duration greater than 1 hour and ≤ 2 hours [Group 2 infants],
32 (15.4%) of infants were born after 2nd stage duration greater than 2 hours [Group 3 infants].

Table 3.2b shows demographic characteristics of the studied infants with differential duration of second stage of labour (n=208) exposure status. There were significant differences between the three groups with respect to mode of delivery by intrapartum antibiotic prophylaxis ($p<0.004$), parity ($p=0.001$) and infant gender ($p=0.018$) whereas other covariates of interest did not show significant differences based on duration of second stage categories.

Among the infants born to **obese mothers**, the mean duration of active 1st stage was 317.6 minutes (SD=355.0 minutes). 152 infants had complete information on active first stage of labour and were distributed as follows [Fig. 3.3]:

99 (65.1%) were born after active 1st stage duration ≤ 6 hours [Group 1 infants = Reference group],
40 (26.3%) were born after active 1st stage duration > 6 to ≤ 13 hours [Group 2 infants], and
13 (8.6%) of infants were born after active 1st stage duration >13 hours [Group 3 infants].

Table 3.3a shows the demographic characteristics of the studied infants with differential duration of active 1st stage of labour (n=152) exposure status. There were significant differences between the three groups with respect to mode of delivery by intrapartum antibiotic prophylaxis ($p=0.001$) and parity ($p=0.049$). No significant differences were detected in other co-variates of interest such as infant gender ($p=0.481$), infant diet ($p=0.794$), maternal ethnicity ($p=0.165$) etc.

For the second stage, infants (n=157) of obese mothers were distributed as follows:

118 (75.2%) were born after 2nd stage duration ≤ 1 hour [Group 1 infants = Reference group],
18 (11.5%) were born after 2nd stage duration greater than 1 hour and ≤ 2 hours [Group 2 infants],
21 (13.4%) of infants were born after 2nd stage duration greater than 2 hours [Group 3 infants].

Table 3.3b shows demographic characteristics of the studied infants with differential duration of second stage of labour (n=157) exposure status. There were significant differences between the three groups with respect to mode of delivery by intrapartum antibiotic prophylaxis status (p=0.007), parity (p<0.001), duration after membrane rupture (p=0.013) and infant's length of hospital stay (p=0.028). No significant differences were detected in other co-variates of interest such as infant gender (p=0.695), infant diet (p=0.226), maternal ethnicity (p=0.313) etc.

3.3.2 Fecal microbiota composition, richness and diversity

3.3.2A Infants born to women with normal pre-pregnancy weight (BMI <25)

I) Effect of duration of active 1st stage of labour

Table 3.4 summarizes the significant (p<0.05) changes in median relative abundance of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to duration of active first stage of labour among infants born to **women with normal pre-pregnancy weight (BMI <25).**

Among all delivery modes (vaginal and C-section), we observed underrepresentation of phylum Actinobacteria and family *Bifidobacteriaceae* (p<0.05) among infants born after active 1st stage >6 to ≤13 hours, but not after active 1st stage > 13 hours [Table 3.5]. Upon stratification of vaginally delivered infants by intrapartum antibiotic prophylaxis (IAP), we saw this change persisted only in infants who received IAP [Table 3.7]. Among C-section births, no significant change was observed in infant gut bacterial composition based on duration of active 1st stage.

We conducted multivariate logistic regression to further explore the association of duration of active 1st stage of labour and gut microbiota profile. Colonization with genus *Bifidobacterium* tended to decrease with longer active 1st stage [active 1st stage >6 to ≤13 hours: aOR = 0.59 (95%CI = 0.39-0.89), p = 0.012; active 1st stage >13 hours: aOR = 0.64 (95%CI = 0.34-1.19), p = 0.155]. Likewise, a 53% decrease in likelihood of colonization with genus

Lactobacillus was observed in infants born after active first stage > 13 hours [aOR = 0.47 (95%CI = 0.23-0.97), p = 0.041].Additionally, family *Veillionellaceae* showed 1.6 times higher likelihood of colonization when active 1st stage was between > 6 to ≤ 13 hours [[active 1st stage >6 to ≤ 13 hours: aOR = 1.60 (95%CI = 1.05-2.44), p = 0.028; active 1st stage > 13 hours: aOR = 1.10 (95%CI = 0.59-2.06), p = 0.770]. These associations were independent of mode of delivery and intrapartum antibiotic prophylaxis (IAP), breastfeeding, parity and membrane rupture duration greater than 18 hours [Table 3.14].

II) **Effect of duration of 2nd stage of labour**

Table 3.9 summarizes the significant (p<0.05) changes in median relative abundance of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to duration of second stage of labour among infants born to **women with normal weight (BMI <25).**

Among all delivery modes (vaginal and C-section), we observed underrepresentation of phylum Actinobacteria and family *Bifidobacteriaceae* (p<0.05) among infants born after second stage > 2 hours [Table 3.10]. Upon stratification of vaginally delivered infants by intrapartum antibiotic prophylaxis (IAP), we saw that only IAP-free infants retained this change, in addition to overrepresentation of *Clostridiaceae* [Table 3.11].

Multivariate logistic regression revealed that infant gut colonization with genus *Bifidobacterium* tended to decrease with longer second stage [2nd stage >1 to ≤2 hours: aOR = 0.76 (95%CI = 0.44-1.29), p = 0.304; 2nd stage > 2 hours: aOR = 0.57 (95%CI = 0.35-0.93), p = 0.024]. Similarly, infants born after 2nd stage > 2 hours had 49% decreased likelihood of colonization with genus *Lactobacillus* [aOR = 0.51 (95%CI = 0.30-0.87), p = 0.014]. Besides, family *Veillionellaceae* showed 1.7 times higher likelihood of increased colonization when 2nd stage was longer than 2 hours [2nd stage >1 to ≤ 2 hours: aOR = 1.45 (95%CI = 0.85-2.48), p = 0.175; 2nd stage > 2 hours: aOR = 1.70 (95%CI = 1.03-2.76), p = 0.037] [Table 3.15].

Microbial richness and diversity did not show significant change in adjusted models according to active 1st or 2nd stage labour durations in infants born to normal weight mothers [Table 3.16].

3.3.2B Infants born to women with pre-pregnancy overweight (BMI ≥25 to <30)

I) Effect of duration of active 1st stage of labour

Table 3.17 outlines the summary of the significant ($p<0.05$) changes in median relative abundance of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the duration of active first stage of labour among infants born to **women with pre-pregnancy overweight (BMI ≥25 to <30).** Among these infants, we saw decreased abundance of family *Bifidobacteriaceae* with after active 1st stage >6 to ≤13 hours (but not after active 1st stage > 13 hours) regardless of IAP exposure [Table 3.17, 3.19, 3.20].

Multivariate logistic regression showed that in infants born with active 1st stage > 6 to ≤ 13 hours, likelihood of gut colonization with phylum Actinobacteria decreased significantly [active 1st stage >6 to ≤ 13 hours: aOR = 0.40 (95%CI = 0.19-0.87), p = 0.030; active 1st stage > 13 hours: aOR = 0.75 (95%CI = 0.23-2.48)], independent of mode of delivery, intrapartum antibiotic prophylaxis (IAP), breastfeeding, parity, membrane rupture duration greater than 18 hours and infant sex [Table 3.26a]. Changes in microbial richness and diversity were not significant.

II) **Effect of duration of 2nd stage of labour**

Table 3.21 outlines the summary of the significant ($p<0.05$) changes in median relative abundance of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the duration of second stage of labour among infants born to **women with pre-pregnancy overweight (BMI ≥25 to <30).** Among these infants, we saw decreased abundance of family *Bifidobacteriaceae* when 2nd stage was longer than 2 hours in vaginally delivered, IAP-free infants [Table 3.23].

Multivariate logistic regression revealed that genus *Bacteroides* (of phylum Bacteroidetes) decreased with 2nd stage >1 to ≤ 2 hours but not after > 2 hours [2nd stage >1 to ≤ 2 hours: aOR = 0.29 (95%CI = 0.10-0.84), p = 0.023; 2nd stage > 2 hours: aOR = 0.62 (95%CI = 0.22-1.71)], after adjustment for mode of delivery and intrapartum antibiotic prophylaxis (IAP),

breastfeeding, parity, membrane rupture duration greater than 18 hours and infant sex [Table 3.27b]. In addition, microbial richness (Chao 1) decreased with 2nd stage >1 to ≤ 2 hours [aOR: 0.28 (95%CI:0.10-0.80), p=0.018)] but not with 2nd stage > 2 hours, whereas Shannon diversity decreased with 2nd stage > 2 hours [aOR: 0.30 (95%CI:0.11-0.801, p=0.017) [Table 3.28].

3.3.2C Infants born to women with pre-pregnancy obesity (BMI ≥ 30)

I) Effect of duration of active 1st stage of labour

Table 3.29 summarizes the significant ($p<0.05$) changes in median relative abundance of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the duration of active first stage of labour among infants born to **women with pre-pregnancy obesity (BMI ≥ 30).** Among all delivery modes (vaginal and C-section), we observed underrepresentation of phylum Actinobacteria and family *Bifidobacteriaceae* ($p<0.05$) among infants born after active 1st stage longer than 6 hours. Firmicutes also showed decreased abundance with active 1st stage longer than 13 hours. In contrast, there was an overrepresentation of phylum Bacteroidetes after longer durations of active 1st stage [Table 3.30]. Upon stratification of vaginally delivered infants by intrapartum antibiotic prophylaxis (IAP), we saw decreased abundance of family *Bifidobacteriaceae* with after active 1st stage > 13 hours was retained only in infants who did not receive IAP [Table 3.31]. Among C-section births, we did not observe statistically significant changes in infant gut bacterial composition based on active 1st stage duration in infants of obese mothers.

Multivariate logistic regression revealed a decreasing trend for colonization with phyla Actinobacteria and Firmicutes, and an increasing trend for colonization with phyla Bacteroidetes and Proteobacteria, according to increasing durations of active 1st stage in infants born to obese mothers [Table 3.38]. Colonization with genus *Bifidobacterium* tended to decrease with longer active 1st stage [active 1st stage >6 to ≤ 13 hours: aOR = 0.52 (95%CI = 0.22-1.22), p = 0.001; active 1st stage > 13 hours: aOR = 0.20 (95%CI = 0.04-0.97), p = 0.001] [Table 3.26]. Also, 76%

reduced likelihood of colonization with family *Veillionellaceae* was seen with active 1st stage > 13 hours [aOR: 0.24; 95% CI: 0.06-0.97] [Table 3.38]. These changes were independent of mode of delivery, intrapartum antibiotic prophylaxis (IAP), breastfeeding, parity and membrane rupture duration greater than 18 hours.

II) **Effect of duration of 2nd stage of labour**

Table 3.33 summarizes the significant ($p<0.05$) changes in median relative abundance of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the **duration of second stage of labour** among infants born to **women with pre-pregnancy obesity (BMI ≥ 30).** Among these infants, decreased abundance of *Bifidobacteriaceae* with 2nd stage > 2 hours was seen among IAP-free, vaginal births [Table 3.35]. Multivariate logistic regression showed infants delivered after 2nd stage longer than 2 hours showed 80% reduced likelihood of colonization with phylum Actinobacteria [2nd stage > 2 hours: aOR = 0.20 (95%CI = 0.05-0.76), p = 0.001] and 88% reduced likelihood of colonization with *Bifidobacterium* [2nd stage > 2 hours: aOR = 0.12 (95%CI = 0.03-0.58), p = 0.001] [Table 3.39], after accounting for mode of delivery, intrapartum antibiotic prophylaxis (IAP), breastfeeding, parity and membrane rupture duration greater than 18 hours.

Lastly, a trend in reduction of Shannon diversity was seen in the adjusted model with active 1st stage > 6 to ≤ 13 hours [aOR: 0.37 (95%CI=0.16-0.88)] but not when active 1st stage was > 13 hours, and also with 2nd stage > 1 to ≤ 2 hours [aOR: 0.37 (95%CI=0.16-0.88)] but not when 2nd stage was > 2 hours [Table 3.40].

The following table summarizes significant associations between labour durations and infant gut microbial composition among infants born to different maternal BMI categories [Table 3.41].

Table 3.41 Infants born to women with:	Active 1st stage (Hours) Ref: ≤6		2nd stage (Hours) Ref: ≤1	
	>6 to≤13 aOR (95% CI); p-value	> 13 aOR (95% CI); p-value	>1 to≤2 aOR (95% CI); p-value	> 2 aOR (95% CI); p-value
***BMI <25**				
Actinobacteria	**0.57 (0.37-0.86); p=0.007**	0.62 (0.33-1.16); p=0.137	0.72 (0.42-1.23); p=0.231	**0.57 (0.35-0.93); p=0.024**
f_Veillionellaceae	**1.60 (1.05-2.44); p=0.028**	1.10 (0.59-2.06); p=0.770	1.45 (0.85-2.48); p=0.175	**1.70 (1.03-2.76); p=0.037**
g_Bifidobacterium	**0.59 (0.39-0.89); p=0.012**	0.64 (0.34-1.19); p=0.155	0.76 (0.44-1.29); p=0.304	**0.57 (0.35-0.93); p=0.024**
g_Lactobacillus	1.12 (0.73-1.70); p=0.613	**0.47 (0.23-0.97); p=0.041**	0.92 (0.53-1.59); p=0.758	**0.51 (0.30-0.87); p=0.014**
†BMI ≥25 to <30				
Actinobacteria	**0.40 (0.19-0.87); p=0.020**	0.75 (0.23-2.48); p=0.637	0.78 (0.27-2.21); p=0.639	0.56 (0.22-1.45); p=0.235
g_Bacteroides	1.104 (0.49-2.22) p=0.911	1.39 (0.40-4.76) p=0.603	**0.29 (0.10-0.84) p=0.022**	0.62 (0.22-1.71) p=0.369
***BMI ≥30**				
Actinobacteria	0.49 (0.21-1.14); p=0.097	0.48 (0.13-1.80); p=0.278	0.78 (0.26-2.31); p=0.781	**0.20 (0.05-0.76); p=0.018**
f_Veillionellaceae	0.63 (0.27-1.47); p=0.286	**0.24 (0.06-0.97); p=0.045**	0.84 (0.29-2.44); p=0.744	0.67 (0.23-1.95); p=0.672
g_Bifidobacterium	0.52 (0.22-1.22); p= 0.133	**0.20 (0.04-0.97); p=0.046**	0.67 (0.23-1.95); p=0.498	**0.12 (0.03-0.58); p=0.008**

* Odd ratios adjusted for mode of delivery by intrapartum antibiotic prophylaxis, exclusive breastfeeding, parity and duration after membrane rupture > 18 hours.

†Odd ratios adjusted for mode of delivery by intrapartum antibiotic prophylaxis, exclusive breastfeeding, parity, duration after membrane rupture > 18 hours and infant sex.

Significant associations are **bold-faced.** (aOR =adjusted odds ratio)

3.4 Discussion

The gut microbiota can influence host energy harvest and host metabolic phenotype and through fermentation of short chain fatty acids (SCFAs) (39) (40), and altered composition of gut microbiota is associated with higher risk of childhood obesity (14) (15). Meanwhile, elevated maternal BMI is a powerful predictor of weight gain in the progeny (5) (6) (7) (8). Since overweight/obese pregnant women not only possess atypical changes in their gut microbiota (17) (18) (19) but also suffer protracted course of labour (22) (23) (24) 25), we probed the infant gut microbial composition differences at 3-4 months of age according to increasing lengths of labour in infants born to normal weight, overweight and obese women. To our knowledge, this is the first study to investigate the association between labour duration and infant gut microbiota according to differential maternal BMI categories.

In the present study cohort of 921 healthy infants, we found alterations in gut microbiota composition in relation to increasing duration of labour and born to 556 normal weight, 208 overweight and 157 obese mothers. Among infants born to normal weight women, we observed that longer durations of active first stage and second stage of labour was associated with reduced tendency of infant gut colonization with *Bifidobacterium* (of phylum Actinobacteria). This change persisted in infants of women with higher BMI born after longer labour durations, and was more severe among the infants of obese mothers. We theorize that higher oxidative stress associated with longer labour durations (41) (42), and the inefficiency some strains of anaerobic *Bifidobacterium* spp. to cope with increased production reactive oxygen species (ROS) (43) (44), probably accounts for reduced vertical transfer of *Bifidobacterium* and lower gut colonization seen in the infants. For infants of obese mothers, presence of atypical maternal gut microbial composition with lower abundance of maternal gut *Bifidobacterium* could have intensified the effect. Studies show that abundance of gut *Bifidobacterium* negatively correlates with obesity (45) (17), and this also true for pregnant women with higher BMI (17) (18) (19). As compared to infants of normal weight mothers, we observed more drastic reduction in fecal *Bifidobacterium* associated with longer labour durations in the infants of obese mothers, and this finding is concerning as reductions in gut *Bifidobacterium* during infancy is correlated with higher risk of increased weight gain in later childhood (14).

In addition, fecal *Lactobacillus* (of phylum Firmicutes) was also observed to be significantly reduced in association with longer labour durations in infants of normal weight mothers. Similar trend for decreased colonization with *Lactobacillus* was observed after longer active first stage in infants of overweight mothers, and after longer active 1st stage and 2nd stage in infants of obese mothers, although statistical significance was not achieved. Gut *Lactobacilli* appear to have influential role in weight alterations, and murine models show that reduced abundance of *Lactobacillus* is associated with obese host phenotype (46). Dietary supplementation with *Lactobacillus* spp. has been shown to reduce adiposity in adult humans (47) (48) suggesting that reduction of *Lactobacillus* spp. may aggravate metabolic disorders. In addition, *Lactobacilli* promote integrity of epithelial tight-junctions in the gut (49), and their reduced abundance could facilitate metabolic endotoxemia ("leaky gut") that in turn initiates inflammation and adiposity (50). On the other hand, a metanalysis of 17 human RCTs concluded that *Lactobacillus* associated weight gain in adult humans is strain and species specific (51). To add, evidence on influence of gut Lactobacillus on childhood obesity is limited. In a double-blind randomized trial, infants fed with *Lactobacillus rhamnosus GG* enriched formula showed significant weight gain compared to controls fed regular formula (52). Another study comparing overweight/obese children to normal weight controls found higher abundance of *Lactobacillus spp.* in obese children (49). In the same study, *Lactobacillus spp*. showed a positive association with plasma C-reactive protein (53), possibly indicating a role of *Lactobacillus* in "low-grade" inflammation, a recognized pathophysiological feature of obesity.

Another finding of interest in our study was the alteration in abundance of family *Veillionellaceae* (of phylum Firmicutes) associated with longer durations of labour in infants born to mothers of different pre-pregnancy weight categories. Among infants born to normal weight mothers, we observed an increased tendency of colonization with *Veillionellaceae* associated longer durations of active first stage and second stage of labour. The *Veillionellaceae* family, as lactate-utilizing bacteria, has a unique metabolic role in the gut (54). Prolonged labour often leads to depletion of the uterine myometrial glycogen stores and ATP, accompanied with decreased cellular ability to handle protons (H^+) and accumulation of lactate (55) (56). We suggest that higher availability of lactate during protracted labour states may favor overgrowth of maternal gut *Veillionellaceae*, thereby setting the foundation for higher prevalence in infants later. In addition, upregulation of Veillionellaceae abundance during prolonged labour may proffer metabolic

advantage during periods of prolonged labour. Gut bacteria aid host energy harvest by fermenting dietary fiber to generate short chain fatty acids (SCFAs). The major SCFAs are acetate, propionate and butyrate, and their production depends upon diet and the abundance and species of cecal and colonic microbiota. *Veillionellaceae* are unique in their ability to convert lactate to propionate through the acrylate pathway (54), and propionate acts as a substrate for hepatic gluconeogenesis. Intense exercise states demand increased hepatic glucose production to maintain optimum glycemia. When hepatic glycogen stores are depleted during prolonged period of exercise, the liver switches to increased gluconeogenesis to meet the energy demand (57). Since protracted labour mimics a period of prolonged exercise, *Veillionellaceae* may have a role in providing the liver with adequate energy substrate for gluconeogenesis by converting lactate to propionate during prolonged labour.

In contrast to infants of normal weight mothers, infants born to obese mothers showed reduced likelihood of gut colonization with *Veillionellaceae* associated longer active first stage. Based on limited available literature, abundance of *Veillionellaceae* appears to have positive association with adiposity and high-fat diet intake (46) (58). Whether reduction in gut *Veillonellaceae* influences the regulation of overall metabolic balance in infants and their risk of weight gain at a later age remains to be investigated. Therefore, further research is needed to elucidate the role and significance of decreased abundance of *Veillionellaceae* associated with longer labour in infants born of obese mothers.

Further, among infants born to overweight mothers, we observed that longer labour duration was associated with decreased trend of colonization with phylum Actinobacteria and genus *Bacteroides* at 3-4 months of age. This association was independent of mode of delivery and intrapartum antibiotic prophylaxis (IAP), breastfeeding, parity, membrane rupture duration greater than 18 hours and infant sex. From available evidence on infants of this age category, reduced gut *Bacteroides* spp. could indicate higher risk of childhood overweight (21). Finally, a reduced trend for microbial diversity (Shannon diversity) was observed with longer labour durations in infants born to overweight and obese mothers, but not among infants of normal weight mothers. Low gut microbial diversity is linked to weight gain (10) (46). Additionally, pregnant women with elevated BMI show low gut microbial diversity (25), and breastmilk of obese mothers

has been shown to possess less diverse bacterial community than normal weight mothers (59). Since our results were independent of breastfeeding, the implication for longer labour and reduced gut microbial diversity with regards to risk of weight gain is emphasized.

3.5 Strengths and limitations

Our study was conducted in a population based longitudinal cohort that recruited pregnant women their third trimester and followed the children up to early life years. Therefore, the results are generalized to the population, and the precedence of exposure (duration of labour) the development of the outcome (changes in infant gut microbiota) is ascertained which allows us to suggest a causal relation. In addition, study of infant belonging to mothers of different BMI categories allowed us to unmask the changes in infant gut microbiota in relation to labour that is devoid of influence of maternal weight, thus providing novel insights to early life factors that influence development of infant gut microbiota. Finally, the use of high throughput gene sequencing technique presents the benefit of high accuracy and reliability to our gut microbiota measures.

One major limitation of our study is the smaller sample size of women in overweight and obese category. This may have limited our study's ability to detect statistical significance of some of the changes in microbiota measures. Home births were excluded from our study. Therefore, our study is unable to characterize the association between duration of labour and infant gut microbiota in the infants delivered at home, which is likely to be different from hospital delivered infants. Finally, our study is limited to gut microbiota changes at 3-4 months of age and we did not study the influence of duration of labour on infant gut microbiota at an older age. Thus, future studies could be directed towards these efforts.

3.6 Conclusion

This study provides further insight into the association between exposure to longer durations of labour at birth among infants born to mothers with different pre-pregnancy weight categories and the changes to infant gut microbial composition at the first 3-4 months of life.

Infant gut dysbiosis is associated with higher risk of childhood obesity. Higher gut Bifidobacteria abundance is shown to be protective of adiposity whereas Lactobacillus-associated weight gain is species-specific. Therefore, longer labour associated under-representation of gut Bifidobacteria and alterations in Lactobacilli observed in infants born to mothers of all BMI categories in this study, but more pronounced in infants of obese mothers, may suggest longer labour duration as a possible indicator for pediatric weight gain. Further, differential alterations of Veillionellaceae associated with longer labour in infants born mothers of different BMI categories provides new insight into possible metabolic role of this bacteria in protracted labour while inviting more investigation to fully comprehend the influence of birth and labour-related events on development of infant gut microbiota.

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Table 3.1a

Population characteristics by duration of active 1st stage of labour among normal weight women (n = 531)				
	Duration of 1st stage ≤ 6 hours [Group 1]	**Duration of 1st stage > 6 to ≤ 13 hours** [Group 2]	**Duration of 1st stage > 13 hours** [Group 3]	p-value (x^2)
	N (%)	N (%)	N (%)	
Row percentages	**n=322 (60.6%)**	**n=156 (29.4%)**	**n=53(10.0%)**	
Baby's gender (n = 531)				0.999
Male	170 (60.7%)	82 (29.3%)	28 (10.0%)	
Female	152 (60.6%)	74 (29.5%)	25 (10.0%)	
Delivery mode (n = 525)				**<0.001**
Vaginal without IAP (n=293)	170 (58.0%)	94 (32.1%)	29 (9.9%)	
Vaginal with IAP (n=108)	40 (37.0%)	48 (44.4%)	20 (18.5%)	
Elective C-section(n=52)	52 (100.0%)	0	0	
C-section with labour (n=72)	60 (83.3%)	10 (13.9%)	2 (2.8%)	
Term gestation (n= 531)				**0.031**
No	14 (93.3%)	1 (6.7%)	0	
Yes	308 (59.7%)	156 (29.4%)	53 (10.0%)	
Infant diet 3 months (n= 526)				0.972
EBF = Yes	189 (59.8%)	96 (30.4%)	31 (9.8%)	
EBF= Partial	91 (62.3%)	40 (27.4%)	15 (10.3%)	
EBF= No	39 (60.9%)	18 (28.1%)	7 (10.9%)	
Parity (n=531)				**<0.001**
Primipara	95 (48.5%)	69 (35.2%)	32 (16.3%)	
Multipara	227 (67.8%)	87(26.0%)	21(6.3%)	
Membrane rupture >18 Hours (n=522)				0.058
No	286(62.3%)	131(28.5%)	42(9.2%)	
Yes	30(47.6%)	23(36.5%)	10(15.9%)	
Length of hospital stay (n=512)				0.064
24 hours of less	81(56.3%)	48(33.3%)	15(10.4%)	
2-3 days	185(59.9%)	92 (29.8%)	32(10.4%)	
4 days or more	46(78.0%)	9(15.3%)	4(6.8%)	
Maternal ethnicity (n=527)				0.891
Caucasian	238(61.0%)	113(29.0%)	39(10.0%)	
Other	33 (55.0%)	20(33.3%)	7(11.7%)	
Asian	49 (63.6%)	21(27.3%)	7(9.1%)	
Prenatal smoke exposure (n=518)				0.205
No	297(59.4%)	152(30.4%)	51(10.2%)	
Yes	14(77.8%)	2(11.1%)	2(11.1%)	
Maternal asthma (n= 531)				0.299
No	258(61.0%)	127(30.0%)	38(9.0%)	
Yes	64(59.3%)	29(26.9%)	15(13.9%)	

IAP = Intrapartum Antibiotic Prophylaxis; EBF = Exclusive breastfeeding
Comparison made by Chi square test. p-value <0.05 are in boldface type.

Table 3.1b

Table 1. Population characteristics by duration of 2nd stage of labour among normal weight women (n = 556)				
Row percentages	Duration of 2nd stage ≤ 1 hour [Group 1] N (%) 379 (68.2%)	Duration of 2nd stage > 1 to ≤ 2 hours [Group 2] N (%) 75 (13.55%)	Duration of 2nd stage > 2 hours [Group 3] N (%) 102 (18.3%)	p-value (x^2)
Baby's gender (n = 556)				0.684
Male	197 (66.8%)	43 (14.6%)	55 (18.6%)	
Female	182 (69.7%)	32 (12.3%)	47 (18.0%)	
Delivery mode (n = 549)				**<0.001**
Vaginal without IAP (n=310)	212 (68.4%)	48 (15.5%)	50 (16.1%)	
Vaginal with IAP (n=115)	54 (47.0%)	24 (20.9%)	37 (32.2%)	
Elective C-section (n=52)	52 (100.0%)	0	0	
C-section with labour (n=72)	56 (77.8%)	1 (1.4%)	15 (20.8%)	
Term gestation (n= 556)				0.589
No	12 (80.0%)	1 (6.7%)	2 (13.3%)	
Yes	367 (67.8%)	74 (13.7%)	100 (18.5%)	
Infant diet 3 months (n= 551)				0.983
EBF = Yes	223 (67.8%)	43 (13.1%)	63 (19.1%)	
EBF= Partial	105 (67.7%)	22 (14.2%)	28 (18.1%)	
EBF= No	47 (70.1%)	74 (13.4%)	102 (16.4%)	
Parity (n=556)				**<0.001**
Primipara	100 (48.1%)	41 (19.7%)	67 (32.2%)	
Multipara	279 (80.2%)	41 (19.7%)	67 (32.2%)	
Membrane rupture >18 Hours (n= 545)				**<0.001**
No	343 (72.2%)	62 (13.1%)	70 (14.7%)	
Yes	27 (38.6%)	12 (17.1%)	31 (44.3%)	
Length of hospital stay (n=536)				0.805
24 hours of less	108 (72.0%)	19 (12.7%)	23 (15.3%)	
2-3 days	216 (66.5%)	47 (14.5%)	62 (19.1%)	
4 days or more	41 (67.2%)	8 (13.1%)	12 (19.7%)	
Maternal ethnicity (n=550)				0.955
Caucasian	278 (68.6%)	52 (12.8%)	75 (18.5%)	
Other	44 (65.7%)	11 (16.4%)	12 (17.9%)	
Asian	53 (67.9%)	11 (14.1%)	14 (17.9%)	
Pre-natal smoke exposure (n= 539)				0.350
No	352 (67.6%)	70 (13.4%)	99 (19.0%)	
Yes	14 (77.8%)	3 (16.7%)	1 (5.6%)	
Maternal asthma (n= 556)				0.474
No	308 (69.4%)	58 (13.1%)	78(17.6%)	
Yes	71 (63.4%)	17(15.2%)	24(21.4%)	

IAP = Intrapartum Antibiotic Prophylaxis; EBF = Exclusive breastfeeding
Comparison made by Chi square test. p-value <0.05 are in boldface type.

Table 3.2a

Population characteristics by duration of active 1st stage of labour among overweight women (n = 201)				
	Duration of 1st stage ≤ 6 hours [Group 1]	**Duration of 1st stage > 6 to ≤ 13 hours** [Group 2]	**Duration of 1st stage > 13 hours** [Group 3]	p-value (x^2)
	N (%)	N (%)	N (%)	
Row percentages	**n=120 (59.7%)**	**n=62 (30.8%)**	**n=19 (9.5%)**	
Baby's gender (n = 200)				**0.019**
Male	59 (54.6%)	42 (38.9%)	7(6.5%)	
Female	60 (65.2%)	20 (21.7%)	19 (9.5%)	
Delivery mode (n = 196)				**<0.001**
Vaginal without IAP (n=103)	50 (48.5%)	40(38.8%)	13 (12.6%)	
Vaginal with IAP (n= 43)	19 (44.2%)	19(44.2%)	5 (11.6%)	
Elective C-section(n=26)	26 (100.0%)	0	0	
C-section with labour(n=24)	22 (91.7%)	2 (8.3%)	0	
Term gestation (n= 200)				**0.031**
No	6 (75.0%)	2 (25.0%)	0	
Yes	113 (58.9%)	60 (31.3%)	19 (9.9%)	
Infant diet 3 months (n= 201)				0.853
EBF = Yes	64 (61.0%)	33 (31.4%)	8 (7.6%)	
EBF= Partial	40 (60.6%)	19 (28.8%)	7 (10.6%)	
EBF= No	16 (53.3%)	10 (33.3%)	4 (13.3%)	
Parity (n=201)				0.114
Primipara	34 (50.0%)	25 (30.85)	9 (13.2%)	
Multipara	86 (64.7%)	37 (27.8%)	10 (7.5%)	
Membrane rupture >18 Hours (n=195)				0.227
No	103 (61.3%)	51 (30.4%)	14 (8.3%)	
Yes	12 (44.4%)	11 (40.7%)	4 (14.8%)	
Length of hospital stay (n=192)				0.227
24 hours of less	28 (65.1%)	11 (25.6%)	4 (9.35)	
2-3 days	67 (54.0%)	44 (35.5%)	13 (10.5%)	
4 days or more	19 (76.0%)	5 (20.0%)	1 (4.0%)	
Maternal ethnicity (n=198)				0.900
Caucasian	92 (59.7%)	48 (31.2%)	14 (9.1%)	
Other	14 (60.9%)	6 (26.1%)	3 (13.0%)	
Asian	13 (61.9%)	7(33.3%)	1 (4.8%)	
Prenatal smoke exposure (n=196)				0.070
No	114 (60.3%)	60 (31.7%)	15 (7.9%)	
Yes	3 (42.9%)	1 (14.35)	3 (42.9%)	
Maternal asthma (n= 200)				0.218
No	86 (61.4%)	44 (31.4%)	10 (7.1%)	
Yes	33 (55.0%)	18 (30.0%)	9(15.0%)	

IAP = Intrapartum Antibiotic Prophylaxis; EBF = Exclusive breastfeeding
Comparison made by Chi square test. p-value <0.05 are in boldface type.

Table 3.2b

Table 1. Population characteristics by duration of 2nd stage of labour among overweight women (n=208)				
Row percentages	Duration of 2nd stage ≤ 1 hour [Group 1]	Duration of 2nd stage > 1 to ≤ 2 hours [Group 2]	Duration of 2nd stage > 2 hours [Group 3]	p-value (x^2)
	N (%)	N (%)	N (%)	
	150 (72.1%)	26 (12.5%)	32 (15.4%)	
Baby's gender (n = 208)				**0.018**
Male	72 (64.9%)	20 (18.0%)	19(17.1%)	
Female	78 (80.4%)	6 (6.2%)	13 (13.4%)	
Delivery mode (n = 202)				**0.004**
Vaginal without IAP (n=109)	72 (66.1%)	20 (18.3%)	17 (15.6%)	
Vaginal with IAP (n=43)	28 (65.1%)	5 (11.6%)	10 (23.3%)	
Elective C-section (n=26)	26 (100.0%)	0	0	
C-section with labour (n=24)	20 (83.3%)	0	4 (16.7%)	
Term gestation (n= 208)				0.460
No	7 (87.5%)	1 (12.5%)	0	
Yes	143 (71.5%)	25 (12.5%)	32 (16.0%)	
Infant diet 3 months (n= 208)				0.296
EBF = Yes	74 (69.8%)	18 (17.0%)	14 (13.2%)	
EBF= Partial	53 (76.8%)	5 (7.2%)	11 (15.9%)	
EBF= No	23 (69.7%)	3 (9.1%)	7 (21.2%)	
Parity (n=208)				**<0.001**
Primipara	34 (49.3%)	14 (20.3%)	21 (30.4%)	
Multipara	116 (83.5%)	12 (8.6%)	11 (7.9%)	
Membrane rupture >18 Hours (n= 201)				0.116
No	128 (74.0%)	22 (12.7%)	23 (13.3%)	
Yes	17 (60.7%)	3 (10.7%)	8(28.6%)	
Length of hospital stay (n=199)				0.898
24 hours of less	30 (69.8%)	7 (16.3%)	6 (14.0%)	
2-3 days	92 (70.8%)	16(12.3%)	22 (16.9%)	
4 days or more	20 (76.9%)	3 (11.5%)	3 (11.5%)	
Maternal ethnicity (n=205)				0.788
Caucasian	117 (73.6%)	18 (11.3%)	24 (15.1%)	
Other	17 (68.0%)	4 (16.0%)	4 (16.0%)	
Asian	13 (61.9%)	4 (19.0%)	4 (19.0%)	
Pre-natal smoke exposure (n= 203)				0.984
No	141 (71.9%)	24 (12.25)	31 (15.8%)	
Yes	5 (71.4%)	1 (14.35)	1 (14.3%)	
Maternal asthma (n= 208)				0.723
No	104 (71.2%)	20 (13.7%)	22 (15.1%)	
Yes	46 (74.2%)	6 (9.7%)	10 (16.1%)	

IAP = Intrapartum Antibiotic Prophylaxis; EBF = Exclusive breastfeeding
Comparison made by Chi square test. p-value <0.05 are in boldface type.

Table 3.3a

Population characteristics by duration of active 1st stage of labour among obese women (n = 152)				
	Duration of 1st stage ≤ 6 hours [Group 1]	**Duration of 1st stage > 6 to ≤ 13 hours** [Group 2]	**Duration of 1st stage > 13 hours** [Group 3]	p-value (x^2)
	N (%)	N (%)	N (%)	
Row percentages	**n=99 (65.1%)**	**n=40 (26.3%)**	**n=13 (8.6%)**	
Baby's gender (n = 152)				0.481
Male	55 (69.6%)	18(22.8%)	6 (7.6%)	
Female	44 (60.3%)	22 (30.1%)	7 (9.6%)	
Delivery mode (n = 148)				**0.001**
Vaginal without IAP (n=68)	35 (51.5%)	26 (38.2%)	7(10.3%)	
Vaginal with IAP (n= 30)	16 (53.35)	10 (33.3%)	4 (13.3%)	
Elective C-section(n=19)	19 (100.0%)	0	0	
C-section with labour(n=31)	26 (83.9%)	3 (9.7%)	2 (6.5%)	
Term gestation (n= 152)				--
No	- -	- -	- -	
Yes	99 (65.1%)	40 (26.3%)	13 (8.6%)	
Infant diet 3 months (n= 152)				0.794
EBF = Yes	32 (64.0%)	13 (26.0%)	5 (10.0%)	
EBF= Partial	37 (71.2%)	12 (23.1%)	3 (5.8%)	
EBF= No	30 (60.0%)	15 (30.0%)	5 (10.0%)	
Parity (n=152)				**0.049**
Primipara	30 (53.6%)	21 (37.5%)	5 (8.9%)	
Multipara	69 (71.9%)	19 (19.8%)	8 (8.3%)	
Membrane rupture >18 Hours (n=147)				0.202
No	81 (65.3%)	35 (28.2%)	8 (6.5%)	
Yes	14 (60.9%)	5 (21.7%)	4 (17.4%)	
Length of hospital stay (n=144)				0.087
24 hours of less	7 (43.8%)	7(43.8%)	2 (12.5%)	
2-3 days	65 (64.4%)	27 (26.7%)	9 (8.9%)	
4 days or more	23 (85.2%)	3 (11.1%)	1 (3.7%)	
Maternal ethnicity (n=152)				0.165
Caucasian	84 (66.1%)	32(25.2%)	11 (8.7%)	
Other	15 (68.2%)	6 (27.3%)	1 (4.5%)	
Asian	0	2 (66.7%)	1 (33.3%)	
Prenatal smoke exposure (n=149)				0.258
No	86 (63.2%)	37 (27.2%)	13 (9.6%)	
Yes	11 (84.6%)	2 (15.4%)	0	
Maternal asthma (n= 152)				0.891
No	65 (65.7%)	25 (25.3%)	9 (9.1%)	
Yes	34 (64.2%)	15 (28.3%)	4 (7.5%)	

IAP = Intrapartum Antibiotic Prophylaxis; EBF = Exclusive breastfeeding
Comparison made by Chi square test. p-value <0.05 are in boldface type.

Table 3.3b

Table 1. Population characteristics by duration of 2nd stage of labour among obese women (n=157)				
Row percentages	Duration of 2nd stage ≤ 1 hour [Group 1]	Duration of 2nd stage > 1 to ≤ 2 hours [Group 2]	Duration of 2nd stage > 2 hours [Group 3]	p-value (x^2)
	N (%)	N (%)	N (%)	
	118 (75.2%)	18 (11.5%)	21 (13.4%)	
Baby's gender (n = 157)				0.695
Male	62 (74.7%)	11 (13.3%)	10 (12.0%)	
Female	56 (75.7%)	7 (9.5%)	11 (14.9%)	
Delivery mode (n = 153)				**0.007**
Vaginal without IAP (n=71)	51 (71.85)	10 (14.1%)	10 (14.1%)	
Vaginal with IAP (n=32)	18 (56.3%)	8(25.05)	6 (18.8%)	
Elective C-section (n=19)	19 (100.0%)	0	0	
C-section with labour (n=31)	26 (83.95)	0	5 (13.7%)	
Term gestation (n= 157)				--
No	--	--	--	
Yes	118 (75.2%)	18 (11.5%)	21 (13.4%)	
Infant diet 3 months (n= 157)				0.226
EBF = Yes	36 (69.2%)	10 (19.2%)	6 (11.5%)	
EBF= Partial	44 (81.5%)	4 (7.4%)	6 (11.1%)	
EBF= No	38 (74.5%)	4 (7.8%)	9 (17.6%)	
Parity (n=157)				**<0.001**
Primipara	34 (57.6%)	11 (18.6%)	14 (23.7%)	
Multipara	84 (85.7%)	7 (7.1%)	7 (7.1%)	
Membrane rupture >18 Hours (n= 152)				**0.013**
No	100 (77.5%)	17 (13.2%)	12 (9.3%)	
Yes	15 (65.2%)	1 (4.3%)	7 (30.4%)	
Length of hospital stay (n=149)				**0.028**
24 hours of less	11 (64.7%)	5 (29.4%)	1 (5.9%)	
2-3 days	81 (77.1%)	12 (11.4%)	12 (11.4%)	
4 days or more	21 (77.85)	0	6 (22.2%)	
Maternal ethnicity (n=157)				0.313
Caucasian	97 (74.0%)	16 (12.2%)	18 (13.7%)	
Other	20 (87.0%)	1 (4.3%)	2 (8.7%)	
Asian	1 (33.35)	1 (33.3%)	1 (33.35)	
Pre-natal smoke exposure (n= 154)				0.074
No	101 (72.1%)	18 (12.95)	21 (15.0%)	
Yes	14 (100.0%)	0	0	
Maternal asthma (n= 157)				0.369
No	80 (78.4%)	11 (10.8%)	11 (10.8%)	
Yes	38 (69.1%)	7 (12.7%)	10 (18.2%)	

IAP = Intrapartum Antibiotic Prophylaxis; EBF = Exclusive breastfeeding
Comparison made by Chi square test. p-value <0.05 are in boldface type.

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI<25)

Table 3.4
Summary table showing **significant** ($p<0.05$) median relative abundance changes of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the duration of active first stage of labour among infants born to normal weight mothers, and following stratification by mode by IAP

ALL MODES OF BIRTHS (n=531)		
Reference group: Group 1 (n=322)	Group 2 (n=156)	Group 3 (n=53)
Phylum Actinobacteria	↓	--
Bifidobacteriaceae	↓	--
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Lactobacillaceae	--	↓
Phylum Proteobacteria	--	--

Group 1 (Reference group): Active 1st stage ≤ 6 hours

Group 2: Active 1st Stage > 6 to ≤13 hours

Group 3: Active 1st Stage > 13 hours

VAGINAL BIRTHS WITHOUT IAP (n=293)		
Group 1 (Ref) (n=170)	Group 2 (n=94)	Group 3 (n=29)
Phylum Actinobacteria	--	↓
Bifidobacteriaceae	--	↓
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Lactobacillaceae	--	--
Phylum Proteobacteria	--	--

VAGINAL BIRTHS WITH IAP (n=108)		
Group 1 (Ref) (n=40)	Group 2 n=48)	Group 3 (n=20)
Phylum Actinobacteria	↓	--
Bifidobacteriaceae	↓	--
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Ruminococcaceae	↓	--
Phylum Proteobacteria	--	--

C-SECTION WITH ACTIVE 1ST STAGE (n=69)	
Group 1 (Ref) (n=57)	Active 1st stage > 6 hrs (n=12)
Phylum Actinobacteria	--
Bifidobacteriaceae	--
Phylum Bacteroidetes	--
Phylum Firmicutes	--
Ruminococcaceae	--
Phylum Proteobacteria	--

IAP = Intrapartum Antibiotic Prophylaxis
-- indicates no significant change

(Note: Elective C-section excluded from analyses)

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI<25)

Table 3.5
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among all modes of delivery in normal weight mothers, according to the duration of active first stage of labour (n=531)

Bacterial Taxa	1st Stage of labour <= 6 hours [Reference group]	1st Stage of labour > 6 to <=13 hours	p-value	1st Stage of labour > 13 hours	p-value
	(n=322; 60.6%)	(n=156; 29.4%)		(n=53; 10.0%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum	6.775	3.585		3.504	
Actinobacteria	(1.875-16.695)	(0.761-13.265)	**0.025**	(0.474-14.453)	0.157
Bifidobacteriaceae	6.306 (1.669-15.516)	3.487 (0.558-13.191)	**0.037**	3.317 (0.285-13.869)	0.174
Coriobacteriaceae	0.039 (0.008-0.140)	0.031 (0002-0.137)	0.639	0.023 (0.004-0.066)	0.189
g_Bifidobacterium	6.306 (1.669-15.516)	3.487 (0.558-13.191)	**0.037**	3.317 (0.285-13.869)	0.174
Bacteroidetes	7.525 (0.093-56.454)	18.880(0.132-61.441)	0.108	13.817 (0.093-58.459)	0.493
Bacteroidaceae	3.805 (0.068-52.451)	10.042(0.070-58.918)	0.213	13.717 (0.067-57.067)	0.562
Firmicutes	23.330(9.888-47.099)	23.895(8.084-47.094)	0.612	25.008 (7.265-48.272)	0.890
Lactobacillaceae	0.000 (0.000-0.054)	0.000 (0.000-0.037)	0.987	0.000 (0.000-0.000)	**0.007**
Streptoccocaceae	0.690 (0.223-1.887)	0.533 (0.182-1.513)	0.347	0.538 (0.270-1.880)	0.844
Clostridiaceae	0.428 (0.029-3.265)	0.206 (0.023-1.851)	0.175	0.703 (0.074-4.481)	0.446
Lachnospiraceae	2.371 (0.029-10.481)	1.784 (0.046-9.675)	0.978	1.408 (0.019-9.810)	0.424
Ruminococcaceae	0.054 (0.000-2.096)	0.035 (0.000-0.655)	0.160	0.023 (0.000-1.664)	0.367
Veillionellaceae	4.315 (0.755-16.178)	6.486 (0.587-17.063)	0.787	4.092(0.823-22.326)	0.570
g_Lactobacillus	0.000 (0.000-0.054)	0.000 (0.000-0.037)	0.987	0.000 (0.000-0.000)	**0.007**
Proteobacteria	19.718(9.126-41.385)	21.169(9.884-40.226)	0.950	20.257(7.900-41.382)	0.898
Enterobacteriaceae	18.220 (6.543-39.289)	18.828(7.902-37.721)	0.985	18.287 (7.769-39.816)	0.817
Verrucomicrobia	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.497	0.000 (0.000-0.008)	0.939
g_Akkermansia	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.497	0.000 (0.000-0.008)	0.939

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI<25)

Table 3.6
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births without IAP in normal weight mothers, according to the duration of active first stage of labour (n=293)

Bacterial Taxa	1st Stage of labour <= 6 hours [Reference group]	1st Stage of labour > 6 to <=13 hours	p-value	1st Stage of labour > 13 hours	p-value
	(n=170; 58.0%)	(n=94; 32.1%)		(n=29; 9.9%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	8.079 (2.881-19.182)	4.825 (1.417-15.435)	0.080	2.028 (0.219-13.590)	**0.043**
Family *Bifidobacteriaceae*	7.166 (2.530-18.597)	4.538 (1.387-14.870)	0.082	1.974 (0.113-13.528)	**0.051**
Family *Coriobacteriaceae*	0.043 (0.008-0.144)	0.035 (0.000-0.117)	0.452	0.016 (0.000-0.054)	0.151
Genus_*Bifidobacterium*	7.166 (2.518-18.521)	4.538 (1.387-14.870)	0.080	1.974 (0.113-13.528)	**0.051**
Phylum Bacteroidetes	35.083 (0.930-62.249)	28.278 (0.993-60.856)	0.832	34.334 (0.198-66.856)	0.807
Family *Bacteroidaceae*	31.413 (0.570 -60.721)	26.078 (0.176-54.222)	0.394	26.814 (0.183-59.566)	0.840
Phylum Firmicutes	16.720 (7.447-32.061)	20.435 (7.362-45.654)	0.526	15.281 (4.649-45.482)	0.740
Family *Lactobacillaceae*	0.000 (0.000-0.049)	0.000 (0.000-0.049)	0.601	0.000 (0.000-0.008)	0.230
Family *Streptoccocaceae*	0.660 (0.194-1.883)	0.457 (0.130-0.946)	0.083	0.497 (0.118-2.045)	0.654
Family *Clostridiaceae*	0.151 (0.016-1.567)	0.171 (0.023-1.239)	0.872	0.641 (0.066-5.786)	0.116
Family *Lachnospiraceae*	1.817 (0.023-8.851)	1.819 (0.093-8.715)	0.293	2.206 (0.019-9.810)	0.912
Family *Ruminococcaceae*	0.046 (0.000-1.562)	0.081 (0.008-1.052)	0.603	0.008 (0.000-0.519)	0.155
Family *Veillionellaceae*	2.730 (0.527-13.243)	3.949 (0.442-12.525)	0.762	2.477 (0.861-14.733)	0.897
Genus_*Lactobacillus*	0.000 (0.000-0.049)	0.000 (0.000-0.049)	0.601	0.000 (0.000-0.008)	0.230
Genus_*Clostridium*	0.012 (0.000-0.511)	0.015 (0.000-0.403)	0.597	0.101 (0.000-4.018)	0.287
Genus_*Veillionella*	1.805 (0.259-10.975)	2.197 (0.247-8.766)	0.801	2.477 (0.805-12.683)	0.518
Phylum Proteobacteria	16.306 (7.647-36.570)	15.674 (9.602-33.592)	0.591	25.729 (7.900-43.662)	0.241
Family *Enterobacteriaceae*	14.843 (4.620-33.061)	14.734 (7.075-31.039)	0.577	24.350 (7.769-42.174)	0.151
Phylum Verrucomicrobia	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.509	0.000 (0.000-0.001)	0.736
Genus_*Akkermansia*	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.509	0.000 (0.000-0.001)	0.736

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI<25)

Table 3.7
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births with IAP in normal weight mothers, according to the duration of active first stage of labour (n=108)

Bacterial Taxa	1st Stage of labour <= 6 hours [Reference group]	1st Stage of labour > 6 to <=13 hours	p-value	1st Stage of labour > 13 hours	p-value
	(n=40; 37.0%)	(n= 48; 44.4%)		(n=20; 18.5%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	4.912 (2.076-14.877)	2.483 (0.229-7.578)	**0.016**	5.711 (1.210-19.453)	0.962
Family *Bifidobacteriaceae*	4.493 (2.039-12.324)	2.285 (0.054-7.251)	**0.020**	5.101 (0.849-19.205)	0.875
Family *Coriobacteriaceae*	0.019 (0.000-0.122)	0.023 (0.008-0.134)	0.552	0.035 (0.008-0.190)	0.658
Genus_*Bifidobacterium*	4.493 (2.039-12.324)	2.285 (0.054-7.246)	0.019	5.101 (0.849-19.205)	0.875
Phylum Bacteroidetes	5.464 (0.054-68.602)	7.575 (0.046-67.733)	0.887	8.569 (0.049-55.431)	0.707
Family *Bacteroidaceae*	2.290 (0.046-61.340)	3.383 (0.046-64.342)	0.789	6.084 (0.033-53.672)	0.578
Phylum Firmicutes	21.225 (9.713-46.318)	28.908 (8.943-55.611)	0.476	32.654 (13.330-52.666)	0.301
Family *Lactobacillaceae*	0.000 (0.000-0.050)	0.000 (0.000-0.016)	0.885	0.000 (0.000-0.000)	0.062
Family *Streptoccocaceae*	0.562 (0.134-1.574)	0.819 (0.284-2.394)	0.127	0.681 (0.342-1.694)	0.331
Family *Clostridiaceae*	0.124 (0.015-2.393)	0.291 (0.019-6.103)	0.425	0.668 (0.029-3.627)	0.323
Family *Lachnospiraceae*	2.425 (0.039-12.053)	0.533 (0.023-7.128)	0.372	0.833 (0.014-10.601)	0.490
Family *Ruminococcaceae*	0.240 (0.000-3.061)	0.008 (0.000-0.362)	**0.046**	0.015 (0.000-2.844)	0.904
Family *Veillionellaceae*	3.861 (0.479-16.569)	10.679 (1.262-22.634)	0.152	14.638 (0.554-31.406)	0.272
Genus_*Lactobacillus*	0.000 (0.000-0.050)	0.000 (0.000-0.016)	0.885	0.000 (0.000-0.000)	0.062
Genus_*Clostridium*	0.016 (0.000-0.400)	0.023 (0.000-0.505)	0.838	0.198 (0.002-0.856)	0.216
Genus_*Veillionella*	3.584 (0.479-16.565)	10.679 (1.073-21.642)	0.191	9.982 (0.366-27.257)	0.541
Phylum Proteobacteria	14.714 (8.869-41.491)	25.432 (15.577-43.282)	0.149	16.729 (6.358-38.882)	0.742
Family *Enterobacteriaceae*	13.704 (6.571-38.955)	23.253 (9.149-42.469)	0.147	16.299 (4.294-35.137)	0.863
Phylum Verrucomicrobia	0.000 (0.000-0.000)	0.000 (0.000-0.008)	0.626	0.000 (0.000-0.008)	0.665
Genus_*Akkermansia*	0.000 (0.000-0.000)	0.000 (0.000-0.008)	0.626	0.000 (0.000-0.008)	0.665

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis.

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI<25)

Table 3.8
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by C-section with active 1st stage of labour in normal weight mothers, according to the duration of active first stage of labour (n= 69)

Bacterial Taxa	**C-section with 1st Stage of labour <= 6 hours [Reference group]**	**C-section with 1st Stage of labour > 6 to <=13 hours**	p-value
	(n= 57; 82.6%)	(n= 12; 17.4%)	
	Median (IQR)	Median (IQR)	
Phylum Actinobacteria	4.584 (0.437-13.187)	7.436 (0.683-53.076)	0.350
Family *Bifidobacteriaceae*	3.985 (0.200-12.583)	6.686 (0.302-52.346)	0.282
Family *Coriobacteriaceae*	0.039 (0.000-0.109)	0.059 (0.002-0.235)	0.602
Genus_*Bifidobacterium*	3.985 (0.200-12.529)	6.686 (0.302-52.325)	0.350
Phylum Bacteroidetes	0.119 (0.039-1.427)	0.128 (0.037-0.245)	0.669
Family *Bacteroidaceae*	0.101 (0.031-0.647)	0.074 (0.025-0.224)	0.457
Phylum Firmicutes	34.292 (21.792-64.305)	29.662 (21.977-81.857)	0.962
Family *Lactobacillaceae*	0.000 (0.000-0.070)	0.000 (0.000-0.000)	0.205
Family *Streptoccocaceae*	0.887 (0.328-2.364)	0.706 (0.308-3.799)	0.740
Family *Clostridiaceae*	1.614 (0.089-7.550)	1.144 (0.262-1.672)	0.194
Family *Lachnospiraceae*	4.812 (0.016-13.154)	4.068 (0.033-21.331)	0.569
Family *Ruminococcaceae*	0.093 (0.004-3.308)	0.282 (0.008-6.427)	0.775
Family *Veillionellaceae*	8.744 (2.328-28.551)	11.674 (5.463-24.262)	0.580
Genus_*Lactobacillus*	0.000 (0.000-0.070)	0.000 (0.000-0.000)	0.205
Genus_*Clostridium*	0.255 (0.027-2.745)	0.2921 (0.087-0.613)	0.831
Genus_*Veillionella*	8.064 (1.104-25.934)	11.662 (5.463-24.232)	0.393
Phylum Proteobacteria	27.777 (14.315-52.52.369)	13.860 (2.659-36.432)	0.053
Family *Enterobacteriaceae*	25.861 (12.688-51.391)	13.711 (1.633-32.321)	0.074
Phylum Verrucomicrobia	0.000(0.000.008)	0.000 (0.000-0.025)	0.655
Genus_*Akkermansia*	0.000(0.000.008)	0.000 (0.000-0.025)	0.655

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI<25)

Table 3.9

Summary table showing **significant** ($p<0.05$) median relative abundance changes of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the duration of second stage of labour among infants born to normal weight mothers, and following stratification by mode by IAP

ALL MODES OF BIRTHS (n=556)		
Reference group: Group 1 (n=379)	Group 2 (n=75)	Group 3 (n=102)
Phylum Actinobacteria	--	↓
Bifidobacteriaceae	--	↓
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Lactobacillaceae	--	↓
Clostridiaceae	--	↓
Phylum Proteobacteria	--	--

Group 1 (Reference group): 2nd stage <=1 hour

Group 2: 2nd stage > 1 to ≤ 2 hours

Group 3: 2nd stage > 2 hours

VAGINAL BIRHTS WITHOUT IAP (n=293)		
Group 1 (Ref) (n=200)	Group 2 (n=45)	Group 3 (n=48)
Phylum Actinobacteria	--	↓
Bifidobacteriaceae	--	↓
Phylum Bacteroidetes	↓	--
Phylum Firmicutes	↑	--
Clostridiaceae	--	↑
Phylum Proteobacteria	--	--

VAGINAL BIRHTS WITH IAP (n=115)		
Group 1 (Ref) (n=54)	Group 2 n=24)	Group 3 (n=37)
Phylum Actinobacteria	--	--
Bifidobacteriaceae	--	--
Phylum Bacteroidetes	--	--
Phylum Firmicutes	↑	--
Clostridiaceae	--	--
Phylum Proteobacteria	--	--

C-SECTION WITH 2nd STAGE (n=69)	
Group 1 (Ref) (n=53)	2nd stage > 1 hour (n=16)
Phylum Actinobacteria	--
Bifidobacteriaceae	--
Phylum Bacteroidetes	--
Phylum Firmicutes	--
Clostridiaceae	--
Phylum Proteobacteria	--

IAP = Intrapartum Antibiotic Prophylaxis
-- indicates no significant change

(Note: Elective C-section excluded from analyses)

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI<25)

Table 3.10
Median relative abundance of dominant bacterial taxa at different taxonomic level in infant gut microbiota at 3-4 months among *all modes* of delivery in normal weight mothers, according to the duration of second stage of labour (n=556)

Bacterial Taxa	2nd Stage of labour <= 1 hour (n=379; 68.2%) Median (IQR)	2nd Stage of labour > 1 to <=2 hours (n=75; 13.5%) Median (IQR)	p-value	2nd Stage of labour > 2 hours (n=102; 18.3%) Median (IQR)	p-value
Actinobacteria	6.300 (1.797-15.943)	5.104 (0.66-14.227)	0.220	3.511 (0.321-13.815)	**0.033**
Actinomycetaceae	0.023 (0.000-0.100)	0.023 (0.000-0.108)	0.622	0.023 (0.000-0.080)	0.681
Bifidobacteriaceae	5.773 (1.612-15.327)	4.487 (0.489-13.424)	0.211	3.290 (0.076-13.427)	**0.040**
Coriobacteriaceae	0.039 (0.008-0.140)	0.054 (0.000-0.155)	0.951	0.015 (0.000-0.104)	**0.013**
g_Bifidobacterium	5.773 (1.612-15.327)	4.487 (0.489-13.424)	0.211	3.290 (0.076-13.427)	**0.040**
Bacteroidetes	18.252 (0.116-60.293)	6.179 (0.070-56.222)	0.237	2.576 (0.070-53.938)	0.116
Bacteroidaceae	13.588 (0.077-56.419)	3.483 (0.055-50.285)	0.196	1.616 (0.045-50.206)	0.130
Firmicutes	22.304 (7.960-46.034)	27.664 (11.527-50.496)	0.085	23.352 (9.480-49.216)	0.276
Enterococcaceae	0.023 (0.000-0.108)	0.016 (0.000-0.116)	0.875	0.039 (0.000-0.124)	0.466
Lactobacillaceae	0.000 (0.000-0.039)	0.000 (0.000-0.023)	0.956	0.000 (0.000-0.008)	**0.012**
Streptoccocaceae	0.564 (0.209-1.877)	0.757 (0.318-2.543)	0.165	0.649 (0.2107-1.651)	0.916
Clostridiaceae	0.255 (0.023-1.884)	0.574(0.023-4.231)	0.285	1.165 (0.063-6.254)	**0.002**
Lachnospiraceae	2.206 (0.031-9.295)	1.389 (0.039-11.440)	0.661	0.581 (0.023-8.707)	0.241
Ruminococcaceae	0.062 (0.000-1.869)	0.023 (0.000-1.370)	0.530	0.008 (0.000-0.746)	**0.032**
Veillionellaceae	4.073 (0.681-16.870)	4.585 (1.097-15.229)	0.774	7.466 (0.497-20.397)	0.439
g_Lactobacillus	0.000 (0.000-0.039)	0.000 (0.000-0.023)	0.956	0.000 (0.000-0.008)	**0.012**
Proteobacteria	18.951 (8.536-39.933)	26.958 (11.257-42.748)	0.269	21.541 (10.616-42.280)	0.223
Enterobacter_unclss	16.723 (6.573-36.741)	26.865 (8.098-42.355)	0.156	20.146 (10.047-40.683)	0.125
Verrucomicrobia	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.507	0.000 (0.000-0.008)	0.995
g_Akkermansia	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.507	0.000 (0.000-0.008)	0.995

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI<25)

Table 3.11
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births without IAP in normal weight mothers, according to the duration of second stage of labour (n=293)

Bacterial Taxa	2nd Stage of labour <= 1 hour [Reference group]	2nd Stage of labour > 1 to <=2 hours	p-value	2nd Stage of labour > 2 hours	p-value
	(n= 200; 68.3%)	(n= 45; 15.4%)		(n= 48; 16.4%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	7.484 (2.292-17.110)	6.978 (1.009-18.320)	0.369	3.426 (0.1718-13.617)	**0.038**
Family *Bifidobacteriaceae*	6.659 (2.224-16.414)	6.533 (0.817-17.796)	0.402	3.290 (0.275-13.563)	**0.049**
Family *Coriobacteriaceae*	0.039 (0.008-0.155)	0.047 (0.000-0.081)	0.398	0.012 (0.000-0.091)	**0.066**
Genus_*Bifidobacterium*	6.659 (2.224-16.414)	6.533 (0.817-17.796)	0.402	3.290 (0.275-13.563)	**0.048**
Phylum Bacteroidetes	39.043 (4.863-64.734)	3.483 (0.081-51.039)	**0.001**	25.895 (0.269-66.759)	0.279
Family *Bacteroidaceae*	35.785 (2.230-61.803)	1.019 (0.070-42.092)	**0.000**	19.415 (0.109-61.981)	0.288
Phylum Firmicutes	15.505 (6.747-32.052)	27.576 (13.194-47.873)	**0.008**	23.460 (5.655-46.579)	0.194
Family *Lactobacillaceae*	0.000 (0.000-0.054)	0.000 (0.000-0.012)	0.976	0.000 (0.000-0.014)	0.132
Family *Streptoccocaceae*	0.552 (0.144-1.846)	0.721 (0.271-1.526)	0.414	0.379 (0.128-1.257)	0.342
Family *Clostridiaceae*	0.108 (0.0.15-0.746)	0.752 (0.023-4.964)	0.014	0.679 (0.065-7.179)	**0.000**
Family *Lachnospiraceae*	1.943 (0.031-8.338)	1.197 (0.039-16.075)	0.549	1.572 (0.039-9.361)	0.975
Family *Ruminococcaceae*	0.054 (0.000-1.327)	0.100 (0008-2.240)	0.535	0.008 (0.000-0.877)	0.127
Family *Veillionellaceae*	2.640 (0479-11.731)	3.485 (1.333-14.381)	0.360	6.791 (0.298-14.617)	0.599
Genus_*Lactobacillus*	0.000 (0.000-0.054)	0.000 (0.000-0.012)	0.976	0.000 (0.000-0.014)	0.132
Genus_*Clostridium*	0.008 (0.000-0.187)	0.117 (0.000-2.276)	**0.013**	0.031 (0.002-3.108)	**0.010**
Genus_*Veillionella*	1.750 (0.173-9.519)	3.485 (1.212-12.248)	0.066	6.130 (0.229-14.611)	0.332
Phylum Proteobacteria	15.395 (7.146-32.240)	30.422 (11.120-47.721)	0.024	17.448 (10.059-39.098)	0.240
Family *Enterobacteriaceae*	14.666 (5.026-29.593)	29.447 (9.770-44.866)	0.008	14,590 (9.650-33.902)	0.207
Phylum Verrucomicrobia	0.000 (0.000-0.008)	0.000 (0.000-0.012)	0.647	0.000 (0.000-0.008)	0.260
Genus_*Akkermansia*	0.000 (0.000-0.008)	0.000 (0.000-0.012)	0.647	0.000 (0.000-0.008)	0.260

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI<25)

Table 3.12
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births with IAP in normal weight mothers, according to the duration of second stage of labour (n=115)

Bacterial Taxa	**2nd Stage of labour <= 1 hour [Reference group]**	**2nd Stage of labour > 1 to <=2 hours**	p-value	**2nd Stage of labour > 2 hours**	p-value
	(n= 54; 47%)	(n= 24; 20.9%)		(n= 37; 32.2%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	4.257 (1.886-17.070)	3.621 (0.399-13.850)	0.346	3.328 (0.227-10.608)	0.101
Family *Bifidobacteriaceae*	3.812 (1.540-16.876)	3.330 (0.066-10.126)	0.256	3.127 (0.039-9.681)	0.082
Family *Coriobacteriaceae*	0.023 (0.008-0.085)	0.077(0.008-0.258)	0.139	0.015 (0.000-0.121)	0.328
Genus_*Bifidobacterium*	3.812 (1.540-16.876)	3.330 (0.066-10.122)	0.256	3.127 (0.031-9.677)	0.078
Phylum Bacteroidetes	17.805 (0.060-69.400)	3.452 (0.048-57.351)	0.398	0.194 (0.043-48.759)	0.139
Family *Bacteroidaceae*	17.755 (0.052-66.818)	3.437 (0.046-56.812)	0.369	0.177 (0.031-45.257)	0.144
Phylum Firmicutes	20.782 (7.396-43.471)	32.015 (16.076-57.797)	**0.027**	33.328 (9.175-53.222)	0.182
Family *Lactobacillaceae*	0.000 (0.000-0.016)	0.000 (0.000-0.052)	0.838	0.000 (0.000-0.004)	0.139
Family *Streptoccocaceae*	0.508 (0.220-1.958)	0.793 (0.379-4.614)	0.104	0.907 (0.205-1.838)	0.328
Family *Clostridiaceae*	0.124 (0.016-1.315)	0.654 (0.035-5.7840	0.104	1.154 (0.035-7.270)	0.055
Family *Lachnospiraceae*	1.375 (0.023-8.899)	1.328 (0.041-9.370)	0.766	0.257 (0.016-7.417)	0.553
Family *Ruminococcaceae*	0.027 (0.000-2.833)	0.019 (0.000-0.713)	0.566	0.008 (0.000-0.457)	0.424
Family *Veillionellaceae*	5.220 (0.444-18.392)	8.887 (1.256-30.807)	0.176	11.484 (0.805-29.344)	0.238
Genus_*Lactobacillus*	0.000 (0.000-0.016)	0.000 (0.000-0.052)	0.838	0.000 (0.000-0.004)	0.139
Genus_*Clostridium*	0.016 (0.000-0.377)	0.019 (0.000-0.819)	0.757	0.093 (0.004-1.397)	0.130
Genus_*Veillionella*	5.049 (0.348-18.291)	8.075 (0.682-24.160)	0.289	11.484 (0.805-29.340)	0.216
Phylum Proteobacteria	17.249 (8.643-42.195)	23.727 (10.294-39.936)	0.931	27.181 (17.280-46.125)	0.075
Family *Enterobacteriaceae*	14.888 (7.503-39.407)	23.517 (4.294-38.678)	1.000	22.673 (17.208-45.327)	**0.036**
Phylum Verrucomicrobia	0.000 (0.000-0.000)	0.000 (0.000-0.006)	0.560	0.000 (0.000-0.008)	0.097
Genus_*Akkermansia*	0.000 (0.000-0.000)	0.000 (0.000-0.006)	0.560	0.000 (0.000-0.008)	0.097

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI<25)

Table 3.13
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by C-section with labour in normal weight mothers, according to the duration of second stage of labour (n= 69)

Bacterial Taxa	**C-section with 2nd Stage of labour <= 1 hour [Reference group]**	**C-section with 2nd Stage of labour > 1 hour**	p-value
	(n= 53; 76.8%)	(n= 16; 23.2%)	
	Median (IQR)	Median (IQR)	
Phylum Actinobacteria	4.143 (0.437-11.138)	13.820 (0.683-53.076)	0.118
Family *Bifidobacteriaceae*	3.872 (0.200-10.004)	13.630 (0.302-52.346)	0.113
Family *Coriobacteriaceae*	0.039 (0.000-0.097)	0.063 (0.002-0.239)	0.412
Genus_*Bifidobacterium*	3.872 (0.200-10.004)	13.630 (0.302-52.325)	0.143
Phylum Bacteroidetes	0.116 (0.039-1.427)	0.139 (0.037-0.399)	0.765
Family *Bacteroidaceae*	0.095 (0.031-0.647)	0.116 (0.025-0.399)	0.629
Phylum Firmicutes	35.457 (24.622-64.305)	24.907 (16.943-71.202)	0.477
Family *Lactobacillaceae*	0.000 (0.000-0.039)	0.000 (0.000-0.052)	0.652
Family *Streptoccocaceae*	0.887 (0.328-2.080)	0.706 (0.308-4.271)	0.943
Family *Clostridiaceae*	1.568 (0.089-7.550)	1.253 (0.262-2.001)	0.460
Family *Lachnospiraceae*	0.4941 (0.015-13.387)	1.488 (0.027-16.282)	0.744
Family *Ruminococcaceae*	0.046 (0.000-3.589)	0.301 (0.008-5.191)	0.732
Family *Veillionellaceae*	10.654 (3.890-29.895)	8.339 (0.876-18.345)	0.348
Genus_*Lactobacillus*	0.000 (0.000-0.039)	0.000 (0.000-0.052)	0.652
Genus_*Clostridium*	0.209 (0.027-2.362)	0.321 (0.087-0.845)	0.836
Genus_*Veillionella*	8.736 (2.329-28.547)	8.327 (0.859-18.308)	0.522
Phylum Proteobacteria	27.777 (14.315-53.120)	15.669 (4.910-34.667)	0.059
Family *Enterobacteriaceae*	25.861 (12.688-52.725)	15.551 (4.885-32.381)	0.085
Phylum Verrucomicrobia	0.000 (0.000-0008)	0.000 (0.000-0.025)	0.328
Genus_*Akkermansia*	0.000 (0.000-0008)	0.000 (0.000-0.025)	0.328

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

CHAPTER 3

REGRESSION ANALYSES: Active 1st stage

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI <25)

Table 3.14

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of active 1st stage of labour among infants of normal weight mothers (n=531)

Microbiota Measure		**Infant's gut microbiota at 3 to 4 months of age**								
Ref. Group 1 = 1st Stage ≤6 Hrs Group 2 = 1st Stage >6 to ≤13Hrs Group 3 = 1st Stage > 13Hrs		**PHYLUM**				**FAMILY**			**GENUS**	
		Actino-bacteria (below vs above median)	**Bacteroidetes** (below vs above median)	**Firmicutes** (below vs above median)	**Proteo-bacteria** (below vs above median)	**Bifidobacteria-ceae** (below vs above median)	**Clostridia-ceae** (below vs above median)	**Veillonella-ceae** (below vs above median)	**Bifidobacterium** (below vs above median)	**Lactobacillus** (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 1st stage of labour	Grp2	0.62 (0.42-0.91)*	1.25 (0.85-1.84)	0.99 (0.67-1.45)	1.10 (0.75-1.61)	0.64 (0.44-0.95)*	0.73 (0.50-1.08)	1.26 (0.86-1.85)	0.64 (0.44-0.95)*	1.13 (0.76-1.66)
	Grp3	0.63 (0.35-1.13)	1.18 (0.66-2.10)	1.16 (0.65-2.09)	1.03 (0.57-1.84)	0.65 (0.37-1.17)	1.18 (0.66-2.11)	0.98 (0.55-1.77)	0.65 (0.37-1.17)	0.44 (0.22-0.87)*
Adjusted for MODE by IAP	Grp2	0.61 (0.40-0.92)*	0.95 (0.63-1.42)	1.26 (0.84-1.89)	1.23 (0.83-1.83)	0.64 (0.42-0.97)*	0.90 (0.60-1.35)	1.61 (1.08-2.41)	0.64 (0.42-0.97)*	1.06 (0.71-1.57)
	Grp3	0.69 (0.37-1.28)	0.89 (0.48-1.64)	1.46 (0.80-2.68)	1.18 (0.65-2.15)	0.72 (0.39-1.34)	1.44 (0.79-2.65)	1.18 (0.65-2.16)	0.72 (0.39-1.34)	0.45(0.22-0.89)*
Adjusted for gestational age	Grp2	0.59 (0.40-0.87)*	1.23 (0.83-1.81)	1.00 (0.68-1.47)	1.09 (0.74-1.61)	0.61 (0.42-0.91)*	0.75 (0.51-1.10)	1.25 (0.85-1.84)	0.61 (0.42-0.91)*	1.15(0.77-1.70)
	Grp3	0.59 (0.33-1.07)	1.15 (0.64-2.06)	1.18 (0.66-2.13)	1.02 (0.57-1.84)	0.62 (0.34-1.12)	1.20 (0.67-2.16)	0.98 (0.54-1.76)	0.62 (0.34-1.12)	0.45(0.23-0.89)*
Adjusted for infant diet at 3 months	Grp2	0.59 (0.40-0.87)*	1.24 (0.84-1.82)	0.98 (0.67-1.45)	1.10 (0.75-1.65)	0.62 (0.42-0.91)*	0.73 (0.50-1.08)	1.28 (0.87-1.89)	0.62 (0.42-0.91)*	1.16(0.78-1.73)
	Grp3	0.63 (0.35-1.14)	1.18 (0.66-2.12)	1.14 (0.63-2.05)	1.03 (0.57-1.86)	0.66 (0.37-1.18)	1.17 (0.65-2.11)	0.97 (0.54-1.75)	0.66 (0.37-1.18)	0.44(0.22-0.88)*
Adjusted for parity	Grp2	0.63 (0.43-0.94)*	1.32 (0.90-1.95)	0.93 (0.63-1.36)	1.05 (0.71-.55)	0.65 (0.44-0.96)*	0.63 (0.42-0.94)*	1.22 (0.82-1.79)	0.65 (0.44-0.96)*	1.14(0.77-1.68)
	Grp3	0.67 (0.37-1.21)	1.32 (0.73-2.38)	1.01 (0.56-1.84)	0.93 (0.52-1.69)	0.68 (0.37-1.23)	0.89 (0.49-1.64)	0.91 (0.51-1.65)	0.68 (0.37-1.23)	0.45(0.22-0.89)*
Adjusted for ROM >18 hours	Grp2	0.62 (0.42-0.92)*	1.23 (0.83-1.81)	1.01 (0.69-1.49)	1.11 (075-1.63)	0.65 (0.44-0.96)*	0.71 (0.48-1.05)	1.24 (0.84-1.83)	0.65 (0.44-0.96)*	1.14(0.77-1.69)
	Grp3	0.59 (0.33-1.08)	1.15 (0.64-2.08)	1.12 (0.62-2.02)	1.05 (0.58-1.89)	0.63 (0.35-1.13)	1.05 (0.58-1.91)	0.93 (0.51-1.67)	0.63 (0.35-1.13)	0.46(0.23-0.91)*
Adjusted for length of hospital stay	Grp2	0.59 (0.40-0.88)*	1.18 (0.79-1.75)	1.10 (0.74-1.64)	1.09 (0.73-1.61)	0.60 (0.41-0.90)*	0.82 (0.55-1.22)	1.34 (0.90-1.20)	0.60 (0.41-0.90)*	1.01(0.68-1.51)
	Grp3	0.68 (0.37-1.23)	1.12 (0.62-2.04)	1.25 (0.69-2.28)	1.00 (0.55-1.81)	0.70 (0.38-1.27)	1.29 (0.71-2.34)	0.95 (0.52-1.72)	0.70 (0.38-1.27)	0.45(0.23-0.89)
Adjusted for age of stool collection	Grp2	0.73 (0.44-1.23)	1.34 (0.80-2.24)	1.37 (0.81-2.32)	1.16 (0.69-1.95)	0.72 (0.43-1.21)	0.94 (0.56-1.58)	1.60 (0.95-2.72)	0.72 (0.43-1.21)	1.78(1.06-3.00)*
	Grp3	0.56 (0.25-1.25)	1.15 (0.52-2.54)	1.10 (0.50-2.44)	1.58 (0.69-3.60)	0.52 (0.24-1.17)	1.66 (0.75-3.71)	0.91 (0.41-2.02)	0.52 (0.24-1.17)	0.45 (0.18-1.10)

Adjusted for Model 1	Grp2	0.57 (0.37-0.86)*	0.94 (0.62-1.44)	1.22 (0.80-1.86)	1.21 (0.80-1.84)	0.59 (0.39-0.89)*	0.75 (0.49-1.15)	1.60 (1.05-2.44)*	0.59 (0.39-0.89)*	1.12 (0.73-1.70)
	Grp3	0.62 (0.33-1.16)	0.91 (0.48-1.72)	1.27 (0.67-2.39)	1.11 (0.59-2.10)	0.64 (0.34-1.19)	1.00 (0.52-1.90)	1.10 (0.59-2.06)	0.64 (0.34-1.19)	0.47 (0.23-0.97)*
Adjusted for Model 2	Grp2	0.52 (0.29-0.95)*	1.05 (0.57-1.92)	1.97 (1.07-3.61)*	1.47 (0.80-2.69)	0.51 (0.28-0.93)*	1.04 (0.57-1.89)	2.31 (1.24-4.32)*	0.51 (0.28-0.93)*	1.63 (0.89-2.99)
	Grp3	0.40 (0.16-1.01)	1.09 (0.42-2.83)	1.11 (0.44-2.81)	1.87 (0.70-4.94)	0.36 (0.14-0.93)*	1.42 (0.55-3.62)	0.69 (0.26-1.79)	0.36 (0.14-0.93)*	0.44 (0.16-1.19)

MODEL 1: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) at 3 months, parity and ROM> 18 hours

MODEL 2: Adjusted for mode by IAP, GA, exclusive breastfeeding (infant diet) at 3 months, parity, ROM>18hr, infant's length of hospital stay, stool collection age

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval;
IAP = Intrapartum antibiotic prophylaxis; GA = gestational age; ROM = rupture of membranes; Grp=Group

CHAPTER 3

REGRESSION ANALYSES: 2nd stage

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI <25)

Table 3.15

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of 2nd stage of labour among infants of normal weight mothers (n= 556)

Microbiota Measure		**Infant's gut microbiota at 3 to 4 months of age**								
Ref. Group 1 = 2nd Stage ≤1 Hrs Group 2 = 2nd Stage > 1 to ≤2Hrs Group 3 = 2nd Stage >2Hrs		**PHYLUM**				**FAMILY**			**GENUS**	
		Actino-bacteria (below vs above median)	**Bacteroidetes** (below vs above median)	**Firmicutes** (below vs above median)	**Proteo-bacteria** (below vs above median)	**Bifidobacteria-ceae** (below vs above median)	**Clostridia-ceae** (below vs above median)	**Veillonella-ceae** (below vs above median)	**Bifidobacterium** (below vs above median)	**Lactobacillus** (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 2nd stage of labour	Grp2	0.75 (0.46-1.23)	0.83 (0.51-1.37)	1.34 (0.81-2.20)	1.24 (0.75-2.04)	0.81 (0.49-.133)	1.33 (0.81-2.19)	1.10 (0.67-1.81)	0.81 (0.49-1.33)	0.99 (0.60-1.63)
	Grp3	0.60 (0.39-0.94)*	0.70 (0.45-1.10)	1.33 (0.86-1.06)	1.34 (0.86-2.08)	0.61 (0.39-0.94)*	1.99 (1.27-2.11)*	1.59 (1.02-2.48)	0.61 (0.39-0.94)*	0.54 (0.33-0.86)*
Adjusted for MODE by IAP	Grp2	0.75 (0.45-1.25)	0.57 (0.34-0.95)*	1.79 (1.06-2.30)*	1.45 (0.97-2.42)	0.80 (0.48-1.32)	1.75 (1.05-2.94)*	1.37(0.82-2.29)	0.80 (0.48-1.32)	0.89 (0.53-1.49)
	Grp3	0.60 (0.38-0.93)*	0.65 (0.41-1.03)	1.40 (0.89-2.19)	1.38 (0.88-2.15)	0.59 (0.38-0.93)*	2.10 (1.33-3.33)	1.65 (1.05-2.59)	0.59 (0.38-0.93)*	0.52 (0.32-0.84)*
Adjusted for gestational age	Grp2	0.73 (0.44-1.20)	0.82 (0.50-1.35)	1.35 (0.82-2.23)	1.24 (0.75-2.05)	0.79 (0.48-1.29)	1.36 (0.83-2.23)	1.09 (0.66-1.79)	0.79 (0.48-1.29)	0.99 (0.60-1.64)
	Grp3	0.58 (0.37-0.91)*	0.69 (0.44-1.08)	1.34 (0.86-2.09)	1.34 (0.86-2.08)	0.59 (0.38-0.92)*	2.02 (1.29-3.17)	1.58 (1.01-2.47)	0.59 (0.38-0.92)*	0.53 (0.33-0.86)*
Adjusted for infant diet at 3 months	Grp2	0.74 (0.44-1.22)	0.87 (0.53-1.44)	1.36 (0.82-2.62)	1.20 (0.72-1.99)	0.79 (0.48-1.31)	1.38 (0.83-2.28)	1.13 (0.68-1.87)	0.79 (0.48-1.31)	0.96 (0.57-1.62)
	Grp3	0.59 (0.44-1.22)*	0.72 (0.46-1.13)	1.34 (0.86-2.09)	1.29 (0.83-2.02)	0.60 (0.38-0.94)*	2.05 (1.31-3.23)*	1.63 (1.04-2.55)*	0.60 (0.38-0.94)*	0.50 (0.31-0.82)*
Adjusted for parity	Grp2	0.79 (0.47-1.30)	0.89 (0.54-1.49)	1.20 (0.72-2.00)	1.14 (0.68-1.90)	0.83 (0.50-1.37)	1.07 (0.64-1.79)	1.07 (0.64-1.77)	0.83 (0.50-1.37)	0.99 (0.60-1.67)
	Grp3	0.64 (0.40-1.02)	0.78 (0.49-1.23)	1.15 (0.72-1.82)	1.19 (0.75-1.89)	0.62 (0.39-0.99)*	1.47 (0.92-2.38)	1.53 (0.96-2.44)	0.62 (0.39-0.99)*	0.54 (0.33-0.89)*
Adjusted for ROM >18 hours	Grp2	0.70 (0.42-1.15)	0.82 (0.50-1.36)	1.37 (0.83-2.27)	1.25 (0.75-2.07)	0.76 (0.46-1.26)	1.28 (0.77-2.12)	1.11 (0.67-1.84)	0.76 (0.46-1.26)	1.02 (0.61-1.69)
	Grp3	0.55 (0.34-0.87)*	0.71 (0.45-1.12)	1.30 (0.82-2.06)	1.35 (0.75-2.14)	0.57 (0.36-0.91)*	1.70 (0.17-2.70)	1.54 (0.97-2.44)	0.57 (0.36-0.91)*	0.55 (0.33-0.90)*
Adjusted for length of hospital stay	Grp2	0.75 (0.46-1.24)	0.86 (0.52-1.42)	1.39 (0.84-2.30)	1.17 (0.71-1.93)	0.81 (0.49-1.33)	1.39 (0.84-2.31)	1.16 (0.70-1.91)	0.81 (0.49-1.33)	0.97 (0.58-1.61)
	Grp3	0.63 (0.40-0.98)*	0.69 (0.44-1.09)	1.30 (0.83-2.05)	1.29 (0.82-2.02)	0.63 (0.40-0.99)*	1.91 (0.21-3.21)*	1.62 (0.13-2.55)*	0.63 (0.40-0.99)*	0.53 (0.32-0.86)*
Adjusted for	Grp2	0.75 (0.45-1.23)	0.84 (0.51-1.39)	1.40 (0.85-2.32)	1.19 (0.72-1.97)	0.81 (0.49-1.32)	1.39 (0.84-2.29)	1.16 (0.70-1.92)	0.81 (0.49-1.32)	0.97 (0.58-1.60)

age of stool collection	Grp3	0.60 (0.38-0.93)*	0.71 (0.46-1.11)	1.41 (0.91-2.21)	1.27 (0.81-1.98)	0.60 (0.39-0.94)*	2.11 (1.34-3.31)*	1.73 (1.10-2.71)*	0.60 (0.39-0.94)*	0.52 (0.32-0.84)*
Adjusted for Model 1	Grp2	0.72 (0.42-1.23)	0.58 (0.33-0.99)*	1.76 (1.02-3.03)*	1.35 (0.79-2.33)	0.75 (0.44-1.29)	1.45 (0.84-2.52)	1.45 (0.85-2.48)	0.76 (0.44-1.29)	0.92 (0.53-1.59)
	Grp3	0.57 (0.35-0.93)*	0.65 (0.39-1.08)	1.28 (0.78-2.11)	1.24 (0.76-2.05)	0.57 (0.35-0.93)*	1.49 (0.90-2.47)	1.70 (1.03-2.76)*	0.57 (0.35-0.93)*	0.51 (0.30-0.87)*
Adjusted for Model 2	Grp2	0.71 (0.41-1.21)	0.60 (0.35-1.04)	1.83 (1.05-3.19)*	1.30 (0.75-2.27)	0.74 (0.43-1.27)	1.50 (0.86-2.63)	1.53 (0.88-2.64)	0.74 (0.43-1.27)	0.91 (0.52-1.59)
	Grp3	0.57 (0.34-0.94)*	0.63 (0.38-1.07)	1.30 (0.78-2.17)	1.20 (0.72-2.00)	0.57 (0.34-0.94)*	1.47 (0.87-2.47)	1.76 (1.05-2.94)*	0.57 (0.35-0.94)*	0.50 (0.29-0.87)*

MODEL 1: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) at 3 months, parity and ROM> 18 hours

MODEL 2: Adjusted for mode by IAP, GA, exclusive breastfeeding (infant diet) at 3 months, parity, ROM>18hr, infant's length of hospital stay, stool collection age

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval;
IAP = Intrapartum antibiotic prophylaxis; GA = gestational age; ROM = rupture of membranes; Grp=Group

RICHNESS and DIVERSITY

INFANTS BORN TO WOMEN WITH **NORMAL PRE-PREGNANCY WEIGHT** (BMI <25)

Table 3.16

Crude and adjusted likelihood ratio of gut microbiota richness and diversity measures at 3-4 months according to duration of active 1st stage of labour among infants of **normal weight mothers (n=531)**

Ref. Group 1 = 1st Stage <= 6 Hours Group 2 = 1st Stage > 6 to <=13 Hrs Group 3 = 1st Stage > 13 Hrs		**Chao1 richness** (below vs above median) OR (95% CI)	**Shannon diversity** (below vs above median) OR (95% CI)
Crude OR for 1st stage of labour	Group2	0.98 (0.67- 1.44)	0.82 (0.56-1.21)
	Group3	1.05 (0.59-1.88)	1.08 (0.60-1.93)
Adjusted for mode by IAP, infant diet at 3 months, parity, ROM > 18 hours	Group2	0.88 (0.58-1.35)	0.89 (0.58-1.35)
	Group3	0.82 (0.43-1.57)	1.12 (0.59-2.12)
* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval; IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes			

Crude and adjusted likelihood ratio of gut microbiota richness and diversity measures at 3-4 months according to duration of second stage of labour among infants of **normal weight mothers (n= 556)**

Ref. Group 1 = 2nd Stage <= 1 Hour Group 2 = 2nd Stage > 1 to <=2 Hrs Group 3 = 2nd Stage > 2 Hrs		**Chao1 richness** (below vs above median) OR (95% CI)	**Shannon diversity** (below vs above median) OR (95% CI)
Crude OR for 2nd stage of labour	Group2	0.87 (0.53-1.43)	1.16 (0.71-1.91)
	Group3	0.81 (0.52-1.25)	0.85 (0.55-1.31)
Adjusted for mode by IAP, infant diet at 3 months, parity, ROM > 18 hours	Group2	0.77 (0.45-1.34)	1.25 (0.72-2.15)
	Group3	0.73 (0.44-1.21)	0.87 (0.53-1.42)
* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval; IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes			

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.17
Summary table showing **significant** ($p<0.05$) in median relative abundance changes of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the duration of active first stage of labour among infants born to overweight mothers, and following stratification by mode by IAP

ALL MODES OF BIRTHS (n=201)		
Reference group: Group 1 (n=120)	Group 2 (n=62)	Group 3 (n=19)
Phylum Actinobacteria	↓	--
Bifidobacteriaceae	--	--
Phylum Bacteroidetes	--	↑
Phylum Firmicutes	--	--
Lactobacillaceae	↓	--
Streptococcaceae	--	↓
Phylum Proteobacteria	--	↓

Group 1 (Reference group): Active 1st stage ≤6 hours

Group 2: Active 1st Stage > 6 to ≤13 hours

Group 3: Active 1st Stage > 13 hours

VAGINAL BIRHTS WITHOUT IAP (n=103)		
Group 1 (Ref) (n=50)	Group 2 (n=40)	Group 3 (n=13)
Phylum Actinobacteria	↓	--
Bifidobacteriaceae	↓	--
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Clostridiaceae	--	↑
Streptococcaceae	--	↓
Phylum Proteobacteria	--	--

VAGINAL BIRHTS WITH IAP (n=43)		
Group 1 (Ref) (n=19	Group 2 n=19)	Group 3 (n=5)
Phylum Actinobacteria	--	--
Bifidobacteriaceae	↓	--
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Clostridiaceae	↑	--
Streptococcaceae	--	--
Phylum Proteobacteria	--	--

C-SECTION WITH ACTIVE 1ST STAGE (n=19)	
Group 1 (Ref) (n=17)	Active 1st stage > 6 hrs (n=2)
Phylum Actinobacteria	--
Bifidobacteriaceae	--
Phylum Bacteroidetes	--
Phylum Firmicutes	--
Clostridiaceae	--
Streptococcaceae	
Phylum Proteobacteria	--

IAP = Intrapartum Antibiotic Prophylaxis
-- indicates no significant change

(Note: Elective C-section excluded from analyses)

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.18
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among all modes of delivery in overweight mothers, according to the duration of active first stage of labour (n= 201)

Bacterial Taxa	1st Stage of labour <= 6 hours [Reference group]	1st Stage of labour > 6 to <=13 hours	p-value	1st Stage of labour > 13 hours	p-value
	(n=120; 59.7%)	(n=62; 30.8%)		(n=19; 9.5%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	7.000 (2.112-19.334)	3.416 (0.857-12.511)	**0.029**	5.332 (0.976-18.759)	0.500
Bifidobacteriaceae	5.688 (1.313-16.675)	3.303 (0.494-12.330)	0.092	3.325 (0.968-18.417)	0.704
Coriobacteriaceae	0.062 (0.008-0.295)	0.039 (0.008-0.172)	0.242	0.008 (0.000-0.163)	0.074
g_Bifidobacterium	5.688 (1.313-16.675)	3.303 (0.494-12.330)	0.092	3.325 (0.968-18.417)	0.704
Bacteroidetes	2.544 (0.077-63.237)	30.557 (0.228-67.709)	0.130	53.228 (2.286-76.915)	**0.009**
Bacteroidaceae	0.963 (0.062-55.954)	23.556 (0.180-57.650)	0.103	47.489 (2.247-76.915)	**0.005**
Firmicutes	16.215 (7.702-38.262)	16.532 (6.927-34.984)	0.676	14.377 (7.379-39.118)	0.641
Lactobacillaceae	0.000 (0.000-0.068)	0.000 (0.000-0.008)	**0.008**	0.000 (0.000-0.016)	0.419
Streptoccocaceae	0.859 (0.213-2.650)	0.643 (0.232-1.569)	0.393	0.209 (0.102-0.427)	**0.004**
Clostridiaceae	0.263 (0.016-1.879)	0.419 (0.047-1.979)	0.303	0.450 (0.140-2.642)	0.184
Lachnospiraceae	1.681 (0.052-8.603)	2.619 (0.062-8.265)	0.554	4.003 (0.581-6.447)	0.272
Ruminococcaceae	0.144 (0.008-1.829)	0.039 (0.000-0.562)	0.145	0.565 (0116-3.341)	0.104
Veillionellaceae	4.004 (0.870-13.181)	2.376 (0.424-12.728)	0.273	2.578 (0.806-7.955)	0.506
g_Lactobacillus	0.000 (0.000-0.068)	0.000 (0.000-0.008)	**0.008**	0.000 (0.000-0.016)	0.419
Proteobacteria	18.930 (7.962-47.522)	21.297 (9.943-41.161)	0.810	8.180 (4.677-22.815)	**0.016**
Enterobacteriaceae	16.972 (5.039-42.885)	18.771 (8.388-41.088)	0.652	5.947 (2.229-20.413)	**0.012**
Verrucomicrobia	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.925	0.000 (0.000-0.008)	0.788
g_Akkermansia	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.925	0.000 (0.000-0.008)	0.788

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.19
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births without IAP in overweight mothers, according to the duration of active first stage of labour (n=103)

Bacterial Taxa	1st Stage of labour <= 6 hours [Reference group]	1st Stage of labour > 6 to <=13 hours	p-value	1st Stage of labour > 13 hours	p-value
	(n=50; 48.5%)	(n=40; 38.8%)		(n=13; 12.6%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	9.808 (4.121-23.980)	3.634 (0.922-12.078)	**0.007**	6.675 (1.533-15.951)	0.393
Family *Bifidobacteriaceae*	8.913 (2.441-18.695)	3.518 (0.519-11.906)	**0.033**	4.864 (1.451-15.577)	0.530
Family *Coriobacteriaceae*	0.140 (0.045-0.678)	0.027 (0.000-0.168)	**0.005**	0.008 (0.000-0.230)	**0.015**
Genus_*Bifidobacterium*	8.913 (2.441-18.695)	3.503 (0.519-11.906)	**0.034**	4.864 (1.451-15.577)	0.530
Phylum Bacteroidetes	46.613 (1.236-68.114)	34.452 (4.363-68.427)	0.942	53.228 (21.299-73.848)	0.308
Family *Bacteroidaceae*	34.953 (0.497-62.515)	25.545 (3.364-59.636)	0.845	48.020 (21.280-73.848)	0.144
Phylum Firmicutes	11.717 (6.673-23.444)	18.502 (6.925-34.225)	0.262	18.509 (8.214-38.371)	0.221
Family *Lactobacillaceae*	0.000 (0.000-0.041)	0.000 (0.000-0.008)	0.244	0.000 (0.000-0.012)	0.262
Family *Streptoccocaceae*	0.798 (0.207-2.460)	0.458 (0.168-1.299)	0.259	0.179 (0.090-0.342)	**0.011**
Family *Clostridiaceae*	0.086 (0.014-0.632)	0.171 (0.023-1.582)	0.185	0.722 (0.157-7.583)	**0.003**
Family *Lachnospiraceae*	1.784 (0.285-7.538)	2.586 (0.056-7.932)	0.981	3.438 (0.492-6.338)	0.747
Family *Ruminococcaceae*	0.464 (0.008-2.637)	0.031 (0.000-0.660)	**0.041**	0.467 (0.194-2.726)	0.633
Family *Veillionellaceae*	2.305 (0.561-6.413)	4.240 (0.551-15.675)	0.256	3.834 (0.717-14.651)	0.415
Genus_*Streptococcaceae*	0.798 (0.207-2.450)	0.458 (0.168-1.299)	0.259	0.179 (0.090-0.342)	**0.011**
Genus_*Lactobacillus*	0.000 (0.0000-0.041)	0.000 (0.000-0.008)	0.244	0.000 (0.000-0.012)	0.262
Genus_*Clostridium*	0.008 (0.000-0.109)	0.008 (0.000-0.214)	0.890	0.016 (0.000-6.985)	0.384
Genus_*Veillionella*	0.897 (0.114-4.623)	1.847 (0.211-13.337)	0.299	1.368 (0.436-9.888)	0.445
Phylum Proteobacteria	15.629 (5.610-35.087)	18.855 (9.496-41.345)	0.381	8.180 (5.283-21.458)	0.135
Family *Enterobacteriaceae*	13.048 (2.782-33.664)	16.404 (7.571-41.318)	0.180	6.092 (1.499-21.206)	0.203
Phylum Verrucomicrobia	0.000 (0.000-0.015)	0.000 (0.000-0.008)	0.301	0.000 (0.000-0.008)	0.790
Genus_*Akkermansia*	0.000 (0.000-0.015)	0.000 (0.000-0.008)	0.301	0.000 (0.000-0.008)	0.790

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.20
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births with IAP in overweight mothers, according to the duration of active first stage of labour (n=43)

Bacterial Taxa	1st Stage of labour <= 6 hours [Reference group]	1st Stage of labour > 6 to <=13 hours	p-value Exact	1st Stage of labour > 13 hours	p-value Exact
	(n=19; 44.2%)	(n=19; 44.2%)		(n=5; 11.6%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	5.708 (0.679-30.569)	2.494 (0.650-10.133)	0.402	2.451 (0.479-36.935)	0.836
Family *Bifidobacteriaceae*	5.654 (0.503-30.251)	2.414 (0.402-9.231)	0.370	2.118 (0.428-36.714)	0.891
Family *Coriobacteriaceae*	0.031 (0.000-0.109)	0.039 (0.016-0.210)	0.246	0.031 (0.008-0.194)	0.629
Genus_*Bifidobacterium*	4.604 (1.450-14.629)	2.267 (0.058-6.640)	**0.008**	3.670 (0.717-19.039)	0.717
Phylum Bacteroidetes	4.794 (0.054-78.533)	0.265 (0.039-55.870)	0.418	35.928 (0.397-72.037)	0.731
Family *Bacteroidaceae*	0.667 (0.046-74.033)	0.218 (0039-55.613)	0.583	32.361 (0.078-65.831)	0.679
Phylum Firmicutes	12.258 (5.334-37.636)	16.781 (6.942-57.107)	0.339	7.379 (4.593-63.297)	0.891
Family *Lactobacillaceae*	0.000 (0.000-0.116)	0.000 (0.000-0.008)	0.311	0.031 (0.004-0.116)	0.406
Family *Streptoccocaceae*	0.324 (0.109-5.336)	0.818 (0.338-2.868)	0.354	0.427 (0.164-0.915)	1.00
Family *Clostridiaceae*	0.023 (0.008-2.539)	0.825 (0.110-6.270)	**0.022**	0.070 (0.023-0.499)	0.891
Family *Lachnospiraceae*	0.231 (0.031-4.026)	2.309 (0.732-8.798)	0.130	4.003 (0.728-48.142)	0.088
Family *Ruminococcaceae*	0.239 (0.008-1.576)	0.047 (0.008-0.464)	0.452	1.142 (0.341-6.401)	0.103
Family *Veillionellaceae*	3.399 (0.217-6.655)	1.752 (0.317-7.727)	0.977	2.578 (1.927-4.089)	0.836
Genus_*Streptococcaceae*	0.402 (0.109-1.565)	0.794 (0.272-2.394)	0.033	0.574 (0.335-1.381)	0.117
Genus_*Lactobacillus*	0.000 (0.000-0.016)	0.000 (0.000-0.015)	0.643	0.000 (0.000-0.008)	0.553
Genus_*Clostridium*	0.016 (0.000-0.366)	0.051 (0.000-0.536)	0.266	0.035 (0.000-0.571)	0.815
Genus_*Veillionella*	3.300 (0.383-11.163)	6.766 (0.578-16.743)	0.205	2.276 (0.325-21.268)	0.786
Phylum Proteobacteria	15.664 (7.665-27.001)	23.992 (11.765-41.063)	0.096	20.421 (4.493-34.639)	0.945
Family *Enterobacteriaceae*	15.243 (6.844-26.776)	23.693 (9.916-37.359)	0.163	5.259 (4.312-21.614)	0.629
Phylum Verrucomicrobia	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.863	0.000 (0.000-0.004)	0.891
Genus_*Akkermansia*	0.000 (0.000-0.008)	0.000 (0.000-0.008)	0.863	0.000 (0.000-0.008)	0.891

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

CHAPTER 3

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY**

OVERWEIGHT (BMI ≥25 to <30)

Table 3.21
Summary table showing **significant** ($p<0.05$) in median relative abundance changes of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the duration of second stage of labour among infants born to overweight mothers, and following stratification by mode by IAP

ALL MODES OF BIRTHS (n=208)		
Reference group: Group 1 (n=379)	Group 2 (n=75)	Group 3 (n=102)
Phylum Actinobacteria	--	--
Bifidobacteriaceae	--	--
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Phylum Proteobacteria	--	--

Group 1 (Reference group): 2nd stage ≤1 hour

Group 2: 2nd stage > 1 to ≤ 2 hours

Group 3: 2nd stage > 2 hours

VAGINAL BIRHTS WITHOUT IAP (n=109)		
Group 1 (Ref) (n=72)	Group 2 (n=20)	Group 3 (n=17)
Phylum Actinobacteria	--	--
Bifidobacteriaceae	--	↓
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Ruminococcaceae	↓	
genus_Veillionella	--	↑
Phylum Proteobacteria	--	--

VAGINAL BIRHTS WITH IAP (n=43)		
Group 1 (Ref) (n=65)	Group 2 (n=5)	Group 3 (n=10)
Phylum Actinobacteria	--	--
Bifidobacteriaceae	--	--
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Streptococcaceae	--	↓
genus_Veillionella	↓	↓
Phylum Proteobacteria	--	--

C-SECTION WITH 2nd STAGE (n=19)	
Group 1 (Ref) (n=15)	Active 2nd stage > 1 hour (n=4)
Phylum Actinobacteria	--
Bifidobacteriaceae	--
Phylum Bacteroidetes	--
Phylum Firmicutes	--
Ruminococcaceae	--
genus_Veillionella	--
Phylum Proteobacteria	--

IAP = Intrapartum Antibiotic Prophylaxis
-- indicates no significant change

(Note: Elective C-section excluded from analyses)

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.22
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among all modes of delivery in overweight mothers, according to the duration of second stage of labour (n= 208)

Bacterial Taxa	**2nd Stage of labour <= 1 hour [Reference group]**	**2nd Stage of labour > 1 to <=2 hours**	p-value	**2nd Stage of labour > 2 hours**	p-value
	(n= 150;72.1%)	(n= 26; 12.5%)		(n= 32; 15.4%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum					
Actinobacteria	6.076 (1.861-15.408)	8.729 (0.141-25.500)	0.953	3.880 (0.899-14.425)	0.535
Bifidobacteriaceae	5.433 (1.324-14.089)	8.288 (0.108-25.081)	0.872	2.925 (0.572-12.679)	0.280
Coriobacteriaceae	0.054 (0.008-0.259)	0.055 (0.006-0.151)	0.467	0.043 (0.008-0.273)	0.956
g_Bifidobacterium	5.433 (1.324-14.089)	8.288 (0.108-25.081)	0.872	2.925 (0.572-12.679)	0.280
Bacteroidetes	23.868 (0.094-63.324)	25.436 (0.102-61.764)	0.930	41.159 (0.315-74.830)	0.094
Bacteroidaceae	17.349 (0.077-53.370)	22.191 (0.078-61.201)	0.841	28.981 (0.118-70.575)	0.106
Firmicutes	18.613 (7.971-39.067)	12.912 (6.927-34.221)	0.280	13.284 (5.026-26.955)	0.090
Lactobacillaceae	0.000 (0.000-0.054)	0.000 (0.000-0.014)	0.139	0.000 (0.000-0.045)	0.732
Streptoccocaceae	0.644 (0.233-1.885)	0.502 (0.147-2.155)	0.887	0.283 (0.156-1.580)	0.266
Clostridiaceae	0.269 (0.023-1.885)	0.442 (0.014-8.520)	0.753	0.322 (0.035-1.390)	1.00
Lachnospiraceae	1.823 (0.052-9.297)	1.984(0.054-5.955)	0.442	3.795 (0.772-6.392)	0.455
Ruminococcaceae	0.201 (0.008-1.789)	0.016 (0.000-1.302)	0.141	0.109 (0.002-0.976)	0.387
Veillionellaceae	4.426 (0.908-14.402)	1.337 (0.489-8.998)	0.072	1.922 (0.690-6.818)	0.170
g_Lactobacillus	0.000 (0.000-0.054)	0.000 (0.000-0.014)	0.139	0.000 (0.000-0.045)	0.732
Proteobacteria	19.771 (7.768-40.985)	16.956 (7.533-42.926)	0.990	10.267 (4.956-41.345)	0.234
Enterobacteriaceae	16.992 (5.221-37.464)	14.601 (4.132-42.850)	0.977	9.929 (4.270-41.318)	0.520
Verrucomicrobia	0.000 (0.000-0.008)	0.000 (0.000-0.000)	0.219	0.000 (0.000-0.023)	0.071
g_Akkermansia	0.000 (0.000-0.008)	0.000 (0.000-0.000)	0.219	0.000 (0.000-0.023)	0.071

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.23
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births without IAP in overweight mothers, according to the duration of second stage of labour (n=109)

Bacterial Taxa	2nd Stage of labour <= 1 hour [Reference group]	2nd Stage of labour > 1 to <=2 hours	p-value Exact	2nd Stage of labour > 2 hours	p-value Exact
	(n= 72; 66.1%)	(n= 20; 18.3%)		(n= 17; 15.6%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	7.276 (2.422-16.028)	10.751 (0.178-21.008)	0.880	3.846 (1.300-11.848)	0.297
Family *Bifidobacteriaceae*	6.652 (1.925-14.570)	10.425 (0.132-20.947)	0.992	2.856 (0.579-5.737)	**0.052**
Family *Coriobacteriaceae*	0.128 (0.015-0.595)	0.039 (0.000-0.114)	**0.035**	0.054 (0.008-1.028)	0.814
Genus_*Bifidobacterium*	6.652 (1.869-14.570)	10.425 (0.120-20.947)	0.970	2.856 (0.0579-5.737)	**0.052**
Phylum Bacteroidetes	51.639 (7.905-69.746)	29.808 (0.231-59.194)	0.103	54.093 (0.747-72.237)	0.950
Family *Bacteroidaceae*	40.164 (7.814-61.742)	26.399 (0.100-57.552)	0.205	25.601 (0.568-67.510)	0.851
Phylum Firmicutes	13.549 (6.807-28.922)	12.912 (7.093-36.343)	0.784	13.987 (6.497-32.710)	0.738
Family *Lactobacillaceae*	0.000 (0.000-0.021)	0.000 (0.000-0.029)	0.377	0.000 (0.000-0.015)	0.981
Family *Streptoccocaceae*	0.457 (0.167-1.246)	0.549 (0.144-2.323)	0.379	0.317 (0.156-1.664)	0.835
Family *Clostridiaceae*	0.132 (0.16-0.739)	0.415 (0.010-5.848)	0.421	0.142 (0.027-2.011)	0.545
Family *Lachnospiraceae*	1.846 (0.287-8.063)	1.997 (0.062-8.659)	0.733	3.459 (0.785-6.151)	0.770
Family *Ruminococcaceae*	0.307 (0.008-2.262)	0.008 (0.000-1.455)	**0.043**	0.212 (0.012-1.078)	0.600
Family *Veillionellaceae*	2.497 (0.422-12.271)	2.625 (0.730-12.875)	0.865	4.585 (1.665-18.461)	0.207
Genus_*Streptococcaceae*	0.457 (0.167-1.246)	0.549 (0.144-2.323)	0.379	0.317 (0.156-1.664)	0.835
Genus_*Lactobacillus*	0.000 (0.000-0.021)	0.000 (0.000-0.029)	0.377	0.000 (0.000-0.015)	0.981
Genus_*Clostridium*	0.008 (0.000-0.119)	0.016 (0.002-1.096)	0.145	0.008 (0.000-0.055)	0.922
Genus_*Veillionella*	0.830 (0.131-8.350)	0.951 (0.250-11.757)	0.541	4.494 (1.396-18.446)	**0.041**
Phylum Proteobacteria	16.146 (5.211-32.823)	16.956 (8.730-45.833)	0.316	10.586 (8.130-38.075)	0.794
Family *Enterobacteriaceae*	13.710 (2.941-29.263)	14.601 (3.932-45.808)	0.410	9.941 (3.011-37.357)	0.859
Phylum Verrucomicrobia	0.000 (0.000-0.008)	0.000 (0.000-0.006)	0.296	0.008 (0.000-0.089)	0.072
Genus_*Akkermansia*	0.000 (0.000-0.008)	0.000 (0.000-0.006)	0.296	0.008 (0.000-0.089)	0.072

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.24
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births with IAP in overweight mothers, according to the duration of second stage of labour (n=43)

Bacterial Taxa	2nd Stage of labour <= 1 hour [Reference group]	2nd Stage of labour > 1 to <=2 hours	p-value Exact	2nd Stage of labour > 2 hours	p-value Exact
	(n= 28; 65.1%)	(n= 5; 11.6%)		(n= 10; 23.3%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	2.725 (0.776-16.868)	2.808 (0.104-60.434)	0.865	4.475 (0.672-21.181)	0.858
Family *Bifidobacteriaceae*	2.586 (0.469-16.011)	2.414 (0.074-59.380)	0.981	3.875 (0.510-21.098)	0.961
Family *Coriobacteriaceae*	0.035 (0.008-0.091)	0.226 (0.008-0.806)	0.314	0.024 (0.008-0.186)	0.987
Genus_*Bifidobacterium*	2.586 (0.469-16.011)	2.414 (0.070-59.380)	0.942	3.875 (0.510-21.098)	0.961
Phylum Bacteroidetes	2.750 (0.048-63.179)	0.086 (0.032-44.018)	0.575	41.159 (0.228-91.501)	0.116
Family *Bacteroidaceae*	0.581 (0.046-57.682)	0.086 (0.016-43.949)	0.509	35.230 (0.106-91.501)	0.116
Phylum Firmicutes	23.271 (6.157-55.587)	16.781 (7.161-52.213)	0.903	7.848 (4.000-18.334)	0.060
Family *Lactobacillaceae*	0.000 (0.000-0.044)	0.000 (0.000-0.020)	0.903	0.004 (0.000-0.131)	0.482
Family *Streptoccocaceae*	1.165 (0.330-3.514)	0.338 (0.124-7.614)	0.364	0.240 (0.130-0.731)	**0.037**
Family *Clostridiaceae*	0.133 (0.017-4.028)	0.780 (0.023-14.661)	0.609	0.689 (0.039-1.263)	0.782
Family *Lachnospiraceae*	1.620 (0.049-9.895)	2.309 (0.366-4.565)	0.827	2.752 (0.544-8.322)	0.757
Family *Ruminococcaceae*	0.288 (0.019-1.104)	0.239 (0.008-34.917)	0.903	0.070 (0.000-0837)	0.230
Family *Veillionellaceae*	3.734 (1.052-10.498)	0.241 (0.035-2.620)	**0.022**	1.143 (0.269-4.563)	0.116
Genus_*Streptococcaceae*	1.165 (0.290-3.514)	0.338 (0.124-7.614)	0.391	0.240 (0.130-0.731)	**0.040**
Genus_*Lactobacillus*	0.000 (0.000-0.044)	0.000 (0.000-0.020)	0.903	0.004 (0.000-0.131)	0.482
Genus_*Clostridium*	0.012 (0.000-0.290)	0.232 (0.004-11.024)	0.314	0.066 (0.000-0.787)	0.442
Genus_*Veillionella*	3.355 (0.694-10.498)	0.132 (0.027-1.444)	**0.006**	0.557 (0.119-2.293)	**0.034**
Phylum Proteobacteria	19.952 (11.951-37.451)	23.992 (8.221-55.960)	0.827	16.370 (3.963-57.188)	0.708
Family *Enterobacteriaceae*	17.982 (7.853-26.311)	23.992 (7.806-54.284)	0.509	16.366 (3.728-56.941)	0.858
Phylum Verrucomicrobia	0.000 (0.000-0.008)	- -	0.268	0.000 (0.000-0.016)	0.883
Genus_*Akkermansia*	0.000 (0.000-0.008)	- -	0.268	0.000 (0.000-0.016)	0.883

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.25
Median relative abundance of dominant bacterial taxa at the genus level in infant gut microbiota at 3-4 months among overweight mothers with C-section with labour, according to the duration of second stage of labour (n =19)

Bacterial Taxa	2nd Stage of labour <= 1 hour [Reference group]	2nd Stage of labour > 1 hour	p-value Exact
	(n= 15; 78.9%)	(n=4; 21.1%)	
	Median (IQR)	Median (IQR)	
	N (%); IQR	N (%); IQR	
Actinobacteria	5.037 (0.248-16.530)	16.037 (3.540-51.045)	0.307
g_Actinomyces	0.031 (0.000-0.093)	0.078 (0.014-1.868)	0.530
g_Bifidobacterium	3.519 (0.070-16.500)	15.808 (3.225-47.545)	0.411
Bacteroidetes	0.109 (0.039-0.279)	0.288 (0.027-43.529)	0.961
g_*Bacteroides*	0.086 (0031-0.209)	0.269 (0.025-42.506)	1.00
Firmicutes	25.162 (16.201-61.669)	20.916 (11.775-30.931)	0.357
g_Enterococcus	0.015 (0.000-0.147)	0.592 (0.045-1.457)	0.221
g_Lactobacillus	0.000 (0.000-0.077)	0.000 (0.000-.046)	0.530
g_Streptococcus	1.209 (0.551-3.223)	1.457 (0.449-16.662)	0.665
g_Clostridia	0.384 (0.008-2.364)	0.070 (0.006-1.146)	0.665
g_Ruminococcus_L	0.039 (0.000-2.654)	3.969 (0.002-15.931)	0.596
g_Oscillospira	0.008 (0.000-0.031)	0.284 (0.010-0.805)	0.221
g_Veilloinella	9.652 (1.499-16.958)	0.858 (0.398-4.066)	0.080
Proteobacteria	37.253 (13.836-69.584)	39.420 (6.054-74.851)	0.810
g_*Citrobacter*	0.614 (0.062-1.499)	0.070 (0.006-0.446)	0.152
g_Enterobacter_unclss	34.647 (8.891-66.643)	39.068 (5.777-74.689)	0.961
Verrucomicrobia	0.000 (0.000-0.008)	0.000 (0.000-1.582)	0.885
g_*Akkermansia*	0.000 (0.000-0.008)	0.000 (0.000-1.582)	0.885

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

REGRESSION ANALYSES: Active 1st stage

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.26a

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of active 1st stage of labour among infants of overweight mothers (n=201)

Ref. Group 1 = 1st Stage ≤6 Hrs Group 2 = 1st Stage >6 to ≤13Hrs Group 3 = 1st Stage > 13Hrs		**Infant's gut microbiota at 3 to 4 months of age**						
		PHYLUM (below versus above median)				**FAMILY** (below versus above median)		
		Actino-bacteria	**Bacteroidetes**	**Firmicutes**	**Proteo-bacteria**	**Bifidobacte-riaceae**	**Bacteroida-ceae**	**Veillonella-ceae**
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 1st stage of labour	Grp 2	0.61 (0.33-1.13)	1.94 (1.03-3.63)*	1.04 (0.56-1.94)	1.21 (0.66-2.25)	1.94 (1.03-3.63)*	1.94 (1.03-3.63)*	0.72 (0.39-1.34)
	Grp 3	0.62 (0.24-1.64)	3.20(1.08-9.44)*	0.62 (0.22-1.75)	0.43 (0.15-1.21)	3.20 (1.08-9.44)*	3.20(1.08-9.44)*	0.49 (0.18-1.38)
Adjusted for MODE by IAP	Grp 2	0.49 (0.25-0.96)*	1.16(0.58-2.32)	1.46 (0.74-2.89)	1.63(0.83-3.18)	1.16 (0.58-2.32)	1.16(0.58-2.32)	0.99 (0.51-1.93)
	Grp 3	0.56 (0.20-1.58)	1.66(0.53-5.20)	0.97 (0.33-2.89)	0.63(0.21-1.85)	1.66 (0.53-5.20)	1.66(0.53-5.20)	0.75 (0.25-2.20)
Adjusted for infant diet	Grp 2	0.60 (0.32-1.13)	1.98(1.04-3.78)*	1.04 (0.56-1.93)	1.25(0.66-2.36)	1.98 (1.04-3.78)*	1.98(1.04-3.78)*	0.71 (0.38-1.33)
	Grp 3	0.69 (0.25-1.91)	3.00(0.99-9.11)	0.61 (0.22-1.71)	0.47(0.16-1.35)	3.00 (0.99-9.11)	3.00(0.99-9.11)	0.46 (0.16-1.31)
Adjusted for parity	Grp 2	0.62 (0.33-1.15)	1.92(1.02-3.61)*	1.15 (0.61-2.17)	1.25(0.67-2.33)	1.92 (1.02-3.61)*	1.92(1.02-3.61)*	0.73 (0.39-1.36)
	Grp 3	0.64 (0.24-1.71)	3.16(1.06-9.37)*	0.72 (0.25-2.05)	0.45(0.16-1.27)	3.16 (1.06-9.37)*	3.16(1.06-9.37)*	0.50 (0.18-1.40)
Adjusted for ROM >18 hours	Grp 2	0.59 (0.32-1.12)	1.96(1.04-3.70)*	1.00 (0.53-1.87)	1.18(0.63-2.21)	1.96(1.04-3.70)*	1.96(1.04-3.70)*	0.66 (0.35-1.24)
	Grp 3	0.66 (0.24-1.81)	3.04(1.01-9.15)*	0.66 (0.23-1.89)	0.44(0.15-1.26)	3.04 (1.01-9.15)*	3.04(1.01-9.15)*	0.47 (0.16-1.36)
Adjusted for infant gender	Grp 2	0.59 (0.31-1.09)	1.80 (0.95-3.41)	1.09 (0.58-2.04)	1.24 (0.66-2.32)	0.66 (0.35-1.23)	2.06 (1.09-3.91)	0.76 (0.41-1.44)
	Grp 3	0.63 (0.24-1.68)	3.41 (1.15-10.16)	0.61 (0.22-1.71)	0.43 (0.15-1.20)	0.60 (0.22-1.59)	3.91 (1.31-11.66)	0.47 (0.17-1.33)

MODEL 1	Grp 2	**0.45 (0.22-0.95)***	1.06 (0.50-2.23)	1.80 (0.86-3.75)	1.85 (0.90-3.84)	1.06 (0.50-2.23)	1.06 (0.50-2.23)	0.95 (0.47-1.92)
	Grp 3	0.70 (0.21-2.29)	1.16 (0.34-3.94)	1.56 (0.48-5.13)	0.84 (0.26-2.72)	1.16 (0.34-3.94)	1.16 (0.34-3.94)	0.75 (0.24-2.36)
MODEL 2	Grp 2	**0.40 (0.19-0.87)***	0.95 (0.44-1.03)	1.88 (0.90-3.96)	1.86 (0.89-3.89)	0.50 (0.24-1.07)	0.95 (0.44-1.03)	0.99 (0.48-2.03)
	Grp 3	0.75 (0.23-2.48)	1.28 (0.37-4.47)	1.49 (0.45-4.94)	0.83 (0.25-2.74)	0.72 (0.22-2.39)	1.28 (0.37-4.47)	0.71 (0.22-2.29)

MODEL 1: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity and ROM> 18 hours

MODEL 2: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity, ROM> 18 hours and infant gender

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval;
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes; Grp=Group

REGRESSION ANALYSES: Active 1st stage
INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT**
(BMI ≥25 to <30)

Table 3.26b

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of active 1st stage of labour among infants of overweight mothers (n=201)

Ref. Group 1 = 1st Stage ≤6 Hrs Group 2 = 1st Stage >6 to ≤13Hrs Group 3 = 1st Stage > 13Hrs		**Infant's gut microbiota at 3 to 4 months of age**		
		GENUS		
		Bifidobacterium (below vs above median)	**Bacteroides** (below vs above median)	**Lactobacillus** (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 1st stage of labour	Grp2	1.94 (1.03-3.63)*	1.94 (1.03-3.63)*	0.44(0.23-0.84)*
	Grp3	3.20(1.08-9.44)*	3.20(1.08-9.44)*	0.78(0.29-2.07)
Adjusted for MODE by IAP	Grp2	1.16(0.58-2.32)	1.16(0.58-2.32)	0.48(0.24-0.95)*
	Grp3	1.66(0.53-5.20)	1.66(0.53-5.20)	0.92(0.33-2.58)
Adjusted for infant diet	Grp2	1.98(1.04-3.78)*	1.98(1.04-3.78)*	0.44(0.23-0.84)*
	Grp3	3.00(0.99-9.11)	3.00(0.99-9.11)	0.83(0.31-2.24)
Adjusted for parity	Grp2	1.92(1.02-3.61)*	1.92(1.02-3.61)*	0.45(0.23-0.87)*
	Grp3	3.16(1.06-9.37)*	3.16(1.06-9.37)*	0.81(0.30-2.18)
Adjusted for ROM >18 hours	Grp2	1.96(1.04-3.70)*	1.96(1.04-3.70)*	0.44(0.23-0.85)*
	Grp3	3.04(1.01-9.15)*	3.04(1.01-9.15)*	0.69(0.25-1.93)
Adjusted for infant gender	Grp2	0.66 (0.35-1.23)	2.06(1.09-3.91)*	0.44 (0.22-0.85)*
	Grp3	0.60 (0.22-1.59)	3.91 (1.31-11.66)	0.78 (0.29-2.08)

MODEL 1	Grp2	1.80 (0.95-3.41)	1.80 (0.95-3.41)	0.49 (0.24-1.01)
	Grp3	3.41 (1.15-10.16)	3.41 (1.15-10.16)	0.96 (0.32-2.95)
MODEL 2	Grp2	0.50 (0.24-1.07)	1.104 (0.49-2.22)	0.48 (0.23-1.00)
	Grp3	0.72 (0.22-2.39)	1.39 (0.40-4.76)	0.98 (0.32-3.02)

MODEL 1: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity and ROM> 18 hours

MODEL 2: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity, ROM> 18 hours and infant gender

* $p < 0.05$; ** $p < 0.005$; OR = odds ratio; CI = confidence interval;
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes; Grp=Group

REGRESSION ANALYSES: 2nd stage

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.27a

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of 2nd stage of labour among infants of overweight mothers (n= 208)

Ref. Group 1 = 2nd Stage ≤1 Hrs Group 2 = 2nd Stage > 1 to ≤2Hrs Group 3 = 2nd Stage >2Hrs		**Infant's gut microbiota at 3 to 4 months of age**						
		PHYLUM (below versus above median)				**FAMILY** (below versus above median)		
		Actino-bacteria	**Bacteroidetes**	**Firmicutes**	**Proteo-bacteria**	**Bifidobac-teriaceae**	**Bacteroid aceae**	**Veillonella-ceae**
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 2nd stage of labour	Grp 2	1.16 (0.50-2.70)	1.05 (0.46-2.42)	0.61 (0.25-1.44)	0.88 (0.38-2.01)	1.05 (0.46-2.42)	1.05 (0.46-2.42)	0.46 (0.19-1.11)
	Grp 3	0.66 (0.31-1.43)	1.50 (0.68-3.28)	0.52 (0.23-1.17)	0.60 (0.28-1.30)	1.50 (0.68-3.28)	1.50 (0.68-3.28)	0.70 (0.32-1.53)
Adjusted for MODE by IAP	Grp 2	1.00 (0.42-2.41)	0.52 (0.21-1.27)	0.82 (0.33-2.02)	1.21 (0.51-2.91)	0.52 (0.21-1.27)	0.52 (0.21-1.27)	0.63 (0.25-1.58)
	Grp 3	0.67 (0.31-1.46)	1.22 (0.51-2.90)	0.57 (0.25-1.31)	0.69 (0.31-1.50)	1.22 (0.51-2.90)	1.22 (0.51-2.90)	0.82 (0.37-1.82)
Adjusted for infant diet at 3 months	Grp 2	1.00 (0.42-2.37)	1.22 (0.52-2.88)	064 (0.27-1.53)	0.75 (0.32-1.77)	1.22 (0.52-2.88)	1.22 (0.52-2.88)	0.49 (0.20-1.20)
	Grp 3	0.71 (0.32-1.55)	1.42 (0.64-3.17)	0.50 (0.22-1.14)	0.63 (0.28-1.39)	1.42 (0.64-3.17)	1.42 (0.64-3.17)	0.67 (0.31-1.47)
Adjusted for parity	Grp 2	1.22 (0.52-2.91)	1.00 (0.43-2.36)	0.76 (0.31-1.87)	0.92 (0.39-2.17)	1.00 (0.43-2.36)	1.00 (0.43-2.36)	0.47 (0.19-1.16)
	Grp 3	071 (0.32-1.60)	1.41 (0.62-2.22)	0.71 (0.30-1.70)	0.64 (0.28-1.46)	1.41 (0.62-2.22)	1.41 (0.62-2.22)	0.71 (0.32-1.64)
Adjusted for ROM >18 hours	Grp 2	1.29 (0.54-3.07)	0.93 (0.40-2.18)	0.60 (0.25-1.45)	0.95 (0.41-2.25)	0.93 (0.40-2.18)	0.93 (0.40-2.18)	0.46 (0.19-1.14)
	Grp 3	0.57 (0.25-1.25)	1.42 (0.63-2.16)	0.53 (0.23-1.22)	0.49 (0.22-1.12)	1.42 (0.63-2.16)	1.42 (0.63-2.16)	0.66 (0.30-1.46)
Adjusted for infant gender	Grp 2	1.10 (0.47-2.60)	0.90 (0.38-2.12)	0.63 (0.26-1.52)	0.90 (0.38-2.10)	1.13 (0.48-2.67)	0.90 (0.38-2.12)	0.98 (0.42-2.30)
	Grp 3	0.65 (0.30-1.40)	1.42 (0.64-3.13)	0.53 (0.23-1.19)	0.61-1.32)	0.52 (0.24-1.15)	1.42 (0.64-3.13)	1.35 (0.62-2.95)

MODEL 1	Grp2	0.91 (0.34-2.48)	0.38 (0.14-1.06)	1.38 (0.50-3.82)	1.27 (0.47-3.39)	1.06 (0.39-2.89)	0.38 (0.14-1.06)	0.82 (0.30-2.21)
	Grp3	0.62 (0.25-1.57)	0.75 (0.27-2.03)	1.01 (0.39-2.61)	0.65 (0.26-1.66)	0.58 (0.23-1.48)	0.75 (0.27-2.03)	0.89 (0.36-2.21)
Model 2	Grp2	0.78 (0.27-2.21)	**0.29 (0.10-0.83)***	1.52 (0.53-4.32)	1.20 (0.44-3.32)	0.87 (0.31-2.49)	**0.29 (0.10-0.84)***	0.45 (0.15-1.35)
	Grp3	0.56 (0.22-1.45)	0.63 (0.23-1.74)	1.06 (0.41-2.75)	0.69 (0.27-1.79)	0.53 (0.20-1.37)	0.62 (0.22-1.71)	1.36 (0.54-3.43)

MODEL 1: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity and ROM> 18 hours

MODEL 2: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity, ROM> 18 hours and infant gender

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval;
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes; Grp=Group

REGRESSION ANALYSES: 2nd stage
INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT**
(BMI ≥25 to <30)

Table 3.27b

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of 2nd stage of labour among infants of overweight mothers (n= 208)

Ref. Group 1 = 2nd Stage ≤1 Hrs Group 2 = 2nd Stage > 1 to ≤2Hrs Group 3 = 2nd Stage >2Hrs		**Infant's gut microbiota at 3 to 4 months of age** **GENUS**		
		Bifidobacterium (below vs above median)	**Bacteroides** (below vs above median)	**Lactobacillus** (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 2nd stage of labour	Grp2	1.23 (0.53-2.84)	1.05 (0.46-2.42)	0.48 (0.19-1.22)
	Grp3	0.54 (0.25-1.18)	1.50 (0.68-3.28)	0.90 (0.41-1.94)
Adjusted for MODE	Grp2	1.16 (0.48-2.78)	0.52 (0.21-1.27)	0.57 (0.22-1.48)
	Grp3	0.57 (0.26-1.26)	1.22 (0.51-2.90)	0.97 (0.44-2.13)
Adjusted for infant diet at 3 months	Grp2	1.03 (0.43-2.47)	1.22 (0.52-2.88)	0.42 (0.16-1.08)
	Grp3	0.56 (0.25-1.27)	1.42 (0.64-3.17)	0.95 (0.43-2.09)
Adjusted for parity	Grp2	1.26 (0.53-2.99)	1.00 (0.43-2.36)	51 (0.20-1.32)
	Grp3	0.56 (0.25-1.28)	1.41 (0.62-2.22)	0.93 (0.43-2.24)
Adjusted for ROM >18 hours	Grp2	1.37 (0.57-3.26)	0.93 (0.40-2.18)	0.41 (0.15-1.09)
	Grp3	0.51 (0.23-1.15)	1.42 (0.63-2.16)	1.00 (0.45-2.22)
Adjusted for infant gender	Grp2	1.13 (0.48-2.67)	0.98 (0.42-2.30)	0.49 (0.19-1.24)
	Grp3	0.52 (0.24-1.15)	1.35(0.62-2.95)	0.90 (0.41-1.96)
MODEL 1	Grp2	1.06 (0.39-2.89)	0.38 (0.14-1.06)	0.46 (0.16-1.33)
	Grp3	0.58 (0.23-1.48)	0.75 (0.27-2.03)	1.34 (0.54-3.35)

MODEL 2	Grp2	0.87 (0.31-2.49)	**0.29** **(0.10-0.84)*** p=0.023	0.45 (0.15-1.35)
	Grp3	0.53 (0.20-1.37)	0.62 (0.22-1.71)	1.36 (0.54-3.43)

MODEL 1: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity and ROM> 18 hours

MODEL 2: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity, ROM> 18 hours and infant gender

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval;
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes; Grp=Group

RICHNESS and DIVERSITY

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OVERWEIGHT** (BMI ≥25 to <30)

Table 3.28

Crude and adjusted likelihood ratio of gut microbiota richness and diversity measures at 3-4 months according to duration of active 1st stage of labour among infants of overweight mothers (n=201)

Ref. Group 1 = 1st Stage <= 6 Hours Group 2 = 1st Stage > 6 to <=13 Hrs Group 3 = 1st Stage > 13 Hrs	**Chao1 richness** (below vs above median)		**Shannon diversity** (below vs above median)
	OR (95% CI)		OR (95% CI)
Crude OR for 1st stage of labour	Group2	0.96 (0.52-1.78)	0.65 (0.35-1.21)
	Group3	0.94 (0.36-2.48)	0.62 (0.23-1.64)
MODEL 1	Group2	0.86 (0.42-1.76)	0.52 (0.26-1.07)
	Group3	0.67 (0.21-2.12)	0.55 (0.18-1.70)
MODEL 2	Group2	0.85 (0.41-1.75)	0.52 (0.25-1.07)
	Group3	0.69 (0.22-2.22)	0.54 (0.17-1.71)

MODEL 1: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity and ROM> 18 hours
MODEL 2: Adjusted for mode by IAP, exclusive breastfeeding status, parity, ROM> 18 hours and infant gender

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval;
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes

Crude and adjusted likelihood ratio of gut microbiota richness and diversity measures at 3-4 months according to duration of second stage of labour among infants of **overweight mothers (n= 208)**

Ref. Group 1 = 2nd Stage <= 1 Hour Group 2 = 2nd Stage > 1 to <=2 Hrs Group 3 = 2nd Stage > 2 Hrs	**Chao1 richness** (below vs above median)		**Shannon diversity** (below vs above median)
	OR (95% CI)		OR (95% CI)
Crude OR for 2nd stage of labour	Group2	0.34 (0.14-0.83)*	0.51 (0.22-1.18)
	Group3	0.98 (0.46-2.12)	0.37 (0.16-0.83)*
MODEL 1	Group2	**0.31 (0.11-0.85)***	0.57 (0.21-1.53)
	Group3	0.53 (0.21-1.35)	**0.34 (0.13-0.89)***
MODEL 2	Group2	**0.28 (0.10-0.80)*** p=0.018	0.57 (0.21-1.56)
	Group3	0.45 (0.17-1.18)	**0.30 (0.11-0.81)*** p=0.017

MODEL 1: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity and ROM> 18 hours
MODEL 2: Adjusted for mode by IAP, exclusive breastfeeding status, parity, ROM> 18 hours and infant gender

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval;
IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥30)

Table 3.29
Summary table showing **significant** ($p<0.05$) median relative abundance changes of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the duration of active first stage of labour among infants born to obese mothers, and following stratification by mode by IAP

ALL MODES OF BIRTHS (n=152)		
Reference group: Group 1 (n=99)	Group 2 (n=40)	Group 3 (n=13)
Phylum Actinobacteria	↓	↓
Bifidobacteriaceae	↓	↓
Phylum Bacteroidetes	↑	↑
Phylum Firmicutes	--	↓
Lactobacillaceae	--	--
Phylum Proteobacteria	--	--

Group 1 (Reference group): Active 1st stage <= 6 hours

Group 2: Active 1st Stage > 6 to ≤13 hours

Group 3: Active 1st Stage > 13 hours

VAGINAL BIRHTS WITHOUT IAP (n=68)		
Group 1 (Ref) (n=35)	Group 2 (n=26)	Group 3 (n=7)
Phylum Actinobacteria	--	--
Bifidobacteriaceae	--	↓
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Lactobacillaceae	--	--
Phylum Proteobacteria	--	--

VAGINAL BIRHTS WITH IAP (n=30)		
Group 1 (Ref) (n=16)	Group 2 (n=10)	Group 3 (n=4)
Phylum Actinobacteria	--	--
Bifidobacteriaceae	--	--
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Ruminococcaceae	--	--
Phylum Proteobacteria	--	--

C-SECTION WITH ACTIVE 1ST STAGE (n=19)	
Group 1 (Ref) (n=17)	Active 1st stage > 6 hrs (n=2)
Phylum Actinobacteria	--
Bifidobacteriaceae	--
Phylum Bacteroidetes	--
Phylum Firmicutes	--
Ruminococcaceae	--
Phylum Proteobacteria	--

IAP = Intrapartum Antibiotic Prophylaxis
-- indicates no significant change

(Note: Elective C-section excluded from analyses)

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥30)

Table 3.30
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among all modes of delivery in infants born to obese mothers, according to the duration of active first stage of labour (n= 152)

Bacterial Taxa	1st Stage of labour <= 6 hours [Reference group]	1st Stage of labour > 6 to <=13 hours	p-value	1st Stage of labour > 13 hours	p-value
	(n=99; 65.1%)	(n=40;26.3%)		(n=13;8.6%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	6.314 (2.791-18.029)	3.566 (0.228-11.478)	**0.016**	2.112 (0.597-8.177)	**0.034**
Bifidobacteriaceae	5.748 (2.142-17.068)	2.814 (0.103-9.937)	**0.014**	1.851 (0.046-6.451)	**0.014**
Coriobacteriaceae	0.078 (0.008-0.511)	0.031 (0.000-0.225)	0.129	0.008 (0.000-1.221)	0.234
g_Bifidobacterium	5.748 (2.142-17.068)	2.814 (0.103-9.937)	**0.015**	1.851 (0.046-6.451)	**0.014**
Bacteroidetes	7.600 (0.101-55.309)	47.377 (0.205-76.583)	**0.037**	50.280 (9.842-74.443)	**0.036**
Bacteroidaceae	6.086 (0.069-47.833)	37.429 (0.198-75.316)	**0.021**	48.508 (9.761-74.443)	**0.014**
Firmicutes	27.689 (13.666-48.550)	22.298 (12.169)	0.266	14.577 (4.110-28.349)	**0.015**
Lactobacillaceae	0.000 (0.000-0.008)	0.000 (0.000-0.000)	0.107	0.000 (0.000-0.000)	0.128
Streptoccocaceae	0.579 (0.162-2.086)	0.476 (0.197-2.679)	0.974	0.479 (0.175-1.823)	0.646
Clostridiaceae	0.537 (0.085-2.390)	0.603 (0.079-1.903)	0.993	0.233 (0.031-1.254)	0.340
Lachnospiraceae	4.438 (0.985-10.719)	4.382 (0.553-11.749)	0.970	2.600 (0.015-7.965)	0.229
Ruminococcaceae	0.728 (0.008-3.616)	0.449 (0.008-1.589)	0.291	0.101 (0.012-2.772)	0.924
Veillionellaceae	6.646 (1.116-21.094)	4.073 (1.129-15.404)	0.180	3.017 (0.225-10.523)	0.091
g_Lactobacillus	0.000 (0.000-0.008)	0.000 (0.000-0.000)	0.107	0.000 (0.000-0.000)	0.128
Proteobacteria	12.961 (4.593-34.660)	9.235 (4.917-20.536)	0.596	16.360 (8.957-39.989)	0.437
Enterobacteriaceae	11.272 (2.875-33.300)	8.230 (4.047-19.221)	0.748	13.980 (5.050-38.234)	0.608
Verrucomicrobia	0.000 (0.000-0.023)	0.000 (0.000-0.008)	0.429	0.000 (0.000-0.008)	0.640
g_Akkermansia	0.000 (0.000-0.023)	0.000 (0.000-0.008)	0.429	0.000 (0.000-0.008)	0.640

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥ 30)

Table 3.31
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births without IAP in obese mothers, according to the duration of active first stage of labour (n=68)

Bacterial Taxa	1st Stage of labour <= 6 hours [Reference group]	1st Stage of labour > 6 to <=13 hours	p-value Exact	1st Stage of labour > 13 hours	p-value Exact
	(n=35; 51.5%)	(n=26; 38.2%)		(n=7;10.3%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	5.773 (2.907-19.550)	4.536 (0.251-22.848)	0.199	3.084 (1.094-5.983)	0.085
Family *Bifidobacteriaceae*	5.532 (2.496-18.917)	4.199 (0.126-20.631)	0.246	2.112 (0.031-3.164)	**0.017**
Family *Coriobacteriaceae*	0.124 (0.008-1.066)	0.047 (0.000-0.520)	0.323	0.015 (0.000-2.710)	0.552
Genus_*Bifidobacterium*	5.532 (2.496-18.917)	4.199 (0.126-20.631)	0.246	2.112 (0.031-3.164)	**0.017**
Phylum Bacteroidetes	45.728 (8.549-75.219)	30.097 (5.026-75.905)	0.560	58.016 (11.101-77.551)	0.741
Family *Bacteroidaceae*	40.987 (2.057-58.751)	26.688 (4.208-74.648)	0.782	49.017 (10.977-77.551)	0.597
Phylum Firmicutes	19.767 (8.670-32.331)	20.543 (12.079-43.560)	0.759	16.141 (4.157-33.515)	0.446
Family *Lactobacillaceae*	0.000 (0.000-0.016)	0.000 (0.000-0.000)	0.100	0.000 (0.000-0.000)	0.287
Family *Streptoccocaceae*	0.756 (0.149-1.893)	0.408 (0.167-3.014)	0.896	0.479 (0.070-2.755)	0.792
Family *Clostridiaceae*	0.257 (0.062-2.052)	0.272 (0.029-1.426)	0.988	0.233 (0.155-2.411)	0.766
Family *Lachnospiraceae*	3.993 (0.985-8.446)	4.287 (0.702-10.186)	0.610	1.793 (0.015-6.165)	0.217
Family *Ruminococcaceae*	0.949 (0.000-2.451)	0.174 (0.006-1.577)	0.167	2.326 (0.070-2.999)	0.487
Family *Veillionellaceae*	6.037 (1.102-16.416)	4.073 (1.438-11.577)	0.771	3.997 (0.147-15.060)	0.597
Genus_*Streptococcaceae*	0.756 (0.149-1.893)	0.408 (0.167-3.014)	0.896	0.472 (0.070-2.755)	0.792
Genus_*Lactobacillus*	0.000 (0.000-0.016)	0.000 (0.000-0.000)	0.100	0.000 (0.000-0.000)	0.287
Genus_*Clostridium*	0.008 (0.000-0.085)	0.019 (0.000-0.204)	0.741	0.139 (0.000-0.196)	0.644
Genus_*Veillionella*	2.896 (0.599-14.629)	1.625 (0.218-8.292)	0.307	0.302 (0.031-5.423)	0.243
Phylum Proteobacteria	7.041 (3.756-17.937)	11.281 (5.258-21.269)	0.166	16.075 (3.925-19.669)	0.353
Family *Enterobacteriaceae*	5.710 (2.420-17.913)	8.934 (4.646-20.756)	0.119	11.826 (3.254-19.661)	0.487
Phylum Verrucomicrobia	0.000 (0.000-0.008)	0.004 (0.000-0.016)	0.249	0.000 (0.000-43.597)	0.620
Genus_*Akkermansia*	0.000 (0.000-0.008)	0.004 (0.000-0.016)	0.249	0.000 (0.000-43.597)	0.620

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥ 30)

Table 3.32
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births with IAP in obese mothers, according to the duration of active first stage of labour (n= 30)

Bacterial Taxa	1st Stage of labour <= 6 hours [Reference group]	1st Stage of labour > 6 to <=13 hours	p-value Exact	1st Stage of labour > 13 hours	p-value Exact
	(n=16; 53.3%)	(n=10; 33.3%)		(n=4; 13.3%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	4.882 (1.550-11.178)	1.995 (0.244-3.384)	0.201	1.842 (0.497-25.569)	0.617
Family *Bifidobacteriaceae*	4.650 (1.383-10.307)	1.796 (0.032-3.166)	0.097	1.757 (0.480-23.548)	0.682
Family *Coriobacteriaceae*	0.047 (0.008-0.313)	0.019 (0.000-0.157)	0.391	0.050 (0.002-1.474)	0.963
Genus_*Bifidobacterium*	4.650 (1.383-10.307)	1.796 (0.032-3.166)	0.097	1.757 (0.480-23.548)	0.682
Phylum Bacteroidetes	46.882 (5.762-74.715)	71.610 (38.139-85.404)	0.182	48.237 (11.821-82.796)	0.820
Family *Bacteroidaceae*	35.302 (5.211-64.109)	68.179 (31.442-78.215)	0.109	47.351 (11.821-82.353)	0.437
Phylum Firmicutes	19.708 (10.953-31.599)	19.483 (5.950-38.598)	0.856	7.215 (3.885-19.978)	0.099
Family *Lactobacillaceae*	0.000 (0.000-0.000)	0.000 (0.000-0.004)	0.816	0.000 (0.000-0.006)	0.820
Family *Streptoccocaceae*	0.190 (0.097-0.503)	0.414 (0.219-3.236)	0.135	0.548 (0.289-6.525)	0.099
Family *Clostridiaceae*	0.411 (0.128-1.990)	0.633 (0.304-0.778)	0.856	0.031 (0.017-0.903)	0.178
Family *Lachnospiraceae*	4.346 (0.553-11.188)	2.601 (0.384-20.954)	0.979	3.616 (0.264-8.305)	0.554
Family *Ruminococcaceae*	0.910 (0.046-4.074)	1.271 (0.022-3.223)	0.816	0.004 (0.000-0.660)	0.080
Family *Veillionellaceae*	5.376 (0.195-9.072)	9.113 (0.114-16.261)	0.623	1.222 (0.457-3.040)	0.437
Genus_*Streptococcaceae*	0.190 (0.097-0.503)	0.414 (0.219-3.236)	0.135	0.548 (0.289-6.508)	0.099
Genus_*Lactobacillus*	0.000 (0.000-0.000)	0.000 (0.000-0.004)	0.816	0.000 (0.000-0.006)	0.820
Genus_*Clostridium*	0.151 (0.002-1.012)	0.160 (0.029-0.307)	0.856	0.019 (0.004-0.029)	0.249
Genus_*Veillionella*	1.150 (0.074-9.072)	5.697 (0.050-16.085)	0.897	1.195 (0.440-2.040)	0.963
Phylum Proteobacteria	10.682 (1.848-21.026)	7.149 (2.098-17.351)	0.551	40.313 (9.845-47.991)	0.249
Family *Enterobacteriaceae*	9.250 (1.149-17.686)	4.609 (1.521-10.405)	0.586	25.000 (2.178-47.989)	0.554
Phylum Verrucomicrobia	0.008 (0.000-6.541)	0.000 (0.000-0.008)	0.077	0.004 (0.000-0.008)	0.385
Genus_*Akkermansia*	0.008 (0.000-6.541)	0.000 (0.000-0.008)	0.077	0.004 (0.000-0.008)	0.385

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥ 30)

Table 3.33
Summary table showing **significant** ($p<0.05$) median relative abundance changes of the dominant bacterial taxa of infant gut microbiota at 3-4 months according to the duration of second stage of labour among infants born to obese mothers, and following stratification by mode by IAP

ALL MODES OF BIRTHS (n=157)		
Reference group: Group 1 (n=118)	Group 2 (n=18)	Group 3 (n=21)
Phylum Actinobacteria	--	↓
Bifidobacteriaceae	--	↓
Phylum Bacteroidetes	--	--
Phylum Firmicutes	--	--
Lactobacillaceae	--	--
Clostridiaceae	--	--
Phylum Proteobacteria	--	--

Group 1 (Reference group): 2nd stage <=1 hour

Group 2: 2nd stage > 1 to ≤ 2 hours

Group 3: 2nd stage > 2 hours

VAGINAL BIRHTS WITHOUT IAP (n=71)		
Group 1 (Ref) (n=51)	Group 2 (n=10)	Group 3 (n=10)
Phylum Actinobacteria	--	--
Bifidobacteriaceae	--	↓
Phylum Bacteroidetes	↑	--
Phylum Firmicutes	↓	--
Clostridiaceae	↓	--
Phylum Proteobacteria	--	--

VAGINAL BIRHTS WITH IAP (n=32)		
Group 1 (Ref) (n=18)	Group 2 (n=8)	Group 3 (n=6)
Phylum Actinobacteria	--	--
Bifidobacteriaceae	--	--
Phylum Bacteroidetes	↓	--
Phylum Firmicutes	↑	--
Veillionellaceae	↑	--
Phylum Proteobacteria	--	--

C-SECTION WITH 2nd STAGE (n=27)	
Group 1 (Ref) (n=22)	2nd stage > 1 hour (n=5)
Phylum Actinobacteria	--
Bifidobacteriaceae	--
Phylum Bacteroidetes	--
Phylum Firmicutes	--
Clostridiaceae	--
Phylum Proteobacteria	--

IAP = Intrapartum Antibiotic Prophylaxis
-- indicates no significant change

(Note: Elective C-section excluded from analyses)

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥ 30)

Table 3.34
Median relative abundance of dominant bacterial taxa in infant gut microbiota at 3-4 months among all modes of delivery in obese mothers, according to the duration of second stage of labour (n= 157)

Bacterial Taxa	**2nd Stage of labour <= 1 hour [Reference group]**	**2nd Stage of labour > 1 to <=2 hours**	p-value	**2nd Stage of labour > 2 hours**	p-value
	(n= 118; 75.2%)	(n= 18; 11.5%)		(n= 21; 13.4%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum	5.584 (2.534-19.128)	4.081 (1.672-6.899)	0.116	2.030 (0.414-5.103)	**0.004**
Actinobacteria	5.111 (1.744-18.180)	2.497 (1.219-6.184)	0.071	1.851 (0.093-4.199)	**0.004**
Bifidobacteriaceae	0.062 (0.008-0.436)	0.055 (0.000-0.860)	0.604	0.023 (0.004-0.101)	0.085
Coriobacteriaceae	5.111 (1.744-18.180)	2.497 (1.219-6.184)	0.071	1.851 (0.093-4.199)	**0.004**
g_Bifidobacterium					
Bacteroidetes	15.992 (0.109-58.894)	48.517 (24.615-81.598)	0.068	50.280 (0.125-73.516)	0.335
Bacteroidaceae	9.819 (0.083-50.710)	44.328 (24.615-72.575)	0.049	37.098 (0.125-73.492)	0.207
Firmicutes	26.473 (12.288-47.833)	17.325 (5.106-30.741)	0.103	19.633 (12.472-40.558)	0.374
Lactobacillaceae	0.000 (0.000-0.008)	0.000 (0.000-0.017)	0.928	0.000 (0.000-0.000)	0.198
Streptoccocaceae	0.655 (0.216-2.591)	0.229 (0.106-1.368)	0.140	0.467 (0.257-1.028)	0.347
Clostridiaceae	0.582 (0.105-2.108)	0.188 (0.019-1.312)	0.127	0.541 (0.079-3.965)	0.883
Lachnospiraceae	4.619 (0.469-10.885)	3.009 (0.517-5.432)	0.214	6.410 (0.782-13.097)	0.860
Ruminococcaceae	0.570 (0.008-1.391)	0.521 (0.000-2.188)	0.590	0.814 (0.031-2.498)	0.645
Veillionellaceae	6.118 (0.986-19.543)	5.171 (1.421-16.261)	0.676	4.212 (0.546-16.191)	0.457
g_Lactobacillus	0.000 (0.000-0.008)	0.000 (0.000-0.017)	0.928	0.000 (0.000-0.000)	0.198
Proteobacteria	12.353 (4.484-32.931)	12.167 (4.280-25.698)	0.729	17.604 (10.316-53.364)	0.083
Enterobacteriaceae	10.793 (3.240-32.527)	5.267 (3.536-25.675)	0.644	14.255 (6.920-53.353)	0.110
Verrucomicrobia	0.000 (0.000-0.023)	0.000 (0.000-0.018)	0.612	0.000 (0.000-0.008)	0.072
g_Akkermansia	0.000 (0.000-0.023)	0.000 (0.000-0.018)	0.612	0.000 (0.000-0.008)	0.072

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥ 30)

Table 3.35
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births without IAP in obese mothers, according to the duration of second stage of labour (n= 71)

Bacterial Taxa	2nd Stage of labour <= 1 hour [Reference group]	2nd Stage of labour > 1 to <=2 hours	p-value Exact	2nd Stage of labour > 2 hours	p-value Exact
	(n= 51; 71.8%)	(n= 10; 14.1%)		(n= 10; 14.1%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	5.558 (2.636-21.948)	4.777 (1.801-8.514)	0.436	2.071 (0.671-5.431)	0.064
Family *Bifidobacteriaceae*	5.014 (1.943-19.325)	3.400 (1.640-7.869)	0.447	1.911 (0.108-3.892)	**0.048**
Family *Coriobacteriaceae*	0.063 (0.008-0.595)	0.043 (0.000-1.474)	0.390	0.055 (0.000-1.083)	0.598
Genus_*Bifidobacterium*	5.014 (1.943-19.325)	3.400 (1.640-7.869)	0.447	1.911 (0.108-3.892)	**0.048**
Phylum Bacteroidetes	41.143 (6.598-67.439)	73.837 (37.964-85.015)	**0.018**	57.953 (17.898-77.624)	0.311
Family *Bacteroidaceae*	26.576 (2.057-56.869)	63.955 (36.457-82.169)	**0.019**	51.428 (7.734-77.553)	0.259
Phylum Firmicutes	23.968 (12.347-40.658)	8.400 (4.707-23.996)	**0.010**	17.546 (6.143-30.062)	0.320
Family *Lactobacillaceae*	0.000 (0.000-0.008)	0.000 (0.000-0.258)	0.401	0.000 (0.000-0.002)	0.384
Family *Streptoccocaceae*	0.756 (0.209-2.536)	0.166 (0.087-1.368)	0.149	0.516 (0.256-0.960)	0.459
Family *Clostridiaceae*	0.467 (0.085-2.052)	0.058 (0.000-0.195)	**0.012**	0.290 (0.142-2.707)	0.815
Family *Lachnospiraceae*	4.438 (0.735-9.567)	2.720 (0.461-3.414)	0.205	3.077(0.131-8.629)	0.675
Family *Ruminococcaceae*	0.619 (0.008-2.175)	0.229 (0.000-0.945)	0.202	1.344 (0.174-2.474)	0.463
Family *Veillionellaceae*	5.985 (1.102-17.190)	3.426 (0.993-6.928)	0.330	7.674 (0.643-15.625)	0.868
Genus_*Streptococcaceae*	0.756 (0.209-2.536)	0.166 (0.087-1.368)	0.149	0.516 (0.256-0.960)	0.459
Genus_*Lactobacillus*	0.000 (0.000-0.008)	0.000 (0.000-0.258)	0.401	0.000 (0.000-0.002)	0.384
Genus_*Clostridium*	0.016 (0.000-0.163)	0.000 (0.000-0.027)	0.130	0.085 (0.023-0.223)	0.241
Genus_*Veillionella*	2.198 (0.382-11.084)	2.644 (0.467-3.771)	0.785	2.066 (0.176-11.770)	0.682
Phylum Proteobacteria	8.217 (4.593-20.803)	5.329 (3.175-25.698)	0.360	16.217 (10.470-31.161)	0.144
Family *Enterobacteriaceae*	7.018 (3.254-19.661)	4.203 (2.823-25.675)	0.330	13.311 (6.325-30.535)	0.167
Phylum Verrucomicrobia	0.000 (0.000-0.015)	0.000 (0.000-0.018)	0.914	0.008 (0.000-0.012)	0.713
Genus_*Akkermansia*	0.000 (0.000-0.015)	0.000 (0.000-0.018)	0.914	0.008 (0.000-0.012)	0.713

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥ 30)

Table 3.36
Median relative abundance of dominant bacterial taxa at different taxonomic levels in infant gut microbiota at 3-4 months among infants born by VAGINAL births with IAP in obesity mothers, according to the duration of second stage of labour (n= 32)

Bacterial Taxa	2nd Stage of labour <= 1 hour [Reference group]	2nd Stage of labour > 1 to <=2 hours	p-value Exact	2nd Stage of labour > 2 hours	p-value Exact
	(n=18; 56.3%)	(n=8; 25%)		(n=6; 18.8%)	
	Median (IQR)	Median (IQR)		Median (IQR)	
Phylum Actinobacteria	2.939 (0.123-14.154)	2.811 (0.467-8.070)	0.849	2.062 (1.375-2.997)	0.673
Family *Bifidobacteriaceae*	2.846 (0.109-13.782)	2.497 (0.021-5.866)	0.429	1.996 (1.313-2.945)	0.721
Family *Coriobacteriaceae*	0.031 (0.000-0.269)	0.058 (0.002-0.711)	0.567	0.019 (0.008-0.041)	0.673
Genus_*Bifidobacterium*	2.846 (0.109-13.782)	2.497 (0.021-5.866)	0.429	1.996 (1.313-2.945)	0.721
Phylum Bacteroidetes	69.503 (27.852-81.586)	37.637 (0.033-49.679)	**0.035**	58.450 (3.069-75.665)	0.454
Family *Bacteroidaceae*	52.600 (12.556-76.442)	37.360 (0.023-45.260)	0.115	42.803 (3.069-73.374)	0.673
Phylum Firmicutes	11.938 (5.196-24.226)	29.937 (15.739-66.534)	**0.047**	21.866 (8.731-31.881)	0.454
Family *Lactobacillaceae*	0.000 (0.000-0.000)	0.000 (0.000-0.000)	0.849	0.000 (0.000-0.002)	0.923
Family *Streptoccocaceae*	0.241 (0.091-1.595)	0.473 (0.165-2.423)	0.367	0.290 (0.128-1.597)	0.820
Family *Clostridiaceae*	0.563 (0.190-1.141)	0.768 (0.295-3.707)	0.338	0.079 (0.012-1.985)	0.224
Family *Lachnospiraceae*	4.107 (0.062-7.122)	3.353 (0.456-28.063)	0.765	11.877 (1.051-18.728)	0.251
Family *Ruminococcaceae*	0.910 (0.006-2.324)	0.733 (0.126-5.559)	0.644	1.157 (0.000-5.814)	0.820
Family *Veillionellaceae*	2.243 (0.102-7.425)	14.840 (3.489-22.878)	**0.035**	3.879 (0.667-11.433)	0.415
Genus_*Streptococcaceae*	0.241 (0.091-1.595)	0.473 (0.165-2.423)	0.367	0.290 (0.128-1.597)	0.820
Genus_*Lactobacillus*	0.000 (0.000-0.000)	0.000 (0.000-0.000)	0.849	0.000 (0.000-0.002)	0.923
Genus_*Clostridium*	0.124 (0.006-0.449)	0.147 (0.021-0.235)	0.935	0.016 (0.000-1.853)	0.537
Genus_*Veillionella*	0.532 (0.061-6.211)	12.559 (0.402-22.878)	0.196	1.613 (0.666-11.433)	0.280
Phylum Proteobacteria	5.631 (1.965-18.458)	17.599 (9.317-40.551)	0.080	22.939 (1.220-55.613)	0.310
Family *Enterobacteriaceae*	5.153 (0.911-13.927)	16.831 (4.104-40.545)	0.070	7.622 (1.210-55.039)	0.494
Phylum Verrucomicrobia	0.008 (0.000-0.027)	0.000 (0.000-0.100)	0.495	0.000 (0.000-0.008)	0.199
Genus_*Akkermansia*	0.008 (0.000-0.027)	0.000 (0.000-0.100)	0.495	0.000 (0.000-0.008)	0.199

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type. IAP = Intrapartum Antibiotic Prophylaxis

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥ 30)

Table 3.37
Median relative abundance of dominant bacterial taxa at the genus level in infant gut microbiota at 3-4 months among obese mothers with C-section with labour, according to the duration of second stage of labour (n = 27)

Bacterial Taxa	2nd Stage of labour <= 1 hour [Reference group]	2nd Stage of labour > 1 hour	p-value Exact
	(n= 22; 81.5%)	(n= 5; 18.5%)	
	Median (IQR)	Median (IQR)	
	N (%); IQR	N (%); IQR	
Actinobacteria	9.188 (4.912-22.897)	0.116 (0.066-17.688)	0.165
g_Actinomyces	0.085 (0.021-0.347)	0.031 (0.008-0.236)	0.314
g_Bifidobacterium	8.833 (3.756-19.473)	0.008 (0.000-17.347)	0.129
Bacteroidetes	0.128 (0.070-9.619)	0.070 (0.023-35.745)	0.485
g_*Bacteroides*	0.097 (0.052-9.244)	0.070 (0.023-35.745)	0.786
Firmicutes	47.389 (30.324-52.842)	43.271 (26.210-51.134)	0.485
g_Enterococcus	0.078 (0.014-0.189)	0.109 (0.093-0.278)	0.314
g_Lactobacillus	0.000 (0.000-0.002)	0.000 (0.000-0.093)	1.00
g_Streptococcus	1.590 (0.255-3.527)	0.557 (0.268-2.052)	0.606
g_Clostridia	0.121 (0.014-1.439)	2.654 (0.143-14.651)	0.165
g_Ruminococcus_L	0.269 (0.000-7.183)	0.008 (0.004-3.833)	0.650
g_Oscillospira	0.027 (0.000-2.428)	0.186 (0.000-6.925)	1.00
g_Veilloinella	11.999 (0.941-31.344)	1.928 (0.070-33.255)	0.524
Proteobacteria	23.116 (12.887-38.843)	42.365 (16.955-58.600)	0.165
g_Citrobacter	0.078 (0.000-0.291)	0.054 (0.000-0.558)	0.832
g_Enterobacter_unclss	19.202 (10.945-36.440)	42.187 (16.474-56.978)	0.086
Verrucomicrobia	0.000 (0.000-0.018)	- -	0.232
g_*Akkermansia*	0.000 (0.000-0.018)	- -	0.232

Results are presented as median and interquartile range (IQR) in parentheses. Comparisons were performed using Mann-Whitney U-test. P values **< 0.05** are indicated in boldface type.

CHAPTER 3
REGRESSION ANALYSES: Active 1st stage
INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥ 30)

Table 3.38 Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of active 1st stage of labour among infants of obese mothers (n= 152)

Microbiota Measure		**Infant's gut microbiota at 3 to 4 months of age**								
Ref. Group 1 = 1st Stage ≤6 Hrs Group 2 = 1st Stage >6 to ≤13Hrs Group 3 = 1st Stage > 13Hrs		**PHYLUM**				**FAMILY**			**GENUS**	
		Actinobacteria (below vs above median)	**Bacteroidetes** (below vs above median)	**Firmicutes** (below vs above median)	**Proteo-bacteria** (below vs above median)	**Bifidobacteria-ceae** (below vs above median)	**Clostridia-ceae** (below vs above median)	**Veillonella-ceae** (below vs above median)	**Bifidobacterium** (below vs above median)	**Lactobacillus** (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 1st stage of labour	Grp2	0.42 (0.19-0.90)*	2.23 (1.04-4.77)*	0.74 (0.35-1.54)	0.76 (0.36-1.63)	0.45 (0.21-0.96)*	1.06(0.51-2.22)	0.56 (0.27-1.18)	0.45(0.21-0.96)*	0.50 (0.21-1.21)
	Grp3	0.54 (0.17-1.77)	2.70 (0.78-9.35)	0.33 (0.09-1.14)	1.21 (0.38-3.87)	0.25 (0.07-0.96)*	0.74 (0.23-2.37)	0.28(0.08-0.96)*	0.25(0.07-0.96)*	0.36 (0.08-1.74)
Adjusted for MODE by IAP	Grp2	0.47 (0.21-1.06)	1.02 (0.43-2.46)	1.20(0.54-2.70)	1.31 (0.56-3.10)	0.50 (0.22-1.13)	1.37(0.61-3.06)	0.74(0.33-1.63)	0.50 (0.22-1.13)	0.53 (0.21-1.34)
	Grp3	0.58 (0.18-1.94)	1.81 (0.45-7.30)	0.42(0.11-1.52)	1.74 (0.50-6.05)	0.27 (0.07-1.04)	0.90(0.27-2.98)	0.31(0.09-1.10)	0.27 (0.07-1.04)	0.38 (0.08-1.82)
Adjusted for infant diet at 3 months	Grp2	0.42 (0.19-0.90)*	2.22 (1.04-4.75)*	0.74 (0.35-1.54)	0.78 (0.35-1.73)	0.45 (0.21-0.97)*	1.04 (0.50-2.19)	0.55 (0.26-1.16)	0.45 (0.21-0.97)*	0.50 (0.20-1.24)
	Grp3	0.54 (0.17-1.77)	2.70 (0.78-9.35)	0.33 (0.09-1.14)	1.25 (0.37-4.23)	0.25 (0.07-0.96)*	0.74 (0.23-2.37)	0.27 (0.08-0.95)*	0.25 (0.07-0.96)*	0.35 (0.07-1.71)
Adjusted for parity	Grp2	0.44 (0.20-0.96)*	2.42 (1.10-5.29)*	0.66 (0.31-1.42)	0.72 (0.33-1.58)	0.45 (0.21-0.97)*	1.06 (0.50-2.25)	0.53 (0.25-1.14)	0.45 (0.21-0.97)*	0.45 (0.18-1.11)
	Grp3	0.55 (0.17-1.81)	2.80 (0.80-9.74)	0.31 (0.09-1.09)	1.19 (0.37-3.81)	0.25 (0.07-0.96)*	0.74 (0.23-2.37)	0.27 (0.08-0.94)*	0.25 (0.07-0.96)*	0.35 (0.07-1.67)
Adjusted for ROM >18 hours	Grp2	0.41 (0.19-0.90)*	2.17 (1.01-4.67)*	0.73 (0.35-1.53)	0.80 (0.37-1.73)	0.46 (0.22-0.99)*	1.05 (0.50-2.21)	0.55 (0.26-1.16)	0.46 (0.22-0.99)*	0.49 (0.20-1.19)
	Grp3	0.44 (0.12-1.58)	3.31 (0.84-13.31)	0.24 (0.06-0.95)*	1.17 (0.34-4.03)	0.18 (0.04-0.87)*	0.64 (0.19-2.18)	0.21 (0.05-0.84)	0.18 (0.04-0.87)*	0.40 (0.08-1.96)
Adjusted for MODEL 1	Grp2	0.49 (0.21-1.14)	0.99 (0.40-2.48)	0.99 (0.43-2.31)	1.52 (0.60-3.87)	0.52 (0.22-1.22)	1.29 (0.56-3.01)	0.63 (0.27-1.47)	0.52 (0.22-1.22)	0.45 (0.17-1.23)
	Grp3	0.48 (0.13-1.80)	1.73 (0.39-7.67)	0.31 (0.07-1.30)	2.20 (0.52-9.27)	0.20 (0.04-0.97)*	0.79 (0.22-2.83)	0.24 (0.06-0.97)*	0.20 (0.04-0.97)*	0.38 (0.07-2.04)

MODEL 1: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity and ROM> 18 hours

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval;
IAP = Intrapartum antibiotic prophylaxis; GA = gestational age; ROM = rupture of membranes; Grp=Group

CHAPTER 3

REGRESSION ANALYSES: 2nd stage

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥ 30)

Table 3.39

Crude and adjusted likelihood ratio of abundance of key gut microbiota measures at 3-4 months according to duration of 2nd stage of labour among infants of obese mothers (n= 157)

Microbiota Measure		**Infant's gut microbiota at 3 to 4 months of age**								
Ref. Group 1 = 2nd Stage ≤1 Hrs Group 2 = 2nd Stage > 1 to ≤2Hrs Group 3 = 2nd Stage >2Hrs		**PHYLUM**				**FAMILY**			**GENUS**	
		Actinobacteria (below vs above median)	**Bacteroidetes** (below vs above median)	**Firmicutes** (below vs above median)	**Proteo-bacteria** (below vs above median)	**Bifidobacteriaceae** (below vs above median)	**Clostridia-ceae** (below vs above median)	**Veillonellaceae** (below vs above median)	**Bifidobacterium** (below vs above median)	**Lactobacillus** (below vs above median)
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude OR for 2nd stage of labour	Grp2	0.62 (0.22-1.70)	3.88 (1.21-12.47)*	0.65 (0.24-1.77)	0.93 (0.34-2.56)	0.62 (0.22-1.70)	0.84 (0.28-2.54)	0.74 (0.27-1.99	0.62 (0.22-1.70)	0.84 (0.28-2.54)
	Grp3	023 (0.07-0.72)*	1.80 (0.69-4.66)	0.74 (0.29-1.88)	1.33 (0.52-3.37)	0.23 (0.07-0.72)*	0.52 (0.16-1.64)	0.67 (0.26-1.70)	0.23 (0.07-0.72)*	0.52 (0.16-1.64)
Adjusted for MODE by IAP	Grp2	0.70 (0.25-1.96)	2.31 (0.69-7.76)	0.90 (0.32-2.51)	1.33 (0.46-3.84)	0.70 (0.25-1.96)	0.62 (0.22-1.75)	0.88 (0.32-2.44)	0.70 (0.25-1.96)	0.86 (0.28-2.66)
	Grp3	0.24 (0.08-0.76)*	1.76 (0.60-5.19)	0.79 (0.30-2.08)	1.43 (0.53-3.82)	0.24 (0.08-0.76)*	0.91 (0.35-2.34)	0.67 (0.26-1.73)	0.24 (0.08-0.76)*	0.53 (0.17-1.69)
Adjusted for infant diet at 3 months	Grp2	0.63 (0.23-1.76)	4.12 (1.26-13.45)*	0.64 (0.25-1.77)	0.69 (0.24-2.03)	0.59 (0.21-1.66)	0.55 (0.20-1.53)	0.78 (0.29-2.14)	0.59 (0.21-1.66)	0.65 (0.21-2.04)
	Grp3	0.23 (0.07-0.71)*	1.77 (0.68-4.59)	0.74 (0.29-1.89)	1.51 (0.56-4.03)	0.23 (0.07-0.73)*	0.84 (0.33-2.14)	0.65 (0.26-1.66)	0.23 (0.07-0.73)*	0.54 (0.16-1.76)
Adjusted for parity	Grp2	0.65 (0.23-1.83)	4.77 (1.42-16.01)	0.53 (0.19-1.51)	0.85 (0.30-2.41)	0.60 (0.21-1.69)	0.45 (0.16-1.29)	0.67 (0.24-1.87)	0.60 (0.21-1.69)	0.74 (0.24-2.31)
	Grp3	0.24 (0.07-0.78)*	2.26 (0.82-6.18)	0.59 (0.22-1.57)	1.20 (0.45-3.15)	0.22 (0.07-0.71)	0.78 (0.29-2.04)	0.60 (0.23-1.59)	0.22 (0.07-0.71)	0.44 (0.13-1.47)
Adjusted for ROM >18 hours	Grp2	0.64 (0.23-1.76)	3.91 (1.21-12.63)	0.66 (0.24-1.80)	0.92 (0.33-2.55)	0.63 (0.23-1.74)	0.50 (0.18-1.37)	0.73 (0.27-1.97)	0.63 (0.23-1.74)	0.84 (0.28-2.53)
	Grp3	0.17 (0.05-0.65)*	2.21 (0.77-6.36)	0.74 (0.27-2.01)	1.24 (0.45-3.44)	0.11 (0.03-0.52)*	0.95 (0.35-2.59)	0.72 (0.27-1.95)	0.11 (0.03-0.52)*	060 (0.18-1.99)
Adjusted for MODEL 1	Grp2	0.78 (0.26-2.31)	2.92 (0.82-10.38)	0.71 (0.24-2.09)	0.93 (0.29-2.96)	0.69 (0.23-2.03)	0.60 (0.20-1.79)	0.84 (0.29-2.44)	0.69 (0.23-2.03)	0.59 (0.17-1.99)
	Grp3	0.20 (0.05-0.76)*	2.18 (0.63-7.57)	0.67 (0.23-2.00)	1.77 (0.54-5.86)	0.12 (0.03-0.58)*	0.88 (0.30-2.55)	0.67 (0.23-1.95)	0.12 (0.03-0.58)*	0.65 (0.18-2.35)

MODEL 1: Adjusted for mode by IAP, exclusive breastfeeding (infant diet) status, parity and ROM> 18 hours

* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval;
IAP = Intrapartum antibiotic prophylaxis; GA = gestational age; ROM = rupture of membranes; Grp=Group

CHAPTER 3

RICHNESS and DIVERSITY

INFANTS BORN TO WOMEN WITH **PRE-PREGNANCY OBESITY** (BMI ≥30)

Table 3.40

Crude and adjusted likelihood ratio of gut microbiota richness and diversity measures at 3-4 months according to duration of active 1st stage of labour among infants of **obese mothers (n=152)**

Ref. Group 1 = 1st Stage <= 6 Hours Group 2 = 1st Stage > 6 to <=13 Hrs Group 3 = 1st Stage > 13 Hrs		**Chao1 richness** (below vs above median) OR (95% CI)	**Shannon diversity** (below vs above median) OR (95% CI)
Crude OR for 1st stage of labour	Group2	1.60 (0.74-3.45)	0.43 (0.21-0.91)*
	Group3	0.90 (0.28-2.86)	0.33 (0.10-1.08)
Adjusted for mode by IAP, infant diet at 3 months, parity, ROM > 18 hours	Group2	1.29 (0.54-3.10)	**0.37 (0.16-0.88)***
	Group3	0.82(0.22-2.07)	0.36 (0.10-1.32)
* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval; IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes			

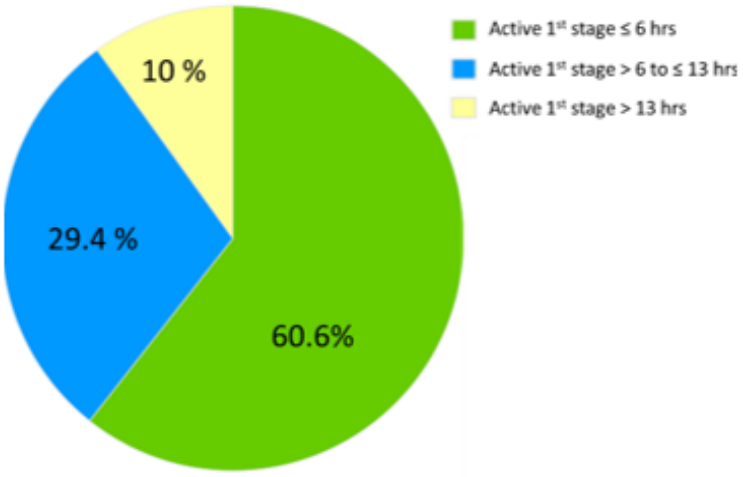
Crude and adjusted likelihood ratio of gut microbiota richness and diversity measures at 3-4 months according to duration of second stage of labour among infants of **obese mothers (n= 157)**

Ref. Group 1 = 2nd Stage <= 1 Hour Group 2 = 2nd Stage > 1 to <=2 Hrs Group 3 = 2nd Stage > 2 Hrs		**Chao1 richness** (below vs above median) OR (95% CI)	**Shannon diversity** (below vs above median) OR (95% CI)
Crude OR for 2nd stage of labour	Group2	1.56 (0.42-3.19)	0.29 (0.10-0.82)*
	Group3	1.84 (0.67-5.07)	0.52 (0.21-1.33)
Adjusted for mode by IAP, infant diet at 3 months, parity, ROM > 18 hours	Group2	1.20 (0.39-3.70)	**0.32 (0.10-0.99)***
	Group3	1.96 (0.56-6.79)	0.59 (0.20-1.74)
* p <0.05; ** p<0.005; OR = odds ratio; CI = confidence interval; IAP = Intrapartum antibiotic prophylaxis; ROM = rupture of membranes			

Figure 3.1

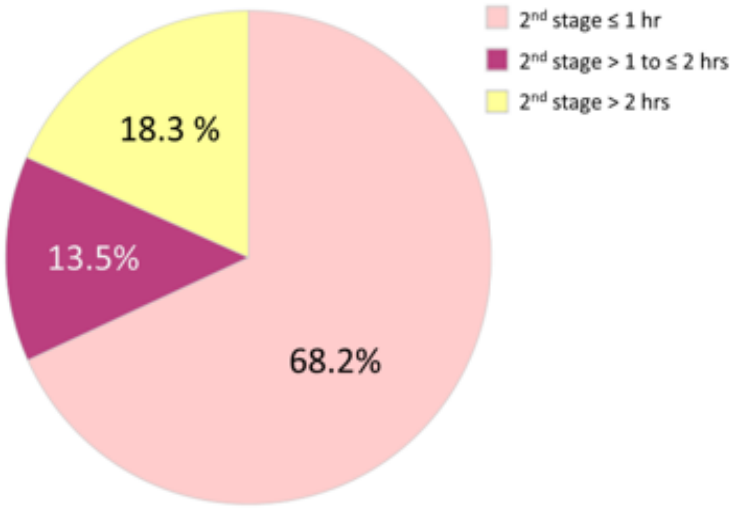
NORMAL WEIGHT PREGNANT WOMEN

a) Distribution of active 1st stage duration (n =531)



Active 1st stage duration	Frequency	Percent
1st Stage ≤ 6Hrs	322	60.6
1st Stage> 6 to ≤ 13 Hrs	156	29.4
1st Stage > 13 Hrs	53	10.0
Total	531	100.0

b) Distribution of second stage duration (n =556)

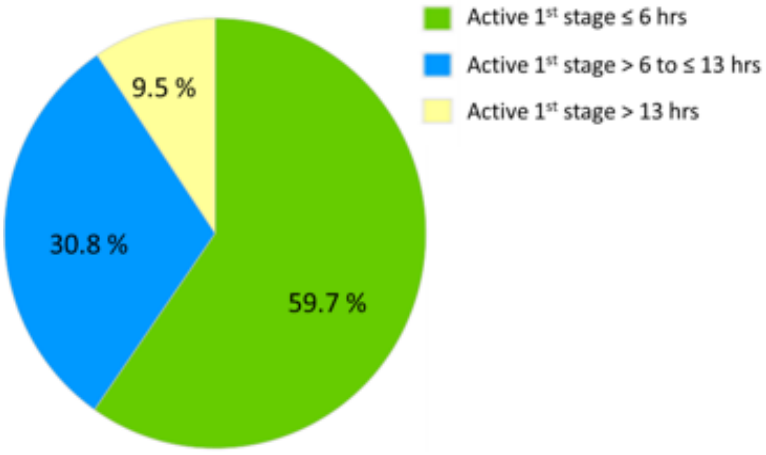


2nd stage duration	Frequency	Percent
2nd Stage <=1Hr	379	68.2
2nd Stage >1 to <=2Hrs	75	13.5
2nd Stage >2 Hrs	102	18.3
Total	556	100.0

Figure 3.2

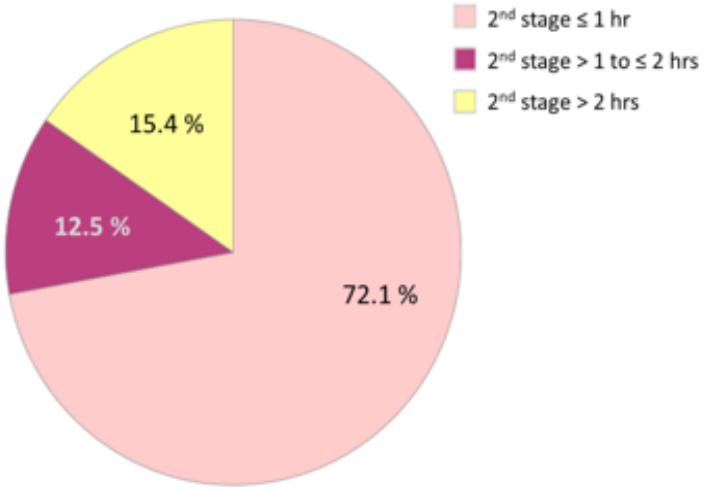
OVERWEIGHT PREGNANT WOMEN

a) Distribution of active 1st stage duration (n =201)



Active 1st stage duration	Frequency	Percent
1st Stage <=6Hrs	120	59.7
1st Stage > 6 to ≤13 Hrs	62	30.8
1st Stage > 13 Hrs	19	9.5
Total	201	100.0

b) Distribution of second stage duration (n = 208)

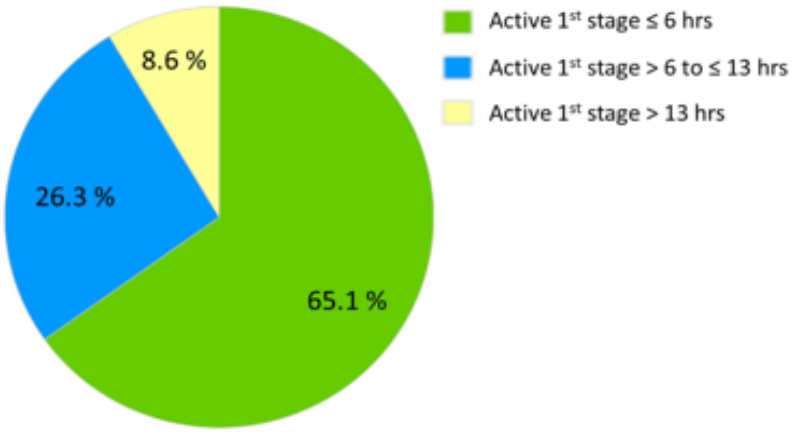


2nd stage duration	Frequency	Percent
2nd Stage <=1Hr	150	72.1
2nd Stage >1 to <=2Hrs	26	12.5
2nd Stage >2 Hrs	32	15.4
Total	208	100.0

Figure 3.3

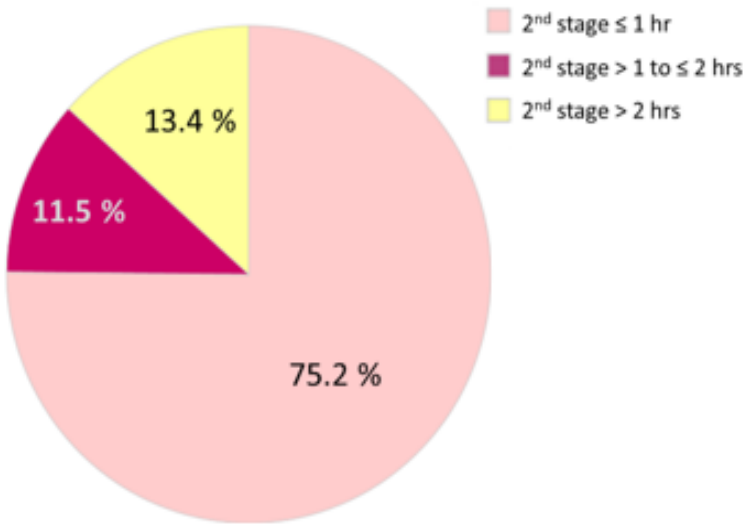
OBESE PREGNANT WOMEN

a) Distribution of active 1st stage duration (n =152)



Active 1st stage duration	Frequency	Percent
1st Stage <=6Hrs	99	65.1
1st Stage > 6 to ≤13 Hrs	40	26.3
1st Stage > 13 Hrs	13	8.6
Total	152	100.0

b) Distribution of second stage duration (n = 157)



2nd Stage duration	Frequency	Percent
2nd Stage <=1Hr	118	75.2
2nd Stage >1 to <=2Hrs	18	11.5
2nd Stage >2 Hrs	21	13.4
Total	157	100.0

CHAPTER 4

General Discussion and Conclusion

4.1 Summary of the Results

Using data from the CHILD longitudinal birth cohort, this thesis explored the influence of duration of labour on infant gut microbiota composition, diversity and richness at 3 to 4 months of age.

Chapter 2 presents the investigation of impact of duration of active first stage and second stage of labour on infant gut microbiota in the general cohort of 999 infants. In infants delivered after active first stage longer than 13 hours and second stage longer than 2 hours, there were statistically significant under-representation phylum Actinobacteria and family *Bifidobacteriaceae* ($p=0.042$) when all delivery modes were considered. When stratified by mode of delivery and intrapartum antibiotic prophylaxis (IAP) exposure, these changes remained significant only among vaginal births without antibiotic exposure where as no differences were observed in infants born vaginally but expose to IAP and by C-section births after labour. Besides, multivariate logistic regression analyses showed that infants born after active first stage longer than 13 hours had a 44% decreased likelihood of colonization with *Bifidobacterium* along with 47% reduced likelihood of colonization with *Lactobacillus.* In addition, when infants were born after 2nd stage longer than 2 hours, they had 52% decreased likelihood of colonization with *Bifidobacterium* and a 37% reduced likelihood of colonization with *Lactobacillus*. These findings were adjusted for delivery mode by IAP use, gestational age, infant diet, parity, duration after rupture of membranes, infant's length of hospital stay and age at stool collection.

Chapter 3 presents a further examination of impact of labour duration on infant gut microbiota in infants born to mothers with different BMI (Body Mass Index) classification. Among infants of normal weight mothers (BMI <25), statistically significant underrepresentation of *Bifidobacterium* was noted when the infants were born after active labour >6 to ≤13 hours, but not after active first stage > 13 hours, and when the infants were born after 2nd stage > 2 hours.

Multivariate logistic regression analyses showed a decreasing trend for colonization with *Bifidobacterium* and *Lactobacillus* with increasing lengths of active first stage and second stage. Interestingly, likelihood of colonization with *Veillionellaceae* increased tended to increase with longer active first stage and reached a 1.7 times increased likelihood of colonization when second stage was longer than 2 hours**.** Among infants born to overweight mother (BMI ≥ 25 to < 30), a trend for underrepresentation of phylum Actinobacteria only in association with longer active 1st stage was noted. Among infants born to overweight mothers, longer labour duration was associated with decreased trend of colonization with phylum Actinobacteria and genus *Bacteroides* at 3-4 months of age, independent of mode of delivery and intrapartum antibiotic prophylaxis (IAP), breastfeeding, parity, membrane rupture duration greater than 18 hours and infant sex. And, among infants of obese mothers (BMI ≥30), underrepresentation of *Bifidobacterium* was observed with active first stage > 13 hours and second stage > 2 hours in vaginally delivered, IAP-free infants. Multivariate logistic regression analyses revealed a more pronounced reduction in colonization likelihood of *Bifidobacterium* with increasing lengths of active first stage and second stage in infants born to obese mothers. Further, a 76% decreased likelihood of colonization with *Veillionellaceae* with active first stage > 13 hours was also observed among these infants. The final estimates were adjusted for delivery mode by IAP use, infant diet, parity and duration after rupture of membranes. Other co-variates of interest such as maternal ethnicity, maternal age etc. were not included in the final model because no significant differences were detected in the distribution of these co-variates according to duration of labour categories by the Chi-square tests. Finally, a reduced trend for microbial diversity (Shannon diversity) was observed with longer labour durations in infants born to overweight and obese mothers, but not among infants of normal weight mothers.

In summary, longer duration of labour was significantly associated with changes in the infant gut microbial composition, including reduced colonization with beneficial *Bifidobacterium* and *Lactobacillus*, at 3 to 4 months of life. When examined within different maternal BMI categories, longer labour duration was associated with more drastic underrepresentation of these probiotic organisms in the infants of obese mothers. Maternal pre-pregnancy BMI also affected

infant gut colonization with *Veillionellaceae* in relation to longer labour durations in the CHILD cohort at 3-4 months of age.

4.2 Strengths of the Study

This study has several strengths. First, this thesis utilized data from participants from three sites (Edmonton, Winnipeg and Vancouver) of the CHILD longitudinal cohort, and is representative of the Canadian general population. Due to its prospective longitudinal cohort study design, the information collected from the participants occurred over time which allows us to measure the changes in the outcome variable over time. It also allows us to associate the changes in one variable to changes in another variable in relation to time, ascertain temporality, reduces recall bias and allows us to hypothesize casual relationships. In addition, data on birth labour durations and birth mode, intra-partum antibiotics prophylaxis (IAP), parity, duration after rupture of membranes, maternal pre-pregnancy BMI, exclusive breastfeeding status in first 3 months were retrieved from hospital birth chart reviews or maternal report at 3-month post-partum or both. All CHILD questionnaires were subjected to an internal validity test, standardized, and validated prior to start of the study. Besides, the large sample size permitted us to conduct stratified analyses to investigate the effect of labour duration independent of delivery mode and IAP use.

Second, high-throughput Illumina sequencing of 16S rRNA gene was employed to profile the infant gut microbiota. 16S rRNA gene is a universal gene for use in bacterial phylogeny and taxonomy, and the most popular housekeeping genetic marker employed in bacterial identification (Janda JM and Abbott SL, 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls. J Clin Microbiol.2007 Sep; 45(9): 2761–2764). The hyper-variable regions (eg. V4) of 16S rRNA gene provide good taxonomic resolution and have sufficient variability between species so as to distinguish each bacterium with high accuracy. The length of 16S rRNA gene (1500 bp) also allow ample availability of sequence data in databases. In addition, Illumina sequencing technology is popularly used in taxonomic studies of the microbiome and allows us to compare the findings of our study with other microbiome research across the globe.

Third, we employed multivariate logistic regression models to establish that the observed associations were attributable to the exposure variable of interest (labour duration), and to account for possible confounders and covariates that could have influenced the observed associations. Unadjusted crude odds ratio was obtained for each covariate, and the final models for research question one (duration of active first stage and second stage of labour) was adjusted for delivery mode by IAP use, gestational age, parity, duration after rupture of membranes, infant's length of hospital stay, infant diet (breastfeeding status), infant age at stool collection and maternal pre-pregnancy weight. The final models for research question two (duration of labour in different maternal BMI categories) were controlled for delivery mode by IAP use, parity, duration after rupture of membranes and infant diet. The rationale for adjusting these covariates was that they have an impact on exposure variable (duration of labour) or the outcome (gut microbiota composition and diversity) or both. In addition, most of these covariates were detected as significant from Chi-squared test. Despite adjusting for those covariates in logistic regression models, the associations between labour duration and gut microbiota dysbiosis, and changes among infants of different maternal BMI categories, remained statistically significant.

4.3 Limitations of the Study

This study also has some limitations. First, majority of participants in the study were recruited from urban areas. Although 80% of Canadian population enjoy urban living (Statistics Canada 2011), it may limit the generalizability and external validity of our results. To add, home deliveries were excluded form our study. Thus, the results from our investigation do not reflect changes in infants born at home.

Second, information on important variables e.g. maternal smoking, breastfeeding status, were obtained from review of self-administered questionnaires. This limits our ability to use objective measurement for those variables of interest.

Third, for the second research question, the sample size infants of mothers with pre-pregnancy overweight and obesity were smaller than that of infants born to normal weight mother.

This may have limited the power of the study to detect significant gut microbiota changes in infants of overweight and obese mothers.

Finally, the high throughput gene sequencing is unable to identify differences among individual species (Jost et al., 2012) and we did not employ qPCR for species identification. Therefore, our findings are limited to reporting at genus level.

4.4 Bias and Confounding

The design of the observational cohort study prevents some bias while allowing a few. First, the very specific inclusion and exclusion criteria from the CHILD study makes the participants of this study very homogenous, thus controlling for many potential confounding. However, the very same specific inclusions and exclusion criteria, along with the fact that most participants were from urban dwellings, could have introduced some *selection bias* in our study because the general population may not have been represented in its entirety. In addition, our second study investigated the outcome (infant gut microbial profile) in relation to exposure (duration of labour) within different maternal BMI categories. Since higher BMI is inversely associated with social economic status and level of education (CIHI 2011: Obesity in Canada), potential socioeconomic disparities between participants of normal weight, overweight and obese categories may have introduced some *selection bias* during identification of the study population for our second study.

Second, the CHILD study collected the information on exposure variable (duration of labour) at the time of baby's birth. Since the hospital personnel recording the length of different stages of labour and other birth parameters were unaware which birth parameters would be investigated in this study, *measurement bias* is reduced. Use of validated CHILD questionnaires, structured interviews, and hospital chart reviews to obtain data for this study also minimized *interviewer bias* in this study. Besides, we deliberately avoided including the *latent* phase of first stage as a measure of our exposure variable because the onset latent phase of first stage is often based on subjective perception, and is thus unreliable. Instead, we only included *active* first stage and second stage of labour as our exposure variable. The objective nature of cut-offs for the

duration of these parameters minimize misclassification of participants, thereby further reducing measurement bias.

In addition, all participating mothers in the study were recruited by CHILD during their pregnancy between the period 2009 and 2012. Although obstetric guidelines have changed over the course of time, and secular trends within medical practice could influence how disease is diagnosed and interventions administered (Paradis 2008), the use of study participants enrolled within narrow span of time and belonging to a prospective cohort reduces potential for *chronology bias* in our study. Some loss of information due to *recall bias* is expected when data is retrieved from self-administered questionnaires to participants. However, this was kept to a minimum in the CHILD study by administering the questionnaires to participants at the most relevant phase of data collection, for instance collection of breastfeeding data at 3 to 4 moths-postpartum home visits etc.

For our second research question, we failed to see significant difference in mean durations of labour between parturients of three different BMI categories (i.e. normal vs. overweight vs. obese). Evidence shows that duration of active first stage is longer as BMI increases, with slowest progression in women with BMI ≥40 (Kominiarek 2011). However, no such difference in duration of active labour by maternal BMI categories was observed in our study. Since obstetricians are aware that overweight and obese mothers are more likely to encounter labour complications such as 'failure to progress' and unplanned C-section, and blinding them to parturients' BMI is virtually impossible, it is likely that overweight/obese women received more closer monitoring of labour and lower threshold for intervention. This could have inadvertently introduced some *performance bias* (measurement bias) on behalf of health-care providers resulting shortened labour lengths in women of elevated BMI in our study.

Finally, the use of high throughput gene sequencing technique imparts high degree of accuracy and reliability in characterizing gut microbiota, and reduces *inter-observer measurement bias*. Since Illumina sequencing is PCR-based technology, some *measurement bias* inherent to PCR-amplification is expected. However, the strictly standard CHILD protocol limits the PCR-amplification to 20 cycles, minimizing this potential bias.

This study has also strived to identify and control for potential confounders so that true association that is devoid of spurious influence by an extrinsic (or a third) factor may be established between the exposure (duration of labour) and outcome (changes in infant gut microbial profile). Covariates that had potential to affect either the exposure or the outcome, or both, and clinically relevant covariates were adjusted for in the analyses. In addition, Chi-square tests were conducted to identify covariates having significant difference in distribution according to exposure categories.

Employing 'stratified analyses' to compare outcome by exposure *separately* for mode of delivery and IAP administration categories was one approach used in this study for controlling confounding effects of birth mode and antibiotic prophylaxis. In addition, for both the first and second research questions, we sought to further minimize the effect of confounding by employing multivariate logistic regression models to control for multiple potential confounders such as mode of delivery by administration of (IAP) (i.e. vaginal delivery without IAP, vaginal delivery with IAP, elective C-section, Emergency C-section), exclusive breastfeeding status, parit and duration after rupture of membranes. Additional covariates adjusted for by logistic regression models for the first question were gestational age, infant's length of hospital stay and age of stool collection as these were also deemed to affect either exposure, outcome, or both.

4.5 Clinical relevance

Balanced development of infant gut microbiota is crucial for immune system maturation and host energy homeostasis. Early life exposures such as mode of delivery (vaginal versus C-section), antibiotic use and breastfeeding (Azad et al., 2015) profoundly influence the infant gut microbial composition and diversity. However, a knowledge void exists on the impact of duration od labour, an inherent element of natural birth, on the development of infant gut microbiota.

Significant associations between duration of labour and changes in infant gut microbiota composition add novel insights to early life factors that influence the development of gut microbiota. Infant gut dysbiosis can impact the development of gut immunity, immune maturation

and host energy harvest, with potential long-term consequences of increased risk of childhood atopy, allergy, and adiposity. Identifying the early life factors, such as protracted duration of labour, that influence the development of infant gut microbiota provides new insights into implementation of early life remedial measures. Besides, results of this study can be implied in favor of healthy pregnancy, promoting ideal maternal weight-maintenance during pregnancy, informed decision making during protracted labour, and to possibly target increment of probiotics to reduce long-term pediatric disease risks.

4.6 Conclusion

In this thesis, the associations between duration of labour and infant gut dysbiosis at 3 to 4 months of age were reported. Depletion of beneficial probiotic organisms was observed with prolonged labour durations. Moreover, these changes were noted to be more severe in infants born to obese mothers and after longer labour durations. Findings from this thesis provides a population-based evidence of influence of labour duration on infant gut microbiota composition and diversity, and adds to our understanding of early life factors that affect balanced growth of infant gut microbiota.

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