UNIVERSITY OF CAPE COAST

GUT MICROBIOME, MALARIA PREVENTION IN PREGNANCY AND MATERNAL HEALTH INDICES AMONG PREGNANT WOMEN IN CAPE COAST

EDWIN YANGNEMENGA

2023

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BY

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Thesis submitted to the Department of Microbiology and Immunology, of the School of Medical Sciences, of the College of Health and Allied Sciences of the University of Cape Coast in partial fulfillment of the requirements for the award of a Master of Philosophy degree in Infection and Immunity

OCTOBER, 2023

DECLARATIONS

Candidate's declaration

I hereby declare that this thesis is the result of my original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature: …… Date…….

Name: Edwin Yangnemenga

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature…….…….. Date……

Name: Dr. Daniel Amoako-Sakyi

Co-Supervisor's Signature…… Date……..

Name: Dr. Faustina Pappoe

ABSTRACT

Gut microbiome during pregnancy offers a 'modulatable' organ to influence pregnancy and pregnancy outcomes. It is as unique at the individual level as it is in the third trimester of gestation. It affects and is affected by hormones, host metabolism and host immunity. Given the enormous changes associated with pregnancy, the gut microbiome structure oscillates between different states, resulting in normal pregnancy and a potential for pathogenic infections and unhealthy outcomes. The study sought to characterize the gut microbiome peculiar to third trimester of gestation and how it relates with IPTp-SP usage and other maternal health indices. Next generation 16S rRNA sequencing techniques were employed to sequence extracted DNA from stool of 22 healthy pregnant women in Cape Coast. Sequences were profiled on the QIIME platform to identify and classify bacterial taxa. Bacterial relative abundances and associations with gestational BMI, IPTp-SP, and deworming status were analysed with several statistical tests using the open-source R software for statistical analysis. The study reports a widely diverse gut bacterial structure with a predominance of *Actinobacteria, Firmicutes, Bacteroidetes* and *Proteobacteria* phyla, and genera *Bacteroides* and *Faecalibacterium.* Three or more doses of [sulfadoxine-pyrimethamine (SP)] were significantly associated with increased gut bacterial diversity (P = 0.031, Kruskal-Wallis) whilst neither gestational BMI nor deworming status influenced bacterial gut diversity ($P > 0.05$). Gut microbiome in the third trimester of gestation is widely diverse and is associated with number of doses of SP taken.

ACKNOWLEDGEMENT

This work is the product of the contributions of numerous actors at various points in time. However, some names deserve a foremost mention. First, my father – Mr. Maurice B.K. Yangnemenga, a retired educationist, whose ideals about education has been the driving force to achieve the highest level possible. To the Head of Department, Prof. Samuel Victor Nuvor, and staff of the Department of Microbiology and Immunology who encouraged me whilst I served in the department and as a student, I owe them a great deal of gratitude. Special thanks to Prof. Dorcas Obiri-Yeboah who stood with me in and out of school, I am most grateful. I specially acknowledge the role of Dr. Daniel Amoako-Sakyi, my supervisor and mentor. He has been instrumental in producing this research piece. To Cynthia Aayaara, who will not give up on me, Dr. Kofi Adu, who kept me on my toes, and to my spiritual family, thank you all for being there for me throughout this period.

DEDICATION

To my parents: Mr. Yangnemenga B.K. Maurice and Madam Susana Yangnemenga

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LIST OF ACRONYMS

BMI –	Body Mass Index
FODMAP –	Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols
IBD –	Inflammable Bowel Disease
IPTp –	Intermittent Preventive Treatment in pregnancy
MBT –	Microbiome-based Therapy
RMNCH –	Reproductive, Maternal, Newborn and Child Health
SP –	Sulfadoxine-Pyrimethamine
WHO –	World Health Organization

CHAPTER ONE

INTRODUCTION

Maternal and infant mortality remains a great health concern for stakeholders in health. An estimated 830 pregnant women die daily from preventable causes (Alkema et al., 2016) with 99% of these deaths recorded in Low- and middle-income countries (LMICs) (Information U.N.D. Millenium Development Goals Report, 2009). In sub-Saharan Africa, maternal mortality has been halved since 1990. Although maternal mortality has generally been on the decline, the risk of maternal death during pregnancy is still disproportionately high in LMICs. For instance, 1 in every 180 maternal deaths are recorded as against 1 in every 4700 in developed countries. Major causes of maternal mortality include postnatal bleeding, preeclampsia, infections, birth complications, and malaria during pregnancy (Oliveira et al., 2014). These major causes are associated with diet, lifestyle, environmental, sociocultural, and economic factors as well as little-talked about factors such as gut microbiome. This study examines the diversity of the gut microbiome in pregnancy and how it relates to maternal health indices.

Background to the Study

Plasmodium parasites are the primary cause of the potentially fatal disease malaria, which has the biggest impact in sub-Sahara Africa (WHO, 2020). Despite the fact that the disease is preventable and curable and is receiving global attention, it remains a public health concern, with an estimated 219 million cases and 435,000 deaths worldwide in 2020. The vast majority of

these fatalities (93%) occurred in Africa (WHO, 2021). Pregnant women and children under the age of five are particularly vulnerable to the disease, accounting for more than 70% of all malaria deaths. Malaria in pregnancy (MiP) is associated with adverse pregnancy and birth comes including, maternal anaemia (Exavery et al., 2014), pre-term delivery, and low birth-weight (Anto et al., 2019). In Ghana, malaria is endemic, with MiP accounting for 17.6% of outpatient visits, 13.7% of hospitalizations, and 3.4% of maternal deaths (National Malaria Control Programme, 2019).

Intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) continues to be an efficient malaria prevention strategy among pregnant women. WHO recommends IPTp using SP to avert the adverse effect of malaria in pregnancy for mother and the unborn (WHO, 2012). IPTp-SP programme involves the administration of a dose of SP starting from the second trimester until delivery. Over the period, the pregnant mother is expected to receive at least 3 doses which has been shown to be optimally beneficial to the mother and foetus (WHO, 2012). The intervention has proven effective in reducing low birth weight, neonatal mortality and maternal anaemia (Exavery et al., 2014). Ghana's National Malaria Control Programme (NMCP) implemented in 2014 recommends a minimum of 5 doses of SP during pregnancy starting from 16 weeks of gestation (President's Malaria Initiative. Ghana – Malaria Operational Plan, 2019).

Sulfadoxine-Pyrimethamine (SP) is essentially an antifolate with considerable antimicrobial activity besides its antiparasitic properties. Antifolates

generally have antibiotic activity against a range of pathogens including *Streptococcus pneumoniae, Escherichia coli, Staphylococcus aureus*, and are thus indicated in the treatment of urinary tract, skin, and soft tissue infections (Capan et al., 2010). IPTp-SP may thus offer important additional public health benefits by treating undetected or previously untreated bacterial infections in pregnant women. Conversely, indiscriminate use of SP may also affect gut normal flora and restructure the composition of the gut microbiome with possible consequences for health and wellbeing in pregnancy. Gut microbiomes and their dysbiosis has been associated with various pregnancy conditions such as preeclampsia, gestational obesity and overweight, preterm birth, gestational diabetes, and also its associations with neonatal neuro and immune development (Crusell et al., 2018; Tabatabaei et al., 2019). In the light of the clinical importance of gut microbiomes, the endemicity of malaria in Ghana, and the enforcement of IPTp-SP recommendations in pregnancy, an understanding of the gut microbiome in pregnancy provides a leverage to influence healthcare.

Statement of the Problem

The importance of gut microbiome in pregnancy cannot be overemphasized. From directly affecting maternal weight gain, diabetes mellitus, preeclampsia, autoimmune diseases, digestive tract diseases, to preterm births, neonatal immune and nervous development, the structure of the gut microbiome during pregnancy serve as an important organ affecting pregnancy and pregnancy outcomes. In recent times, Reproductive, Maternal, Neonatal, and Child Health (RMNCH) interventions leverage on an understanding of pregnancy health and

outcomes to proffer interventions to improve outcomes (Black et al., 2016). The Gut microbiome organ, among others, has become an integral factor to consider in the design of modern RMNCH interventions (Black et al., 2016). This is particularly important because gut microbiomes do not only influence health and disease in pregnancy but may also modulate the success or otherwise of RMNCH interventions and thus must inform the design and implementation of same. However, gut microbiomes are peculiar to the genetic, socio-cultural, religious, economic, climatic, and health systems of societies and even individuals (Spor et al., 2011; Walker et al., 2017). Despite the clinical importance of gut microbiome in pregnancy, in sub-Saharan Africa and particularly in Ghana, the gut microbial structure remains relatively unknown. Thus, the potential role of gut microbiomes in pregnancy and pregnancy outcomes remains unknown.

During pregnancy, the normal physiological changes associated with it greatly affect the diversity and composition of microbial communities in the gut, although some studies have reported conflicting findings (Arumugam et al., 2011; De Filippo et al., 2017). Diet, (Garcia-Mantrana et al., 2018; Olivier-Van Stichelen et al., 2019; Wankhade et al., 2018), pre-pregnancy weight and weight gain during pregnancy and medications during pregnancy (Collado et al., 2008; Gomez-Arango et al., 2016b; Santacruz et al., 2010) contribute to shape the gut microbiome of the pregnant woman. Conversely, Intermittent Preventive Treatment in pregnancy using Sulfadoxine-Pyrimethamine (IPTp-SP) is a recommended intervention during pregnancy aimed at reducing Malaria in Pregnancy (MiP) and its concomitant effects (WHO, 2012). Pregnant women

from second trimester are encouraged to receive a monthly dose of SP until delivery. This intervention, a WHO recommendation, is not only an effective malaria intervention but has also been associated with better RMNCH outcomes that appear to be independent of its antimalarial properties. SP, like all sulfur drugs, has broad spectrum antibiotic activity, aside its basic antiparasitic function (Capan et al., 2010). However, it remains to be understood whether SP with its widely reported antibacterial properties, affects the gut microbiome during pregnancy.

Furthermore, Microbiome-Based Therapies (MBTs) are a potential alternative to traditional preventive and curative medicine because of their ability to modulate microbial communities which in turn affect health through their symbiotic and metabolic activity (Bajaj et al., 2022). However, MBT engineering is hinged mainly on the clear understanding of gut microbial structures in health and in diseases, and factors affecting the modulation of microbial communities (Wong & Levy, 2019). This is particularly important in pregnancy because of the relatively delicate and oscillating immune and metabolic states in pregnancy. The apparent lack in understanding of the microbiome in the Ghanaian population especially during pregnancy hinders the opportunity to ride on the emerging potential alternatives to traditional medicine. This study therefore seeks to explore the gut microbiome in third trimester pregnancy among Ghanaian women and its association with BMI, deworming and IPTp-SP uptake.

Aim of the Study

The purpose of the study is to characterize the gut microbiome and its association with pregnancy health indices in the third trimester of gestation among Ghanaian women.

Research Objectives

1. To characterize the gut microbiome of pregnant women receiving antenatal care at two health facilities in Cape Coast, Ghana.
2. To determine the associations between IPTp-SP uptake and maternal gut microbiome of pregnant women receiving antenatal care at two health facilities in Cape Coast, Ghana.
3. To determine the association between gestational BMI and gut microbiome of pregnant women receiving antenatal care at two health facilities in Cape Coast, Ghana.
4. To determine the association between deworming status and gut microbiome of pregnant women receiving antenatal care at two health facilities in Cape Coast, Ghana.

Significance of the Study

Microbiome greatly influences pregnancy and prenatal development. Attempts to improve pregnancy and pregnancy outcomes will require an understanding of the microbiome during pregnancy and how to modulate that system to achieve the best possible results for both mother and neonate(s). This is the underlying principle on which microbiome-based therapies are developed. MBTs are a potential alternative to traditional preventive and curative medicine

because of their ability to modulate microbial communities which in turn affect health through their symbiotic and metabolic activity. This requires a thorough understanding of the pregnancy microbiome and how it relates to health, and particularly neonatal development. This study shall provide understanding to drive the anticipated and highly effective natural treatment – microbiome-based therapies (MBTs). Our understanding of the classes of microbiota associated with healthy pregnancy will inform the microbial classes to selectively "grow" in pregnancy to promote health and wellness and better RMNCH outcomes. This will translate into better pregnancy outcomes for infants and improved livelihoods.

Microbiomes are thought to be population-specific (De Filippo et al., 2017). Thus, it is inappropriate and potentially harmful for findings of one population to be applied in the development public health and clinical care policies for a different population. The discovery of ethnic and population-specific variation in microbiome composition and diversity is instructive to concepts in microbiome engineering. Studies have reported microbiome variations between rural and urban dwellers; different ethnic groups and different subsistence modes (Gupta et al., 2017). This emphasizes the need for population-specific microbiome studies to better understand the community peculiar microbiome dynamics. This study, the first of its kind among this population in the Central region, will provide relevant data of the population-specific distribution of gut microbiome in pregnancy peculiar to the Ghanaian context.

IPTp-SP interventions have shown great promise in preventing malaria in pregnancy and has also been associated with preventing low birth weight babies amongst others (Eisele et al., 2012). These other benefits are thought to be independent of the antiparasitic properties of SP, and more likely due to the bacteriostatic properties of sulfur drugs. This is evinced in animal models studies where class B antibiotics are shown to reduce microbial diversity and increase maternal weight gain (Khan et al., 2016). Consequently, G6PD deficient pregnant women who are excluded from Sulfur drugs may miss out on the potential benefits of SP as an IPTp intervention. A better understanding of the role of IPTp-SP in gestational microbiome will inform the development of interventions that couple the antiparasitic properties and microbiome-enhancing properties of SP, in formulations that are friendly to G6PD deficient pregnant women.

Delimitations

This is a pilot study designed to explore and inform future large-scale studies in the Ghanaian context. Thus, extrapolations of the findings of this study beyond the study population should be done with caution.

Limitations

The most significant limitation of this study was the relatively small number of participants. Because of our relatively small sample size, the findings of this study must be generalized to the entre Cape Coast pregnant women population with caution. Consequently, to maintain statistical power for the research, the statistical analyses were informed by the sample size and the

findings of this study are interpreted taking into consideration this limitation in sample size.

The highly sensitive and cost effective 16S rRNA metagenomic sequencing method employed in this study has minimal taxonomic resolution, thus does not allow for functional relevance of the genes to be accounted for or provide information on the functional composition of sampled communities. It is not optimum in species level resolution and thus the present study reflects a broad view of microbiome down to the genus level.

Organization of the Study

This thesis is structured as a standard five-chapter thesis according to the recommendations of the University of Cape Coast. Each chapter is dedicated to one of the five major classifications of the parts of the thesis. Chapter one introduces the study. It presents the background context in which the study problem is grounded, the problem studied, the significance of the study, the general and sub objectives of the study, and the definition of the relevant terminologies related to the study. Chapter two examined the empirical literature relevant to the present study. Major headings explored included malaria prevention using IPTp-SP, description of the maternal gut microbiome, the comparison of the gut microbiome in healthy pregnancy and pregnancy complications, the implications of maternal gut microbiome in health and development of both mother and foetus, the association of diet with the maternal gut microbiome, the associations of antimicrobials usage and maternal gut microbiome and the associations of BMI and the gut microbiome. Chapter three

presents the materials and methods employed to achieved the objectives of the study. It describes the study design, the study population, sample, and sample size, the sampling and sampling procedures, the laboratory protocols and the data analysis. It also included ethical considerations. Chapter four presents the results of the study as well as a discussion of the findings in relation to current knowledge and the scientific implications of the findings. Chapter five, the last of the chapters, summarizes the findings of the study, gave scientific conclusions to the study, and proffered recommendations based on the findings.

CHAPTER TWO

LITERATURE REVIEW

Introduction

Pregnancy is a critical window that affects two or more people directly – the mother and foetus(es). The health of the prospective mother and the proper development of the baby(ies) is determined by several factors, including but not limited to the gut microbiome of the pregnant woman. The significance of this bacterial world in the gut has been acknowledged rightly as a pseudo-organ, of a sort. Its intricate connection and interaction with the pregnant mother and developing foetus brings it to the fore in the discussion of maternal and neonatal health. This chapter presents an in-depth review of empirical studies, and relevant literature to the subject under study. It defines RMNCH and its importance in Malaria in Pregnancy (MiP). It also describes the gut microbiome as seen in a healthy non-pregnant person and in pregnancy, the role of the gut in health and disease, the modulators of the gut microbiome in pregnancy, and the effect of the gut microbiome in pregnancy and the developing foetus.

RMNCH: Malaria in Pregnancy and IPTp-SP

Global maternal and neonatal mortality has dropped in the past 30yrs. However, the rate of decline has markedly slowed down in many countries creating doubts on whether they can meet the SDGs (Black et al., 2016). Reproductive, Maternal, Neonatal, and Child Health (RMNCH) has been a matter of priority among civil societies and governments all over the world, especially in Low and Middle-Income-Countries (Black et al., 2016).

Subsequently, governments all over the world signed the Millennium Development Goals (MDGs) with a call on nations to reduce infant and maternal mortality by 67% and 75% respectively by 2015. This was followed by the SDG commitment by world nations with a similar call expected to be delivered by 2030 (Black et al., 2016). RMNCH includes health issues that affect people at all stages of life, from infants and children to adolescent girls and women prior to, during, and after pregnancy and delivery. RMNCH is designed to reduce the burdens of frequent communicable and noncommunicable diseases; unwanted pregnancies; high rates of undernutrition; high maternal, newborn, and child mortality and stillbirths; and loss of human capacity (Black et al., 2016). RMNCH interventions to reduce maternal and newborn morbidity and mortality include preventing postpartum haemorrhage, preeclampsia and eclampsia, obstructed labour; and antenatal interventions such as nutritional interventions and antenatal treatment of maternal infections.

Prevention and prophylactic treatment of malaria in pregnancy, especially, using Insecticide Treated Nets (ITNs) and Intermittent Preventive Treatment in pregnancy with Sulfadoxine-pyrimethamine (IPTp-SP) has specially been outlined as important interventions in improving RMNCH outcomes by reducing malariaassociated morbidity and mortality in pregnancy, low birthweight and neonatal mortality and increasing mean birthweight (Gamble et al., 2006; Radeva-Petrova et al., 2014). Subsequently, in moderate to high Plasmodium falciparum transmission areas, WHO recommends a three-prong approach to dealing with malaria during pregnancy and its effects. These include the

promotion and use of insecticidetreated nets (ITNs), appropriate case management through prompt and effective treatment of malaria in pregnant women and the administration of intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) during pregnancy (WHO, 2004).

IPTp-SP is an integral part of the WHO approach which is initiated as soon as the second trimester sets in. Pregnant women in these endemic areas are advised to receive a dose of SP at least a month apart until delivery. IPTp-SP should ideally be administered as directly observed therapy (DOT) of three tablets sulfadoxinepyrimethamine (each tablet containing 500 mg/25 mg SP) giving the total required dosage of 1500 mg/75 mg SP (WHO, 2014). SP is contraindicated in women receiving Co-trimoxazole or high dose folic acid (≥ 5mg /day) which counteract the antimalarial properties of SP. A 0.4mg/day dose of folic acid however can be coadministered with SP with optimum benefits (Peters et al., 2007).

IPTp-SP protects against the negative effects of malaria in pregnancy on maternal and fetal outcomes namely, clinical malaria, placental infection, fetal anaemia, maternal anaemia, low birth weight and neonatal mortality (Menéndez et al., 2010). In areas with moderate to high malaria transmission, IPTp-SP is a costeffective measure in the prevention of maternal malaria and neonatal mortality resulting from malaria in pregnancy (Sicuri et al., 2010). Amidst the current resistance to SP, and other antimalarials, SP is still effective in reducing neonatal mortality by 18% and low birthweight by 21% (Eisele et al., 2012). A metaanalysis of seven trials evaluating IPTp-SP conducted by WHO (WHO,

2012) demonstrated that compared to two doses of IPTp-SP, three or more doses were related with greater mean birth weight and fewer low birth weight (LBW) newborns. The estimated reduction in risk of LBW was 20% (95% CI, 6-31). This result held true for a variety of SP resistance levels. Additionally, it was discovered that placental malaria was lower in the 3+ dosage group. Between the two groups, there were no differences in the major adverse events (WHO, 2012).

In the light of its enormous benefits for RMNCH, WHO care guidelines for pregnant women include increasing accessibility of IPTp-SP services to every pregnant woman as part of the Focused Antenatal Care (FANC) initiative. The guidelines recommend that each pregnant women receives a dose at least every month after the first trimester. This is to ensure that each pregnant women would have received at least 3 doses of SP during pregnancy (WHO, 2014). DOTs model also ensures that each pregnant women received the doses as prescribed. Although, IPTp-SP programme is prescribed for moderate to high malaria transmission regions, in areas where malaria transmission has not been assessed, WHO recommends that IPTp-SP be administered as in a malaria endemic community. Given the low cost of IPTp-SP and the difficulties of reintroducing IPTp-SP after withdrawal of same, nations are discouraged from withdrawing IPTp-SP programmes without enough data to support it (WHO, 2014).

The widely recommended use of SP is mainly based on its antimalarial properties and the added advantages of reducing low birthweight and neonatal mortality. It goes without saying that SP aside its antimalarial properties also have antibiotic activity against a broad spectrum of bacteria (Capan et al., 2010).

Whilst it has not been reported anywhere, the antibiotic properties of SP may affect gut microbiomes, an important bacterial organ modulating health and disease.

The Human Gut Microbiome

The gut microbiome refers to the estimated 100 trillion microorganisms that colonize the human intestinal tract, forming a symbiotic relationship with their sites of colonization and beyond (Power et al., 2014). It is estimated that there are as many bacterial cells as there are human cells in the body, weighing in total 0.2kg (Sender et al., 2016). In total, genes of the human microbiome are 150-fold more than human genes (Power et al., 2014). Different body sites harbor different microbial populations due to varying levels of pH, oxygen, nutrients, humidity, and temperature (Spor et al., 2011). The human microbiome develops over time, beginning in-utero, through birth till adulthood and death. The development of the gut microbiome in particular is affected by mode of delivery, maternal and infant diet, and maternal oral microbiome, and environmental associations (Brown et al., 2013). While the entire gut begins in the mouth and continues to the anus, the gut microbiome describes the intestinal portion alone, given that the intestine harbors the most diverse and abundant microbial community in the body (Bäckhed et al., 2012). The gastrointestinal tract (GIT) is colonized by a variety of bacteria ranging from bacteria, viruses, protozoans, to Achaeans. The ratio of these microorganisms varies across age groups and other determinants. In adults, however, 80% of these organisms are bacteria of the phyla *Bacteroidetes, Firmicutes* and *Actinobacteria* (Baldassarre

et al., 2018). In contrast, neonatal gut microbiome immediately after birth consist mainly of *Enterococci, Staphylococci*, and *Enterobacteria.* Over the next few days of life, the microbial structure shifts slightly towards adult microbiome and by the first birthday of the infant, the structure of the microbiome takes the form *Lactobacillus, Clostridium, Bifidobacterium*, and *Bacteroides* (Baldassarre et al., 2018).

The Gut Microbiome (GM) performs significant roles in its symbiotic relationship with the human intestines. Among other functions, the gut microbiome aids in the assimilation of minerals and vitamins; assist in digestion of fiber; together with the liver, the gut microbiome detoxifies and excretes xenobiotics and harmful chemicals (Mayer et al., 2014); management of intestinal wall integrity; facilitating systemic and intestinal mucosal immunity (Power et al., 2014). Research showed that GMs play a vital role in human immunity, and are involved in the development of chronic diseases such as diabetes, hypertension, and inflammatory bowel disease (Dolan & Chang, 2017; Hooper et al., 2012; Lezutekong et al., 2018)

Gut microflora ferment non-digestible foods such as dietary fibre to provide substrates for SCFA-producing bacteria (Wong et al., 2006). The main SCFA produced by gut bacteria include butyrate, propionate, and acetate. Butyrate is a multifunction SCFA that is a major energy source for colonocytes, with a capacity to induce apoptosis of colonic cancer cells. It also triggers intestinal gluconeogenesis, and thus essential in glucose homeostasis (De Vadder et al., 2016). During β-oxidation, butyrate plays important role in the epithelial

cells' uptake of large volumes of oxygen. This creates a state of hypoxia in the gut and contribute to maintaining oxygen concentrations and preventing gut microbial dysbiosis (Byndloss et al., 2017). The most abundant SCFA, acetate, is an important metabolite in the growth of other bacteria. It is transferred to peripheral tissues where it is involved in lipogenesis and cholesterol metabolism and is also thought to play an important role in central appetite regulation (Frost et al., 2014). Propionate is transferred to the liver where it regulates gluconeogenesis and satiety signaling through interaction with the gut fatty acid receptors (De Vadder et al., 2016). Randomized control studies showed that increased SCFA production was negatively correlated with insulin resistance (Zhao et al., 2018) and diet-associated obesity (Lin et al., 2012). Butyrate and propionate, but not acetate, seem to control gut hormones and reduce appetite and food intake in mice (Lin et al., 2012).

Other products of the gut microbiome such as indolepropionic acid and trimethylamine directly affect the host metabolism and health. They are produced from carnitine and phosphatidylcholine by the metabolic activities of gut microbiota and thus, levels of these products are directly proportional to the microbial structure of the individual. Trimethylamine is oxidized in the liver to trimethylamine N-oxide which is positively correlated with increased risk of atherosclerosis and major adverse cardiovascular conditions (Chen et al., 2020; Tang et al., 2013). Indolepropionic acid is highly correlated with dietary fibre (De Mello et al., 2017) and is a potent scavenger of free radicals in vitro (Chyan et al., 1999), and thus may reduce the risk of type II diabetes (De Mello et al.,

2017). Gut microfloral enzymes are also necessary in breakdown of bile acid, secondary and unconjugated bile acids. These molecules act as signaling chemicals influencing various host processes (Long et al., 2017).

Gut Microbiome in Pregnancy

Pregnancy is a special period characterized by lot of metabolic, immune and hormonal changes in the body (Zakaria et al., 2022). These changes are closely associated the composition and diversity of the gut microbiome. This in turn affects maternal immunity, metabolism, and digestion and fetal development. Maternal gut microbiome remains largely unchanged in the first trimester of gestation compared to pre-pregnancy period (de Brito Alves et al., 2019). Changes in composition and diversity of the microbiome occur progressively across the trimesters with the highest diversity of gut microbiome observed in the third trimester (Smid et al., 2018).

Typically, the gut microbiome of normal pregnancy is composed of largely of *Bifidobacterium,* but also of *Proteobacteria* and *Actinobacteria* (Koren et al., 2012). The gut microbiome during the first trimester of pregnancy has little to no difference from the healthy pre-pregnancy microbial structure with a predominance of *Firmicutes*, mainly *Clostridiales*, over *Bacteroidetes* (Walters et al., 2014). Over time, the maternal gut microbiome shifts towards *Proteobacteria*, *Bifidobacteria* and lactic acid-producing bacteria dominance and a decline in butyrate-producing bacteria such as *Faecalibacteria* (Koren et al., 2012). Significant difference in microbiome, typical of pregnancy, is observed over the following trimesters, with the third trimester presenting the most distinct

pregnancy microbiome. During this period, there is reduced alpha diversity (individual richness) across microbial flora and increased inter-subject beta diversity as well as relative abundance of opportunistic pathogenic bacteria (Koren et al., 2012). DiGiulio et al., however, did not observed any difference in gestational gut microbiome as compared to nonpregnant periods (DiGiulio et al., 2015). The significance of these contested microbial changes during pregnancy is also not incontrovertible. Whilst some researchers (DiGiulio et al., 2015; Yang et al., 202) have observed no significant impact of these aberrations in microflora, Turjeman et al., showed that transferring gut microbiome of third trimester pregnant women into mice caused weight gain and marked low grade inflammation (Turjeman et al., 2021). Similarly, alterations in maternal gut microbiome influenced maternal metabolism (Fuhler, 2020), and consequently affects the development and priming of neonatal organs and systems (Zakaria et al., 2022). Also, hormonal changes during pregnancy coupled with changes in the gut mucosa immunity induce low-grade inflammation. This facilitates increased diffusion of glucose from the gut epithelium towards the lumen, and thus may induce weight gain while modifying the gut microbiota during normal pregnancies (Gosalbes et al., 2019).

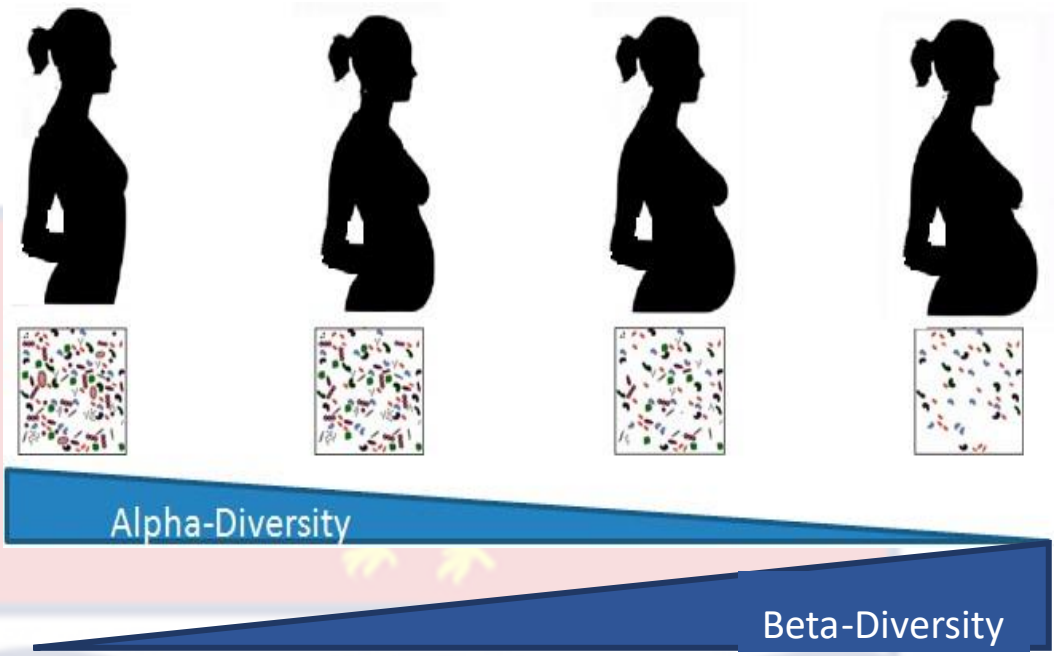

Figure 1.: Alpha and beta diversity profiles as seen in pre-pregnancy, 1st trimester, 2nd trimester and 3rd trimester pregnancies respectively (Mesa et al., 2020).

The structure of gut microbiomes in pregnancy is important in the priming the woman for birth. Women who had preterm deliveries, for example, presented with different beta diversity as compared to women who had normal deliveries (Hiltunen et al., 2022). Furthermore, in spontaneous preterm deliveries, mothers gut showed significantly reduced microbial diversities especially, in *Streptococcus* and *Bifidobacterium* (Dahl et al., 2017). Although these normal florae are nonpathogenic under normal circumstances, their presence in some sterile sites and imbalances in relative abundances of the flora can give rise to intrauterine infections. Women who experience premature membrane ruptures, for example, show the presence of microbes in the otherwise sterile amniotic fluid (Edwards et al., 2017). The mechanisms via which

microorganisms get into the uterus has not been clearly defined. However, two mechanisms have been postulated: (1) microbes in the gut leak into the placenta or uterus; (2) ability of gram-negative bacteria to ascend the vagina into the uterus via their liposaccharide-induced prostaglandins and other inflammatory cytokines production (Edwards et al., 2017).

Also, maternal gut microbiome during pregnancy is an important determinant of infant health and development (Gomez-Arango et al., 2016a; Nyangahu & Jaspan, 2019) with the potential to modulate autoimmune conditions and atopy (Nyangahu & Jaspan, 2019). The gut microbiome is connected to the foetus via the placenta. Although the placenta has its own microbiome, including *Tenericutes, Fusobacteria, Bacteroidetes*, and *Firmicutes,* all nonpathogenic bacteria (Aagaard et al., 2014), the effect of gestational gut microbiome are seen in the placenta, albeit, indirectly. Gestational diabetes, obesity and antibiotic use, all of which are associated with gestational gut microbiome dysbiosis, affect changes in the placental microbiomes (Pelzer et al., 2017). For example, Antony et al., (2015) reported that excessive weight gain during pregnancy is associated with risk factors such as disorders of placental microbiomes and preterm birth. Postpartum, infant gut microbiome appears to be derived largely from breastmilk microbiome of the mother. Pannaraj et al., (2017) observed that the most abundant gut microbiome in the infant in the early days after birth were from the maternal breastmilk, for infants that were breastfed. Thus, gestational microbiome does not only affect the foetus in-utero in the prenatal periods, but also in the early postnatal periods.

Gut Microbiome in health and disease

In health or in disease, gut microbiome appears to play crucial roles. Whilst the mechanisms of these roles are yet to be defined, the apparent associations of distinct microflora with various states of health draws a clear line of relationship. Conditions such as gestational diabetes mellitus, gestational obesity and overweight, preeclampsia, autoimmune diseases and digestive tract diseases have particularly been indicated in gut microbial dysbiosis.

Gestational Diabetes Mellitus

Gestational Diabetes Mellitus (GDM) is the third major type of diabetes after types I and II diabetes. It is a form of impaired glucose tolerance that is only diagnosed during pregnancy (Schneider et al., 2011; American Diabetes Assocation, 2018). Pregnant women with GDM have higher chances of returning to normal glucose metabolism after delivery. GDM however, increases the risk of developing Type II diabetes in later life (Bellamy et al., 2009; Lauenborg et al., 2004). The incidence of GDM globally is estimated to range between 1 and 14% (Sacks et al., 2012; Zhu & Zhang, 2016). Women with GDM present with a peculiar gut microbiome. Whilst the abundance of *Firmicutes, Klebsiella variicola, Collinsella, Rothia, Ruminococcus, Actinobacteria, Parabacteroides distasonis, Desulfovibrio* was increased in women with GDM compared to women with normal blood sugar (Crusell et al., 2018; Ferrocino et al., 2018), *Parabacteroides, Dialister, Akkermansia, Roseburia, Bacteroides, Methanobrevibacter smithii, Eubacterium* species, *Alistipes* species, *Bifidobacterium* species were reduced in GDM women compared with women

with normal blood sugar (Cortez et al., 2019; Kuang et al., 2017). The relative abundance of bacteria of the order *Pseudomonadales* and genus *Acinetobacter* have also been shown to be reduced in women with GDM as compared to their healthy control pregnant women. This typical microbial dysbiosis especially in the third trimester of GDM women persists up to 8 months postpartum.

GDM and other complications of pregnancy such as insulin resistance, inflammation and hyperglycaemia are attributable to leaky gut caused by increased permeability of intestines (Flint et al., 2012; Navab-Moghadam et al., 2017). Normal gut flora contributes to the maintenance of intestinal wall integrity. In the event of gut dysbiosis, metabolic products of non-resident and pathogenic bacteria affects the permeability of the intestinal walls (Navab-Moghadam et al., 2017). Also, reduction in the relative abundance of *Acinetobacter* genus, for example, has been correlated with decreased production of anti-inflammatory cytokines such as IL-10 and metallopeptide inhibitor-3, and eosinophils (Bassols et al., 2016). Although the mechanisms of action of gut microbiomes in GDM is not well explained, it has been severally correlated with GDM and its associated known causes.

Gut microbiome and gestational obesity

The importance of gestational obesity in pregnancy cannot be overemphasized because of its relevance in the development of various conditions during pregnancy. It is defined as the gaining of excess weight during pregnancy such that the total weight of the expectant mother exceeds normal ranges for gestational weight. It may occur as either obesity during pregnancy or as

postpartum weight retention. The categorization of weight gains during pregnancy that are considered gestational obesity has been promulgated by the American Institute of Medical Research (IOM) as shown in Table 2.1. Gestational obesity has not only been indicated in GDM and maternal hypertension during pregnancy but also adversely affect the developing foetus in ways including giant baby, subinvolution of uterus, and neonatal congenital defects (Poston et al., 2016; Zambrano et al., 2016).

Hitherto, gestational obesity has been attributed largely to hormonal imbalance during pregnancy. However, recent evidence suggests gut microbiomes have been implicated in the development of gestational obesity. The gut microbiome has been considered a metabolic organ whose imbalance, like hormonal imbalance, can offset the natural balance of metabolism and cause gestational obesity (Kalliomäki et al., 2008). A prospective follow-up study showed that excessive weight gain during pregnancy was associated with a high concentration of *Bacteroides spp*. in the intestine (Collado et al., 2008). The gut dysbiosis associated with gestational obesity was seen in the significant decreased relative abundance of *Bacteroides* and *Bifidobacterium* and the significant increase in the relative abundance of *Enterobacteriaceae*, *Escherichia coli* and *Staphylococcus* (Santacruz et al., 2010). These bacterial changes were not only correlated with weight gain but also with biochemical parameters such as triglycerides, transferrin, plasma cholesterol, high-density lipoprotein cholesterol and folic acid. Similarly, Collado et al. reported reduced *Bifidobacterium* presence and higher levels of *Akkermansia muciniphila* and

Staphylococcus in the gut of pregnant women compared with non-pregnant women (Collado et al., 2012). In general, gestational obesity or overweight is associated with reduced gut bacterial diversity (Stanislawski et al., 2018) and increased pro-inflammatory bacteria (Zacarias et al., 2018). These studies highlight the role of maternal gut microbiome in regulating maternal lipid metabolisms and subsequently the maintenance, gain or loss of weight during pregnancy.

The mechanisms by which gut microbiomes cause gestational obesity has not been clearly defined. Various researchers have speculated various mechanisms including; (1) gut microbial disorders may affect the epigenetic modifications which control lipid absorption and weight gain; (2) chronic inflammation resulting from bacterial death and subsequent release of bacterial cell wall liposaccharides endotoxins into the intestines may cause insulin resistance and inflammatory responses; (3) bacterial dysbiosis may increase intestinal capacity for absorption, especially of monosaccharides; (4) the metabolic production of secondary bile acids from primary bile acids in the small intestines; (5) the activation of liver endocannabinoid (eCB) and ChREBP/SREBP-1systems; (6) and during fasting, some gut microbiomes ability to produce adipose cytokines which can suppress protein lipase and subsequently cause fat accumulation in peripheral tissues (Collado et al., 2008; Soderborg et al., 2018; Barlow et al., 2015; Gu et al., 2017; Khan et al., 2016; Kumar et al., 2014).

Preeclampsia

Preeclampsia presents with raised blood pressure and proteinuria after 20 weeks of gestation. Symptoms may include dizziness, headache, vomiting, nausea, and epigastric discomfort (M. A. Brown et al., 2018). One (1) in twenty (20) women will develop preeclampsia in pregnancy. Globally, it is the second leading cause of maternal mortality (Ghulmiyyah & Sibai, 2012; Huppertz, 2008; Mol et al., 2016).

Although the causes of preeclampsia are not clear, primiparas, hypertensive, overweight, and women with vascular diseases and a history of preeclampsia are at an increased risk of developing preeclampsia (Ananth et al., 2013; Hutcheon et al., 2011). However, recent studies by Kell and Kenny showed that oxidative stress, antiangiogenic response, abnormal trophoblast invasion into the placenta, and increased proinflammatory cytokines are part of the mechanisms that lead to preeclampsia (Kell & Kenny, 2016). The obstruction of the utero-placental blood flow exacerbates the risk of preterm birth and/or low birth weight foetuses (Carter et al., 2017; Ilekis et al., 2016; Salmani et al., 2014).

In recent times, gut microbiomes have been associated with preeclampsia. The microbial diversity observed in the placenta in preeclampsia has been related to gastrointestinal, respiratory and periodontal infections. *Escherichia, Salmonella, Bacillus*, and *Listeria*, for example, which are found in the placenta in preeclampsia are linked to gastrointestinal infections whilst *Klebsiella* and *Anoxybacillus* are linked to respiratory infections (Amarasekara et

al., 2015). Studies have shown increased abundance of pathogenic bacteria (*Bulleidia moorei* and *Clostridium perfringens*) in the intestines of preeclampsia patients compared to healthy controls. The abundance of probiotics (*Coprococcus catus*) however, decreased in preeclampsia women (Lv et al., 2019). Further studies highlighted the relative abundance of *Fusobacterium* and *Veillonella* and decrease in beneficial *Faecalibacterium* and *Akkermansia* bacteria (X. Chen et al., 2020). Whilst this data does not suggest a clear line of causation between gut microbiome and preeclampsia, it highlights the associations that exist between the two. Modulation of gut microbiome may hold promise for symptomatic treatment of preeclampsia.

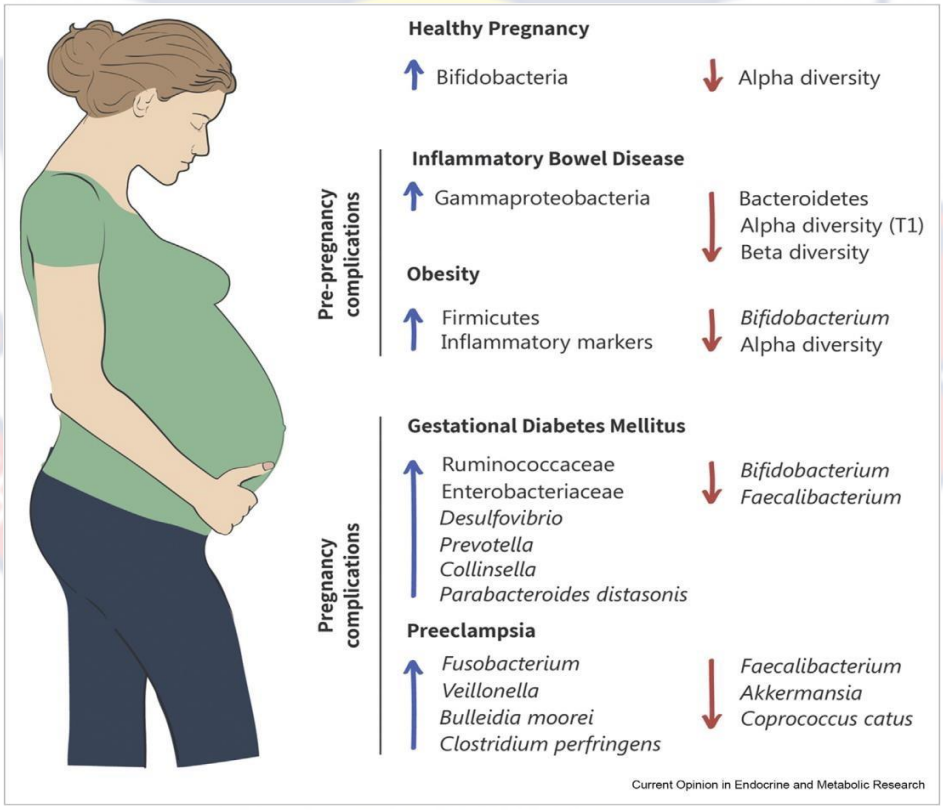

Figure 2.: Gut bacteria in health and in various pregnancy conditions (Turjeman et al., 2021)

Digestive tract disease

Pregnancy presents with frequent gastrointestinal conditions including gastritis and enteritis. Reactions such as nausea, vomiting, esophageal reflux, and constipation are also common (McCarthy et al., 2014). According to Matthew et al., the incidences of nausea and vomiting during pregnancy are 50 – 80% and 50% respectively (Matthews et al., 2015). In a few cases, some pregnant women may experience hyperemesis gravidraum which can result in fluid and metabolic imbalance (Einarson et al., 2013).

These conditions in the gastrointestinal tract appear to have an effect on the bacterial colonies in the tract. For example, whilst the relative abundance of *Collinsella* and *Blautia* decreased with gastrointestinal disorders, the relative abundance of *Acinetobacter, Paenibacillus* and *Enterococci* increased with gastrointestinal disorders (Jin et al., 2020). The abundance of *Collinsella* and *Blautia* are thought to accurately predict digestive disorders that Jin et al., (2020) propose that they be used as biomarkers for digestive disorders. Not only does gastrointestinal disorders affect gut microbiomes, it is conversely affected by gut microbiomes. In inflammatory Bowel Disease (IBD), alpha diversity of the gut microbiome generally reduces, with an increase in *Gamaproteobacteria* and a decrease in *Bacteroidetes* (Torres et al., 2020). Gut microbial dysbiosis is thought to increase prevalence of colitis. In a mice experiment, Xie et al., showed that supplementing pregnant mice with high fat diets altered the intestinal microbiome and subsequently increased dextran sodium sulfate (DSS)–induced colitis in offspring (Xie et al., 2018). Using the DSS model of induced colitis in

adult mice, Yan et al., (2017) showed that colonizing the gut of the mice with *Lactobacillus rhamnosus* (LGG) showed reduced susceptibility to intestinal inflammation and injury and an increase in IgA production.

The impact of the maternal gut microbiome transcends the mother's body to the infant's gut. In population-based studies, antibiotic use during pregnancy was correlated with increased risk of inflammatory bowel disease (IBD) in offspring (Örtqvist et al., 2019). Whilst the mechanisms of action of these relationships are yet to be fully understood, it has been established that maternal gut microbiome affects the health of the maternal gut and to some extend influence the development and health of the neonate's gut. Modulating the maternal gut flora may hold promise for improving the health of the mother during pregnancy and the development and health of the neonate.

Autoimmune diseases

Autoimmune diseases are diseases of the immune system where the body's immune system reacts to autoantigens. This results in damage to the cells that presents the autoantigens. There are about seventy (70) autoimmune diseases including Systemic Lupus Erythromatosus (SLE) and rheumatoid arthritis (RA) (Davidson & Diamond, 2001; Ding et al., 2022). Autoimmune disease prevalence globally is estimated to be about 7%, with females more likely to have autoimmune conditions than males (Cooper et al., 2009). This is thought to arise from female sex hormones modulating immune response via sex hormone receptors (Adams Waldorf & Nelson, 2008; Cai et al., 2019; Tsao et al., 2019). In pregnancy, various physiological changes such as immune, metabolic and

hormonal changes are designed to accommodate and support the developing foetus. These temporary modifications also present possibilities of developing autoimmune conditions.

In recent times, the role of gut microbiomes in autoimmune disease has generated widespread interest in the research community. Dysbiosis of gut microbiomes has been associated with RA and SLE (Mu et al., 2015). In a study of SLE, the authors found that changes in the GMs during pregnancy and lactation interfered with the autoimmune response (Mu et al., 2015). Though there are no studies of RA in pregnant women, animal model studies of RA showed that a bacterium could restore the microflora imbalance which was able to protect the bones of rats with RA. Reasonably, we infer that gut microbiome may influence autoimmune diseases in pregnancy since pregnancy is the window for most autoimmune disease's development in the neonates and that changes in gut microbiome during pregnancy has been well established.

Maternal gut microbiome and offspring immune development

Maternal microbiomes are closely related to offspring microbiomes and the development of the offspring. Various maternal microbiomes influence perinatal development of the neonate via the placenta or play important roles in the early postnatal microbial colonization of the neonate and immune sensitization and development. Maternal microbiomes may be transmitted vertically to neonates via maternal skin, vagina (during vaginal birth), breastmilk, and faeces (Nyangahu et al., 2018). Bacteria of the maternal gut

microbiome appear to be better suited than non-maternally derived flora for survival in the gut of the offspring (Ferretti et al., 2018).

Infant's immune planning and priming is influenced by the changing dynamics of maternal microbiome during gestation (de Agüero et al., 2016). In animal model studies, treating pregnant mice with heavy antibiotic doses, thus altering the gut microbiome, revealed that offspring were deficient in IL-17 producing lymphocytes and IL-17 transcripts in the ileum (Deshmukh et al., 2014). Similarly, in another study, after treating pregnant mice with three antibiotics, the offspring's GMs diversity reduced, and IL-17-IFN-γ–producing CD4+ and CD8+ cells in mesenteric lymph nodes decreased in the baby's enteric immune system (Hu et al., 2001). In the offspring's peripheral immune system, (Gonzalez-Perez et al., 2016) observed that treating dams with antibiotics during gestation and lactation resulted in pups' CD8+ T cells failure to produce IFN-γ. They also observed a change in the distribution of NK and dendritic cell subsets (Gonzalez-Perez et al., 2016). In a mouse model of autoimmunity based on the NLRP3 inflammasome mutation R258W, the maternal microbiomes were required for neonatal IL-1b and tumor necrosis factor-a (TNF-a) responses in the skin (Nakamura et al., 2012).

Maternal microbiomes may be important in the development and priming of neonatal immune cells. Also, perturbation of maternal microbiomes also affects the development of adaptive immune system (Nyangahu et al., 2018). The importance of optimum distribution and diversity of maternal microbiome in the development of cellular and adaptive immune system cannot be overemphasized.

An in-depth understanding of the mechanisms of this relationship will provide significant novel insights into the management of pregnancy to achieve healthy outcomes for both mother and offspring.

Maternal microbiome and neonatal neurodevelopment

Microbiomes at various sites establish symbiotic relationships with their immediate environment and the human body as a whole. Some of these relationships are important for brain and nerve development (Dinan & Cryan, 2017). Consequently, changes in the structure of microbial communities have been associated with some neurological disorders such as stress, anxiety, depression, and autism (Vuong & Hsiao, 2017). Like the role of the environment in the Barker's hypothesis (Barker, n.d.), it has been suggested that perinatal microbiomes modulate the development and priming of adult brain (Codagnone et al., 2019).

Maternal microbiomes are also essential in host neurological development, perhaps via epigenetic modifications. A study of germ-free mice showed that the lack of regulatory effects of microbiomes led to abnormal brain development in mice, such as abnormal growth of microglia, high myelinization of the prefrontal cortex, and increased permeability of the blood-brain barrier (Braniste, n.d.; Clarke et al., 2013; Thion et al., 2018). Furthermore, in mice that were deficient in microbial diversity and abundance, genes for neural development including neurotransmission in the hippocampus and neuronal plasticity, were poorly expressed (J. Chen et al., 2017; Stilling et al., 2015). These changes are important precursors for the development of anxiety, cognitive

deficits, increased stress response, visceral pain response, and changes in fear perception (Hoban et al., 2018; Luczynski et al., 2017).

In addition, studies showed that changes in the structure of perinatal microbiomes regulate gene expression, function, and morphology of progeny microglia, these effects will appear in the early stages of embryo development. Genetic experiments underscore the importance of maternal microbiome in the regulation of microglia. Microglial status can be regulated in microbially depleted mice during adulthood, and three independent studies emphasized the role of maternal microbiomes in guiding embryonic microglial development. What's more, the gene expression changes of germ-free mouse microglia were more obvious in adult microglia than in neonatal microglia compared with the conventional control group (Matcovitch-Natan et al., 2016).

The mechanisms by which maternal microbiome influence neurological development has not been well defined. However, various studies propose possible mechanisms underlying these interactions: (1) Toll-like Receptors (TLRs) signaling on neuronal proliferation: TLRs can perform innate immune recognition on the components of microbiomes; TLRs are expressed in all subtypes of brain resident cells, including intact expression in astrocytes, neurons, and oligodendrocytes (Hanke & Kielian, 2011; Kawai & Akira, 2011). (2) Cytokine signaling on neurogenesis: In the brain, cytokines have different effects on neurodevelopment. For example, IL-4 inhibits the proliferation of mouse embryonic neural precursor cells (NPCs), IL-34 and CSF-1 promote neuronal proliferation, and IL-6 promotes the occurrence of fetal striatum cells;

maternal intestinal microbiomes can fully change germ-free mouse intestinal cytokines distributed (Pronovost & Hsiao, 2019). (3) Complement proteins in synaptic refinement: The complement system contributes to the clarity of cells and humoralmediated pathogenic microbiomes (Ricklin et al., 2016). Synaptic complement proteins play an important role in the early development of neurons. For example, complement protein C3 is localized to the axons of retinal geniculate cells and depends on upstream complement proteins C1 and C4 (Amarasekara et al., 2015)

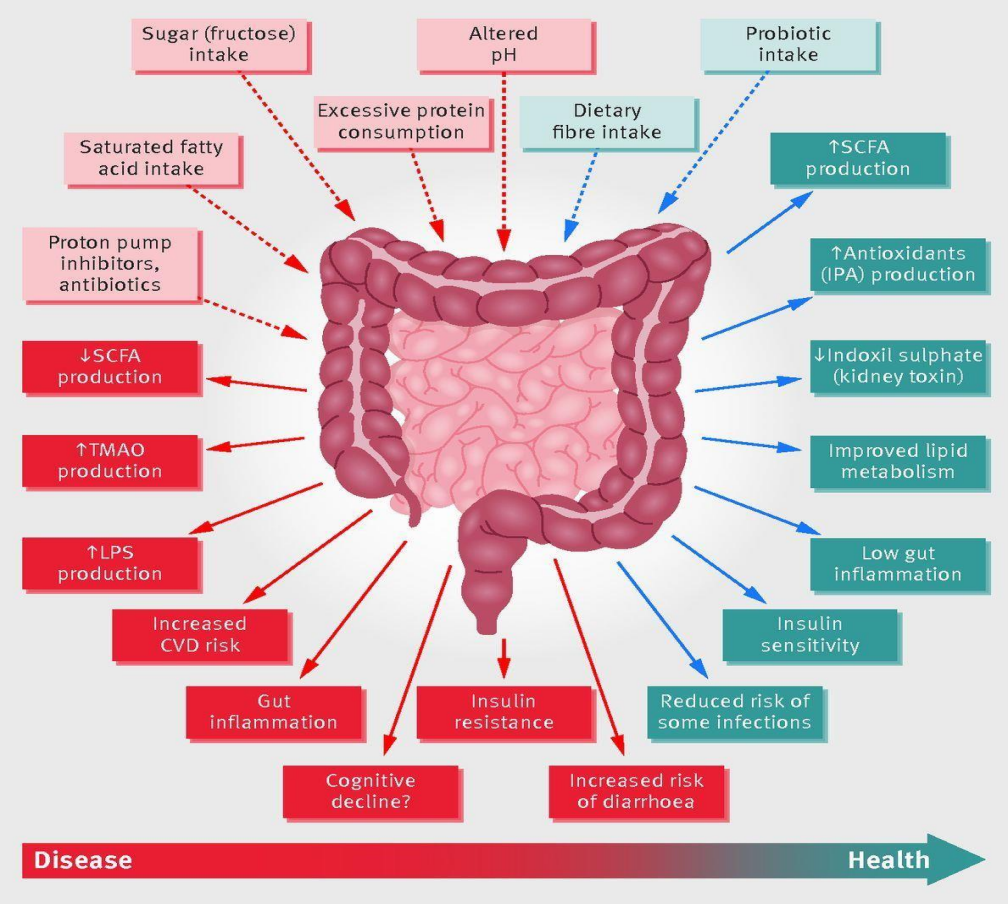

Figure 3: Summary of the role of gut microbiota in health and in disease (Valdes et al., 2018)

Nutrition and the Gut Microbiome

Dietary patterns play important roles in the relative abundances of different gut microbiota. The biochemical properties of various foods and supplements have varying effect on various bacterial colonies. High intensity sweeteners such as aspartame, sucralose, and saccharin though "generally recognized as safe" have been shown in animal studies to disrupt the diversity and balance of normal gut microbiota (Nettleton et al., 2016). After supplementing rats' diet with sucralose for 12 weeks, Abou-Donia et al., (2008)) observed significantly elevated *Clostridia, Bacteroides*, and entire aerobic bacteria in the gut. This commiserated with significantly higher stool pH compared with controlled pairs (Abou-Donia et al., 2008). In a much longer study, Bian et al., fed mice with sucralose for 6months and observed increased expression of gut microbial pro-inflammatory genes and interrupted faecal metabolites (Bian et al., 2017). Also, food additives, including emulsifiers commonly found in processed foods, modulate gut microbiome diversity and balance (Chassaning, Koren, Goodrich et al. 2015). Emulsifiers such as polysorbate–80 and carboxymethylcellulose and when fed to mice was found to be associated with reduced microbial diversity in the gut of the mice as compared to healthy controls. The study animals showed reduced relative abundance of *Verrucomicrobia* and *Bacteroidales* and increased presence of pro-inflammatory *Proteobacteria* (Chassaning et al. 2014).

The typical Western diet, mainly dietary fats, sugars and processed foods (Morrison & Regnault, 2016), promotes gut dysbiosis and weight gain,

conditions that have adverse health outcomes for both mothers and (Dunlop et al., 2015; Morrison & Regnault, 2016). Conversely, certain dietary nutrients, namely low-fat protein (for instance beans, skinless chicken, lean beef), organic proteins and produce (which reduces exposure to dietary antibiotics and pesticides), unsaturated fatty acids (for instance in canola and olive oils, flaxseeds, and salmon), whole grains, and certain strains of probiotics have been found to promote a healthy gut microbiome, enhance intestinal integrity and reduce excessive systemic inflammation (Griffin, 2015; Kashtanova et al., 2016). Fermentation of dietary fibre by gut microbiota produces short chain fatty acids, mainly acetate, butyrate and propionate. These prebiotics are a source of energy for epithelial cells and promote intercellular connections, thus enhancing the integrity of the intestinal wall (David et al., 2014).

Restricted dieting also significantly affects gut microbiomes. Restrictive diet forms include strict vegan diets, gluten-free diets, raw food (clean eating) diets, and low Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols (FODMAP) diets. The debate over who is healthier, vegans or omnivores, goes on unabated. A study of the two groups found striking differences of gut microbes' metabolites in serum but not an equally striking difference in gut microbial communities (Wu et al., 2016). Similarly, in a randomized control experiment, 10 omnivores were fed either a low fat and high fibre or high fat and low fibre diet for 10days. The result showed a modest impression on the composition of the gut microbiome and relatively no observed difference in SCFA production (Wu et al., 2016). Together, these data support a

greater role for diet influencing the bacterial derived metabolome than just the short-term bacterial community. Furthermore, in people with coeliac disease or gluten sensitivity, invitro and animal studies showed that gluten-free bread reduced the microbiome dysbiosis associated with the conditions (Mohan et al., 2016). Most gluten-free dieters however, may not necessarily be gluten intolerant or have coeliac disease.

Gluten-free dieters stand a higher risk of developing heart diseases, perhaps because of the decreased intake of whole grain (Lebwohl et al., 2017). The peculiar gluten-free diet microbiome has been highlighted by an experiment that exposed 21 healthy persons to gluten-free diet for 4 weeks. The result revealed a significantly difference in the microbial communities before and after the experiment, including the reduction in abundance of many beneficial microbes after the experiment (Bonder et al., 2016).

Low FODMAP diet is associated with reduced symptoms of irritable bowel syndrome, perhaps because of its association with the gut microbiome. *Bifidobacterium*, for example, is very sensitive to FODMAP diets. In IBD, the responsiveness of the condition to FODMAP diets can be assessed by assessing the faecal microbial profiles (Bennet et al., 2018). Low FODMAP diets can cause significant changes in the gut microbiome and its resulting metabolome but the extend and significance of these changes remains to be examined (McIntosh et al., 2017).

Gut microbiome change responds within few days after dietary change. After 14days of switching diets, rural Africans and African American showed

significant differences in microbial profiles (O'Keefe et al., 2015). Among the African Americans consuming rural African diets, an observed increase in butyrateproducing bacteria translated into a 2.5-fold increase in serum butyrate production (O'Keefe et al., 2015). David, Maurice, Carmody et al. also observed the same changes a controlled experiment comparing effect of animal-based and plant-based proteins on gut microbiome. The change in microbiome however, was observed as early as day 5 (David et al., 2014). Healthy microbiomes are resilient in the midst of temporal changes arising from dietary modulations, with the potential to adjust back to normal profiles (Korem et al., 2017).

Prebiotics and Dietary Fibre

Dietary fibres are "edible carbohydrate polymers with three or more monomeric units that are resistant to the endogenous digestive enzymes and thus are neither hydrolysed nor absorbed in the small intestine" (Jones, 2014). Some sources of dietary fibre are fermentable, and thus serve as substrate for bacteria of the distal gut (Deehan et al., 2018). Prebiotic foods on the other hand are "food components or ingredients that are not digestible by the human body but specifically or selectively nourish beneficial colonic micro-organisms" (Bindels et al., 2015). Some scientists prefer to refer to them as "microbiota accessible carbohydrates" (Sonnenburg & Sonnenburg, 2014) because they are fermentable dietary fibre that can be used as growth substrate by microbes that can use them (Deehan et al., 2018). "Non-digestible" and fermentable dietary fibre promotes bacteria such as *Bifidobacterium adolescentis, Ruminococcus bromii,* and *Eubacterium rectale* (Martinez et al., 2010; Venkataraman et al., 2016). The type

of dietary fibre to a large extend, dictates the bacterial phyla that are selectively enriched (Martinez et al., 2010). This selectivity may be determined by the chemical structure of the carbohydrate in the fibre and the enzymatic ability of the microbe to access the sugars from the fibre.

Microbiome accessible carbohydrates play an important role in the structure, composition and relative abundances of bacteria because of its "selectivity". They can thus be used as a strategy to increase the populations of beneficial bacteria that are normally minority. These effects of microbiota accessible carbohydrates however, are only present as long as the substrates are available. A withdrawal of the diet containing the accessible carbohydrates will revert the temporal increase in the particular bacterial populations. Some short-term dietary fibre supplementation trials have shown little to no difference in microbial diversity (Zhao et al., 2018), but may still affect body metabolic states via the production of bacterial metabolites such as SCFA (David et al., 2014). Low fibre intake reduces production of SCFA and shifts the gastrointestinal microbiota metabolism to use less favourable nutrients (Gibson et al., 1991), leading to the production of potentially detrimental metabolites (Duncan et al., 2007; Russell et al., 2011). Low fibre that characterizes Western diets has been linked to degrading colonic mucus wall, making way for microbiome populations to encroach, increase susceptibility to pathogens and inflammation. This is thought to explain the link between Western diets and chronic diseases (Desai et al., 2016; Earle et al., 2015). These findings, together with the role of butyrate in preventing oxygen induced gut microbiota dysbiosis (Byndloss et al., 2017),

provide a strong rational to enrich dietary fibre consumption to maintain intact mucosal barrier function in the gut (Ray et al., 2018).

The maternal serum levels of SCFA are negatively correlated with BMI (Gomez-Arango et al., 2016a). Increased maternal serum SCFA levels can positively influence metabolic changes seen in pregnancy: maternal weight gain, glucose metabolism and levels of various metabolic hormones (Koren et al., 2012; Priyadarshini et al., 2014). Gomez-Arango et al. recently evaluated the relationship between maternal gut microbiota, weight, blood pressure and plasminogen activator inhibitor-1 (PAI-1) levels in overweight and obese pregnant women and found that butyrate producing gut microbes were negatively correlated with diastolic and systolic blood pressure and PAI-1concentrations (Gomez-Arango et al., 2016b). These findings support the possible therapeutic role of increasing butyrate producing microbes in reducing blood pressure in obese and overweight pregnant women. Omega-3 long-chain PUFA has also been shown to enhance connection of gut epithelial cells, improving intestinal wall integrity (Li et al., 2008).

Probiotic foods

Probiotics are live bacteria that can be included in drugs, foods or dietary supplements for their health benefits in the body. Common probiotic bacteria include *Lactobacillus* and *Bifidobacteria* species. Despite their loudly trumpeted benefits in the host, probiotics may fail to establish themselves in the GIT and effect of their presence on resident bacterial communities may be nearly nonexistent (Kristensen et al., 2016; Walter et al., 2018). However, probiotic

foods can affect the host through the modulation of host immunity and metabolic states through the production of bacteria metabolomes. An examination of over 300 probiotics trials showed the beneficial role of probiotic supplements in preventing necrotizing enterocolitis, diarrhoea, pulmonary exacerbations in children with cystic fibrosis, acute upper respiratory tract infections, and eczema in children. In recent times, probiotic treatments are being modified using newer bacterial specimen, combining various probiotic bacteria or combining probiotics and prebiotics (a combination known as synbiotics) in therapy (Plovier et al., 2017). Other probiotic therapy also includes personalized bacterial engraftment based on patient's microbial profile in conditions such as inflammation, obesity, cancer, and lipid metabolism (Chua et al., 2017). Stable engraftment of a probiotic *Bifidobacterium longum*, for example, has been shown to depend on individualized features of the gut microbiota, providing a rationale for the personalization of probiotic applications (MaldonadoGómez et al., 2016).

In the light of the vast variations of microbiomes on individual levels, the personalized optimum diet of each person must be tailor-made for the gut microbiome of each person. Zeevi et al. assessed the microbiomes of some 900 people and monitored their diets, blood glucose, and physical activity for 7days. Based on the data, they developed a machine learning algorithm which was able to correctly predict blood glucose levels based on personalized microbiota and clinical data. Subsequently, in a double-blinded randomized trial, using the developed algorithm to propose dietary interventions predictably affected the blood glucose levels (Zeevi et al., 2015). A study on response to bread (Korem et

al., 2017) using a randomized crossover trial of one week long dietary interventions showed significant interpersonal variability in the glycaemic response to different bread types. The type of bread that induced the lower glycaemic response in each person could be predicted based solely on microbiome data collected before the intervention (Korem et al., 2017).

Antimicrobials and Microbiome

The role of infections, stress and antimicrobial use in causing microbial dysbiosis, with a potential to affect maternal health and neonatal development, has been well documented (Bilder et al., 2013; Vela et al., 2015). Medication plays a key role in modulating gut microbiome profiles. Broad spectrum antimicrobials especially, with properties to inhibit or eliminate microorganisms of particular spectrum, affects both pathogenic and non-pathogenic beneficial bacteria in the gut. In a Dutch-Belgian population-wide study, medication including progesterone, osmotic laxatives, TNF-α inhibitors and rupatadine, was the major determinant of microbiota structure (10% of microbial community variations) (Falony et al., 2016). Further, proton pump inhibitors have been shown to have significant impact on the microbial communities in the gut, a phenomenon that is thought to explain the higher incidence of gastrointestinal infections in persons on these medications (Jackson et al., 2016). The role of antibiotics in destabilizing gut microbiome has been employed in achieving microbial dysbiosis-associated weight gain in farm animals and poultry. Routine administration of low dose antibiotics in animals ensures health, increase growth and weight within relatively short periods (Blaser, 2016).

Sulfadoxine-pyrimethamine and gut microbiome

Sulfadoxine-pyrimethamine is a combination therapy mostly used as a prophylactic against asexual forms of *Plasmodium falciparum*. It is recommended by the WHO in medium to high malaria endemic regions especially in sub-Saharan Africa (Ramharter et al., 2007; WHO, 2004). Whilst both individual components are folic acid antagonist, Sulfadoxine, a sulfonamide, inhibit dihydropteroate synthase enzyme, and pyrimethamine inhibits folic acid synthesis by suppressing the activity of dihydrofolate reductase responsible for reducing dihydrofolate to tetrahydrofolate, an important substrate in the survival of malaria in blood cells. SP is traditionally an antimalarial drug, that is administered from second trimester, but also has broad spectrum antibacterial activity (Mehaffey et al., 1995). Recent records show a decreased efficacy and an increased resistance to SP (Briand et al., 2007). However, the benefits of SP in reducing low birthweight are now being highlighted (Desai et al., 2016). This observation has been supported by studies showing that antimalarials such as mefloquine when co-administered with cotrimoxazole in HIV pregnant women was effective at only reducing malarial infections but had no effect on birthweights. It has therefore been hypothesized that the well documented broad spectrum antibacterial activity of SP, independent of its antimalarial properties, is responsible for the observed increased birthweights associated with SP uptake (Dingens et al., 2016; González et al., 2014).

Sulfadoxine-pyrimethamine-induced modulation of gut microbiota has also been suggested as possible mechanisms mediating the SP-associated

maternal and neonatal birthweight observed in pregnant women on SP (Dingens et al., 2016). Thought the mechanism of action is unclear, this hypothesis draws from the wellestablished use of antibiotics in fattening farm animals (Trasande et al., 2013). In animal model studies, antibiotic induced-gut microbial alterations led to changes in cholesterol and lipid metabolism, thus increasing adiposity (Cho et al., 2012).

Similarly, antibiotic use in neonates has been shown to increase BMI (Trasande et al., 2013). Alternatively, Capan et al. hypothesized that by treating unintended bacterial infections, SP could prevent preterm birth and associated low birth weight (Capan et al., 2010). The SP is also thought to influence maternal metabolism and reduce via its effect on the gut microflora and reduce genitourinary tract infections.

The antibiotic activity of SP has been widely examined. In-vitro drug susceptibility study showed a broad spectrum of anti-bacterial activity of SP against Gram-positive and low activity against Gram-negative bacteria (Capan et al., 2010). Unlike Mefloquine with high antibacterial activity against only pneumococci, and like azithromycin with high and broad activity against bacteria including gonococci, SP showed broad bacteriostatic activity across various bacterial phyla. The result of this bactericidal/static activity of SP in the gut is a reorganization of the structure of microbial communities in the gut. This may lead to production of harmful metabolic products, disruption of the intestinal mucus wall integrity, and consequently, leaky gut, and in some cases infection by pathogenic bacterial. Bacterial infections such as *S. agalactiae* and

pneumococcal infections, and bacterial dysbiosis contribute significantly to adverse pregnancy and birth outcomes (Mullick et al., 2005). Conversely, there is evidence of improved maternal and neonatal health with the administration of optimum doses of antimicrobials during pregnancy (Gray et al., 2001). Thus, antimalarials such as SP and azithromycin confer either beneficial or harmful effect on the pregnant mother's gut based on the structure of the gut microflora and the specific antibacterial properties of the given antimalarial.

Deworming and gut microbiome

Deworming during pregnancy has been strongly recommended as a prophylactic against helminths infections during pregnancy (WHO, 2005). Helminth infections in pregnancy have been associated with low birth weight, perinatal mortality and maternal anaemia (WHO, 1994; Christian et al., 2004; Friedman et al., 2007). Despite their reported benefits, deworming is only initiated from the second trimester of gestation because of adverse effects of deworming drugs in first trimesters. In Ghana and most countries, the drug of choice for deworming is albendazole or its analogue, mebendazole.

Albendazole is an antihelminthic drug used in treating helminthic infections and other associated complications. Its active metabolite, albendazole sulfoxide binds the B-tubulin subunit of the helminth's microtubules and thus inhibits microtubule polymerization (Venkatesan, 1998). Albendazole also causes impaired glucose utilization and causes a decrease in the parasite's glycogen stores and at high concentrations, inhibits the Kreb cycle and thus parasite immobilization and death (Venkatesan, 1998). It is indicated in Trichuriasis,

Filiariasis, Ascariasis, metronidazole-resistant Giardiasis, Trichinosis, hookworms, Strongyloidiasis. In communities with infection prevalence above 20%, WHO recommends deworming using albendazole and others from the second trimester of gestation. A recent evaluation of datasets of over 800,000 births showed that deworming was associated with 14% reduced risk of infant mortality within first 4 weeks of birth.

It further suggested that deworming was associated with reduced low birth weights. Albendazole and mebendazole has been reported to be associated with reduced maternal anaemia in Sierra Leone (Torlesse & Hodges, 2001); and with reduced birth weight in Nepal (Christian et al., 2004). These reports were not consistent in a larger study in Peru which observed no association between mebendazole and maternal anaemia or neonatal survival (Larocque et al., 2006).

Albendazole has not been indicated in a bacterial infection. However, despite its many indications for albendazole, its applications are still being investigated and may be more than is currently known, including it implications for gut microbiomes. For example, because of its limited toxicity to normal cells, it is being investigated for potential anticancer properties (Movahedi et al., 2017).

Other factors affecting gut microbial composition in pregnancy

Hormonal changes

Gestation is marked by prominent hormonal shifts with consequent immune and inflammatory effects which affect the gut and the gut microbiome (Brantsæter et al., 2011; Koren et al., 2012). Pregnancy hormones such as

progesterone and estrogen affect bacterial growth and metabolisms, and virulence of pathogenic bacteria, thus remodeling the structure of the gut microflora (Mulak et al., 2014). *Listeria monocytogenes* infection during pregnancy, for example, is partly due to increased levels of progesterone and estrogen in pregnancy, with the potential to cause adverse birth outcomes such as preterm birth and stillbirth (Garcia-Gómez et al., 2013). The peculiar hormonal changes associated with pregnancy also affect gut contractility and transit (Mayer et al., 2014). The increase in transit time may be an adaptive measure in pregnancy to allow for absorption of more nutrients in the gut. This may also explain the weight gain during pregnancy.

Metabolic changes

Metabolic changes are associated with pregnancy. These metabolic changes though normal in pregnancy, are the abnormal findings in metabolic syndrome outside of pregnancy (Chassaing et al., 2014). With no differences in diet, including total energy intake, pregnant women have been found to gain greater adiposity with significantly higher leptin, insulin and insulin resistance measures, cholesterol, and glycated hemoglobin with each trimester of pregnancy compared to their nonpregnant counterparts (Collado et al., 2008). At the start of pregnancy, microbial structure and composition is similar to non-pregnant women (Santacruz et al., 2010). However, with the advent of each trimester, a distinct microflora associated with inflammatory states develop in about 70% of pregnant women (Santacruz et al., 2010). The most significant change in microbiota occurs in the *Firmicutes* and *Bacteroidetes,* with *Firmicutes* showing

higher levels akin to levels in obesity (Santacruz et al., 2010). The metabolic state is also characterized by increase in proinflammatory cytokines (IL-2, IFN-y, TNF-a and IL-6), with adipose tissues, mucosal tissue of GIT, and placental tissue showing low grade inflammation; weight gain; insulin resistance; raised fasting blood glucose; and glucose intolerance (Cani et al., 2012; Nuriel-Ohayon et al., 2016). Whilst these changes may be abnormal in non-pregnant persons, they appear normal and necessary conditions required for the health of the mother and developing foetus (Cani et al., 2012; Chassaing et al., 2014).

Chapter Summary

The gut microbiome peculiar to pregnancy can be thought of as an organ of its own in its distinctiveness, and peculiar functions in healthy pregnancy and pregnancy complications. The pregnancy gut microbiome develops gradually to a more distinct structure in the second and third trimesters of gestation, mainly made up of *Proteobacteria*, *Actinobacteria* and *Bifidobacteria*. The intricate symbiotic relationship between the gut and the resident microflora is relatively stable, with the ability to be restored when it is disturbed. Metabolic, hormonal, immune, diet and physiological changes in pregnancy affects the composition, structure, and diversity of the gut microbiome. Antimalaria drugs with broad spectrum antibiotic activity, like most antibiotics, also modulate the development and selection of gut bacteria. However, this is not a one-way traffic as the gut microbiome also affects these processes directly and/or indirectly through the modulation by bacterial metabolites, maintenance of gut integrity, competitive inhibition of pathogenic bacteria, and forming part of mucosal immunity. The

activities of gut microbiota during pregnancy have been associated with gestational obesity, gestational diabetes mellitus, gastrointestinal disorders, preeclampsia, autoimmune diseases, offspring immune development and neurodevelopment. Consequently, the modulation of gut bacteria through diet, and/or bacterial engraftment and other processes hold great promise of positively affecting the common complications of pregnancy and the developing foetus and newborn.

CHAPTER THREE

RESEARCH METHODS

Introduction

Chapter three explores the materials and scientific methodologies employed in this study. The basis of sound scientific research relies on time-tested and empirically proven methodologies, and procedures. These standard operating protocols may however be modified to reflect the aim of the study and the particular characteristics of the study and study population. This study leverages on these scientific principles in designing the study, selecting the study population, sites, sample size, sampling techniques, data collection, laboratory protocols, and data management and analysis.

Research Design

An exploratory study design was employed to study the gut microbial structures of third trimester pregnant women and their relations with maternal health indices in Cape Coast from November 2019 to August, 2021.

Study Area

The study was conducted in Cape Coast, in the Central Region. Specifically, two healthcare facilities providing Antenatal Care (ANC) services were selected for the study. These were selected for their proximity and the diverse populations they attend to. The University of Cape Coast Hospital serves primarily university staff, students and adjoining communities. The ANC clinic which opens from 8am to 4pm each day from Monday to Friday serves an average 35 clients per day. The clients here are regarded among the elite group

and are most likely to be educated and middle-income earners. The contrast is Ewim Polyclinic, sited metres away from the Kotokuraba market in Cape Coast. It is surrounded by slums, and largely petty traders who ply their trade in the market. Unlike the University Hospital, it is a polyclinic that provide only basic clinical services. Daily average ANC attendance stands at 25. These facilities provide prenatal, antenatal and postnatal care to the women in and around the catchment areas.

Population

The study population consisted of pregnant women in the third trimester specifically from 29 to 38 weeks of gestation.

Sample Size

The study involved 22 pregnant women at their third trimester and receiving antenatal care at UCC Hospital and Ewim Polyclinic. After simulating a set of matrices of pairwise distances for which within-group and between-group distances matched the distribution of distances observed in the HMP stool sample set analyzed by unweighted Jaccard distance, we applied the bootPower command three times to assess PERMANOVA power with a sample size of five, ten or twenty per group, as described by Kelly et al. (2015). This analysis allowed for 90% power to detect an effect size (ω^2) of 0.05, 0.02; and 0.008 for a sample size of 5, ten or twenty per group respectively. Thus, for comparison across two Deworming and three BMI and SP groups, a minimum sample size of 5 per group will detect a moderate effect size of 0.05 at a power of 90% for comparison across BMI and SP groups and an effect size of 0.02 at a power of

90% for comparison across Deworming groups. According to Wu et al., a sample size of ten per group will allow for the detection of an ω^2 of 0.056 and 0.013 for Weighted and Unweighted Unifrac distances respectively. Based on these assumptions, a sample size of 22 will likely affords adequate statistical power for the primary outcome measure.

Inclusion and exclusion criteria

The study included expectant mothers of no apparent health conditions aged 19–40 years, and within the third trimester of pregnancy (27–42) weeks of gestation). Also, women free of chronic diseases (diabetes, hypertension, kidney disease, or cancer), autoimmune disorders, or infections with the human immunodeficiency virus, or hepatitis in preconception and receiving antenatal care in any of the two participating health facilities and expected to give birth in same fit into the inclusion criteria. They were also not to be planning to permanently leave the study area during the period. However, women with multiple pregnancy (pregnant with two or more fetuses), high risk pregnancy or pre-eclampsia, history of chronic diseases or infections that could compromise immune status, or evidence of antibiotic use during pregnancy were excluded from the study.

Sampling Procedure

Consecutive sampling was used in recruiting participants from the two main study sites into the study. This sampling technique allowed for working with multiple samples at convenient times. The first phase of sampling was

conducted at the University Hospital before subsequently sampling at the Ewim Polyclinic.

Participants of the study were recruited from the ANC clinics of selected health facilities. The researcher approached and explained the study in English and Twi at the various Antenatal Care Clinics of the two health facilities on each day of ANC visit. Mothers who voluntarily consented to enroll on the project were further evaluated against the inclusion and exclusion criteria for eligibility and enrolment. The eligible, informed and consenting pregnant women endorsed consent forms. Arrangements were then made for data collection.

Data Collection Procedure

Study personnel administered a data collection instrument to each participant. The instrument was designed to collect socio-demographic data and clinical data relevant to the study. Questionnaires were administered in a language of choice of the participants. Gestational data such as gestational weight and height, IPTp-SP uptake, deworming, contraception use, parity etc data were extracted from the ANC records of each participant. These data were self-reported whilst some were collected by their attending ANC nurses. Participants were then given a sealed prelabelled stool sample plastic container, with a spatula. They were rightly instructed on how to collect an uncontaminated 1gm of freshly passed stool in the morning of their next antenatal visit. The study personnel made follow up calls to participants to remind them of how to collect the samples on the eve of their next ANC visit. The study personnel met and collected the samples within 1 h of sample collection. Collected samples were

sent to the lab within 4 h of collection, aliquoted into clean sterile labelled Eppendorf tubes (1.5 mL) containing normal saline and stored at -80°C until genomic sequencing.

Laboratory protocol

DNA extraction

DNA extraction was performed at the School of Medical Science General Laboratory. The preparation of the sample for DNA extraction involved the mechanical homogenization of 0.2 g of stool samples with a Mini BeadBeater 8 (BioSpec, USA) for 4 min at 5,000 rpm as described by Smith et al. (2011). Genomic DNA was then extracted from each sample using QIAamp DNA stool Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The Simplinano spectrophotometer (Biochrom, Harvard Bioscience Inc., United States) was used to measure the quality and concentration of the extracted bacterial DNA.

16S rRNA sequencing

16S rRNA sequencing was conducted at the Massachusetts Institute Technology (MIT), United States. The V4 region of the 16S rRNA was amplified using multiplex polymerase chain reactions [95◦C for 5 min, followed by 25 cycles of 95◦C for 30 s, 55◦C for 30 s, 72◦C for 40 seconds, and 72◦C for 6 min] using the extracted DNA as template and universal primer (16S Amplicon PCR Forward Primer: 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 16S Amplicon PCR Reverse Primer: 805R (5′-GGACTACHVGGGTWTCTAAT-3′) with barcode sequence. The ends of each primer were integrated with index

adaptors. The resulting PCR products were then mixed with equal amounts of 2× loading buffer and loaded onto a 2.0% agarose gel electrophoresis to detect DNA. Samples with approximately 450 base pairs bands were selected and mixed in isodensity ratios. PCR products mixtures were purified using, GeneJET Gel extraction Kit (Thermo Fisher Scientific, Massachusetts, United States).

Library preparation

The sequence library was prepared following the 16S rRNA metagenomic sequencing guidelines. Sample amplification and subsequent amplicon product quantification were conducted using KAPA HiFi HotStart ReadyMix 2X and Nextera XT Index barcodes. The Bioanalyzer DNA 1000 chip (Agilent Technologies, California, United States) was used to assess the quality of the amplicons. Libraries of pooled samples were then sequenced using Illumina MiSeq sequencer per manufacturers guidelines in the 2 × 300 base pair-end runs (MiSeq Reagent kit v3). The quantity and validity of sequenced libraries were then assessed using Qubit 2.0 Fluorometer (Thermo Fisher, Massachusetts, United States) and Agilent 2100 Bioanalyzer (Agilent Technologies, California, United States), respectively. Finally, samples were sequenced on an Illumina NextSeq platform (Illumina, Inc., California, United States) at the MIT integrated genomics core facility, BioMicro Center.

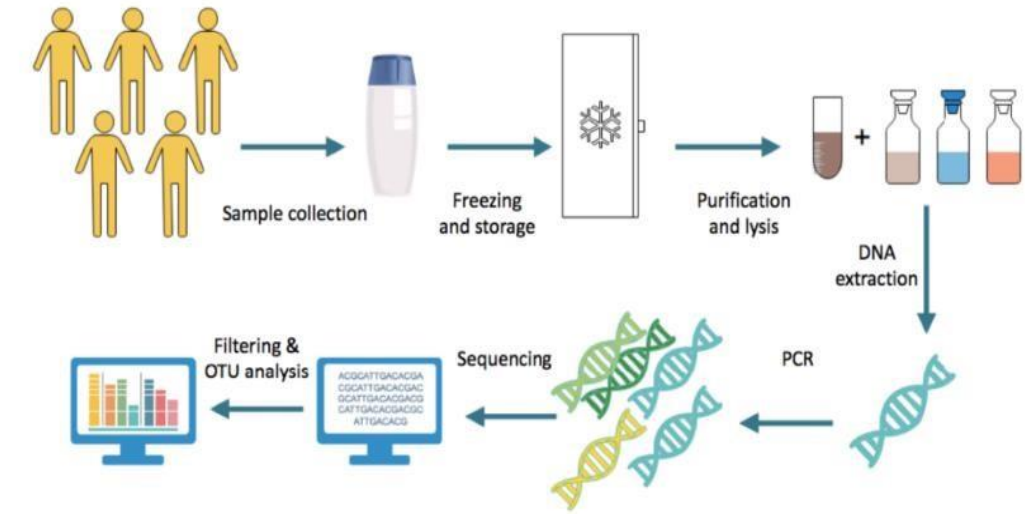

Figure 4.: Summary of the project workflow

Bioinformatic analysis and quality control

The data was imported into Quantitative Insights into Microbial Ecology (QIIME2). Trimming and denoising were done by truncating the forward read lengths at 249bp and reverse read lengths at 225 to preserve all reads with Q >20. The representative sequences were visualized and an all-to-all alignment created to start building a phylogenetic tree. The resulting alignments were filtered with MAFFT to remove highly variable positions, which are known to generate too much noise in trees. The phylogenetic tree was generated from the filtered alignment using FastTree. QIIME2 was used to choose a root for the tree since the tree produced by FastTree was unrooted.

Greengenes 99% Weighted taxonomy classifier was used in the classification of the bacterial species. The weighted classifier gave a superior classification precision for samples coming from a variety of habitats (Kaehler et al., 2019). Taxonomic classification was based on the Greengenes 13.8 reference

database, with a similarity threshold of 97%. These 16S rRNA gene classifiers were trained with weights that take into account the fact that not all species are equally likely to be observed. The extract from the reference database only read sequences that corresponded with the sequencing primers (16s rDNA V4, 515F-805R). The naïve-bayes taxonomic classifier was then trained on the extracted reads. The classifier was then tested and the resulting taxonomic assignments visualized. The ASV table was then exported to R for further data analysis.

Data Processing and Analysis

Statistical analyses were performed using the open-source statistical software R (https://www.r-project.org). The differences between clinical characteristics were calculated using two-tailed T-test, Kruskal-Wallis or Wilcoxon Rank test, where appropriate. To perform alpha and beta diversities and taxa relative abundance analysis, sequence data was first rarefied to allow for even comparison between groups. Alpha diversity metrics namely number of observed ASV, Chao1, Simpson and Shannon diversity measures were employed to investigate the specie richness and evenness. Specifically, Shannon assessed specie richness and evenness whilst Simpson assessed specie richness. Bray-Curtis, Unifrac and Weighted Unifrac, beta diversity metrics, investigated the temporal and spatial changes in species composition and the similarities between bacterial population in relation to ecological metrics between samples. Specifically, whilst weighted UniFrac presented both the different microbial communities present or absent and their respective abundances; Unweighted UniFrac demonstrated the presence or absence of different microbial

communities, Bray Curtis distance quantified microbiota compositional dissimilarity between groups according to counts of every sample across the groups. The association between beta diversities and covariates was assessed using PERMANOVA and ultimately, Omnibus. Omnibus used the minimum *P* values of each beta diversity (obtained by PERMANOVA) as a test statistic, combined with the association evidence from beta diversity measures to determine an overall associated *P* value. A multi-dimensional scaling (MDS) in R was used to generate ordination plots. Bacterial abundance data was first normalized at each taxa level using permutation test to allow for relative abundance and differential taxa abundance analysis at ASV, phylum, class, order, family and genus levels. Taxa with relative abundances of less than 5% were agglomerated into one to reduce number of test taxa. To correct for various testing at every taxonomical level, False discovery rate (FDR) control was used and FDR-adjusted p-values < 0.05 were considered significant.

Ethical Considerations

Ethical clearance for the study was obtained from the University of Cape Coast Institutional Review Board (UCCIRB/EXT/2019/26). Written informed consent was sought from participants before been enrolled into the study. This project was carried out by following the Helsinki declaration for research involving human subjects. Covid-19 preventive measures such as the use of nose masks, constant disinfection of hands before and after interactions with every study participant, and the maintenance of social distancing etc were employed during sample collection and all research related activities. A thorough

explanation of the study protocol, the "costs" and benefits of the study was made to all prospective participants in a language the prospective participant understood. The participants were made to understand that the participation is voluntary and that at any time in the course of the research, she could choose to opt out of the project without any negative consequences to her. It was also ensured that all participants are anonymized and codes instead of names were used to identify various participants

Chapter Summary

This chapter highlighted the various materials and methods used assessing the study objectives. The cross-sectional study was conducted in two health facilities in Cape Coast – Ewim Polyclinic and UCC Hospital, among pregnant women in their third trimester of gestation. The 22 study participants provided 1gm of freshly passed stool that was collected and preserved by the research team until analysis. Food frequency questionnaire were also administered to the respondents. Other sociodemographic and clinical data was extracted from the ANC folders of the participants. DNA was extracted from the participant-provided stool samples, and 16s RNA sequencing done on the extracted DNA. The observed bacterial species were matched against the Greengenes 13.8 reference database to draw taxonomic classes. The bacterial diversity, phylogenetic families and taxonomic classes formed the part of the statistical analysis. The R (version 4.05) software for statistical analysis. Association analysis was done between BMI, IPTp-SP uptake, deworming and gut microbiota.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

In pregnant women, the gut microbiome is likened to a complex and yet stable "organ" that modulates various immune, homeostatic and endocrinal processes with far-reaching consequences for expectant mother and the unborn baby. In Ghana and several other malaria endemic countries, IPTp-SP has been implemented to varying degrees but there are no studies investigating its impact on maternal microbiome. This study attempted to describe the gut microbiome in pregnancy and investigate the effect of IPTp-SP on maternal gut microbiome and other reproductive health indices and this chapter presents the findings and discussion. The first part of this chapter present finding of this study in sequential response to the study objectives. The second part discusses the findings.

Results

Sociodemographic characteristics of participants

The demographic characteristics are summarized in Table 4.1. The mean age of participants was 28.86 with the oldest and youngest being 37 and 20 years old respectively. Primigravida were about half of the participants, whilst parity of the participants was almost evenly distributed for nulliparous, primiparous and multiparous groups. A relatively higher number of our participants were either overweight or obese whilst only one was underweight. Participants on three or more doses of SP were twice as those on only one or no dose of SP. Conversely, only 4 of our study participants were not dewormed.

Table 1.: Participants' characteristics

Variable	Frequency N (%)	Mean (Range)
Age		28.86 (20 - 37)
Marital Status		
1. Single	8 (36.4)	
2. Married	14 (63.6)	
Educational Attainment		
1. Primary	3 (13.6)	
2. JHS/JSS/Middle	10 (45.5)	
3. SHS/SSS/Secondary	6 (27.3)	
4. Tertiary	3 (13.6)	
Number of Pregnancy		
1. Primigravida	7 (31.8)	
Number of births 1.		
Nulliparous	7 (31.8)	
2. Primiparous	6 (27.3)	
3. Multiparous	9 (40.9)	
BMI		
1. Underweight	1 (4.5)	
2. Normal weight	9 (41.0)	
3. Overweight	7 (31.8)	
4. Obese	5 (22.7)	
IPTp-SP Status		
1. None	6 (27.3)	
2. Less than three doses	1 (4.5)	
Deworming		
1. Dewormed	18 (81.8)	
2. Not dewormed	4 (18.2)	

Source: Field data (2021)

Characterization of gut microbiome

To answer the first objective, we characterized 5,283 operational taxonomical units (OTUs) obtained from the 22 samples and sequenced at a sequence-similarity index of 97% to 100% (figures 5, 6 and 7). The observed OTUs belonged to 11 phyla, 17 families, and 26 genera. *Actinobacteria* were the most predominant phyla, followed by *Bacteroidetes, Firmicutes*, and *Proteobacteria* respectively as shown in figure 5. The most abundant bacteria were of the families *Bacteroidaceae* followed by *Lachnospiraceae Lactobacillaceae,* and *Prevotellaceae* respectively as shown in figure 6. At the genus level, *Bacteroides* was the most abundant genus as shown in figure 7.

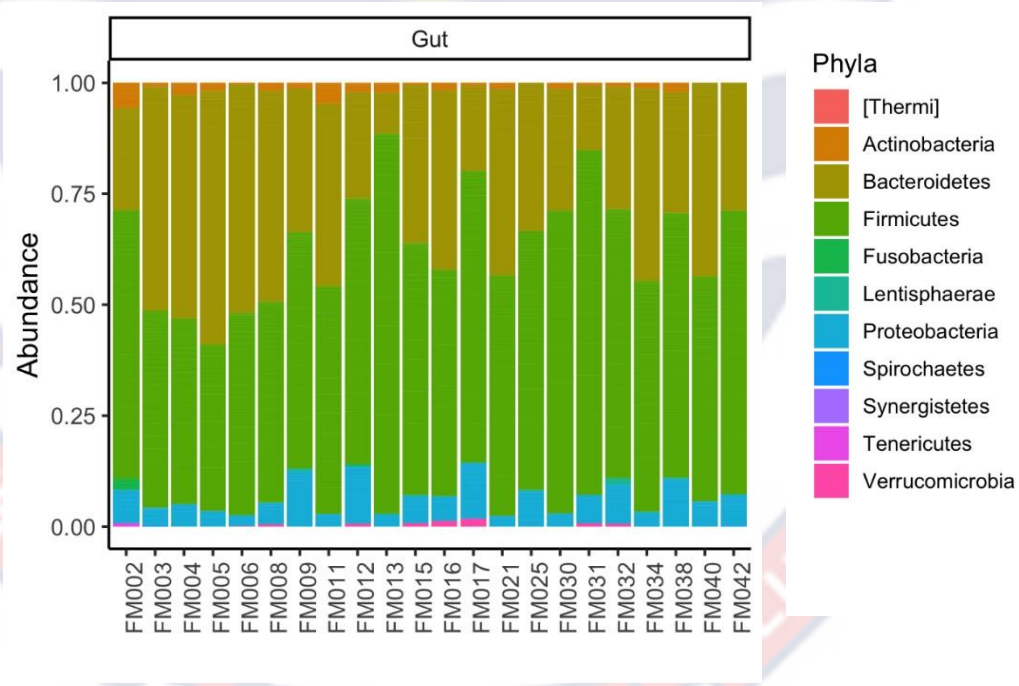

Figure 5: Bar plot showing taxonomic abundance of the bacterial phyla of the gut microbiome of study participants (Source: field data, 2021)

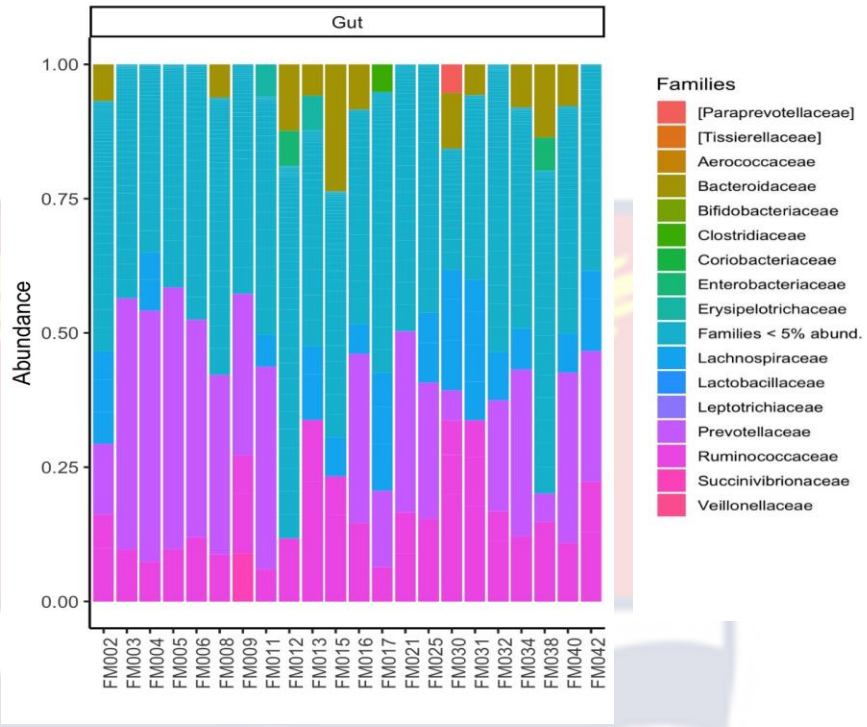

Figure 6: Bar plot showing taxonomic abundance of bacterial families of the gut microbiome of study participants (Source: field data, 2021)

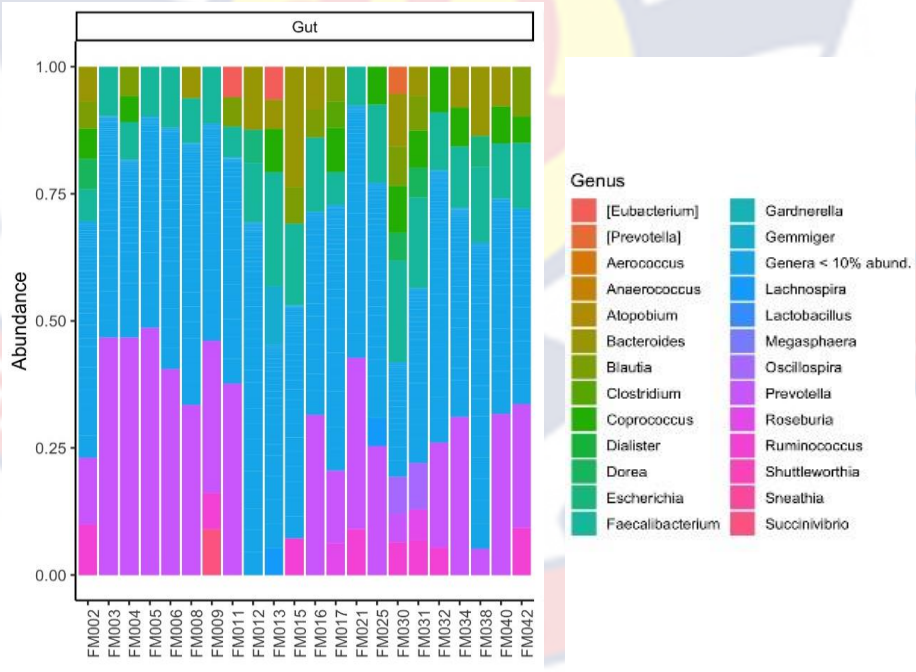

Figure 7: Bar plot showing taxonomic abundance of the bacterial genera of the gut microbiome of study participants (Source: Field data, 2021)

Phylogenetic analysis of bacterial groups

To characterize the relatedness of the observed gut microbiota, phylogenetic analysis of bacterial groups was extracted from the metagenomic data. The results showed increasing relatedness of bacteria down the classification system. Five major phyla captured the top 25 most dominant genera observed in our study. Of these, *Firmicutes* was the most diverse phyla with three bacterial classes whilst phylum *Fusobacteria* had the narrowest lineage as shown in Table 2

Table 2: Phylogenetic lineage of major bacteria genera

Phylum	Class	Order	Family	Genus
Actinobacteria	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Atopobium*
	Actinobacteria	*Bifidobacteriales*	*Bifidobacteriaceae*	*Gardnerella*
Bacteroidetes	*Bacteroidia*		*Provetellaceae*	*Prevotella*
		Bacteroidales	*Bacteroidaceae*	*Bacteroides*
			Eubacteriaceae	*Eubacterium*
			Oscillospiraceae	*Oscillaspira*
				anaerococcus
			Clostridiaceae	*Clostridium*
				Blautia
				Coprococcus
	Clostridia	*Clostridiales*		*Dorea*
Firmicutes			*Lachnospiraceae*	*Lachnospira*
				Roseburia
				Shuttleworthia
				Faecalibacterium
			Ruminococcaceae	*Ruminococcus*
				Dialister
	Negativicutes	*Selenomonadales*	*Veillonellaceae*	*Megasphaera*
	Bacilli	*Lactobacillales*	*Aerococcaceae*	*Aerococcus*
			Lactobacillaceae	*Lactobacillus*
	Gammaproteobacteria	*Enterobacterales*	*Enterobacteriaceae*	*Escherichia*
Proteobacteria		*Aeromonadales*	*Succinivibrionaceae*	*Succinivibrio*
	Alphaproteobacteria	*Hyphomicrobiales*	*Hyphomicrobiaceae*	*Gemmiger*
Fusobacteria	*Polytrichopsida*	*Polytrichales*	*Leptotrichaceae*	*Sneathia*

Source: Laboratory data, 2021

Relationship between gestational BMI and gut microbiome

The results of the relationship between gestational BMI and gut microbiome are presented as alpha diversity plots (described by Shannon diversity index, Simpson diversity index and Chao1 diversity index) and beta diversity plots (described by Bray-Curtis distances, Unifrac and Weighted Unifrac distances).

ANOVA (ASV, Chao1, and Shannon metrics) and Kruskal-Wallis test (Simpson metric) were conducted to determine the differences across group diversities. The Shannon diversity index showed that there was greater microbial richness within Normal BMI participants ($H > 5.60$) than within any of the other groups. This difference however was not statistically significant ($P = 0.76$, ANOVA) when compared with the other groups. In all groups however, there was high microbial richness and evenness ($H > 5.50$, $D < 1.00$) as shown in figure 8.

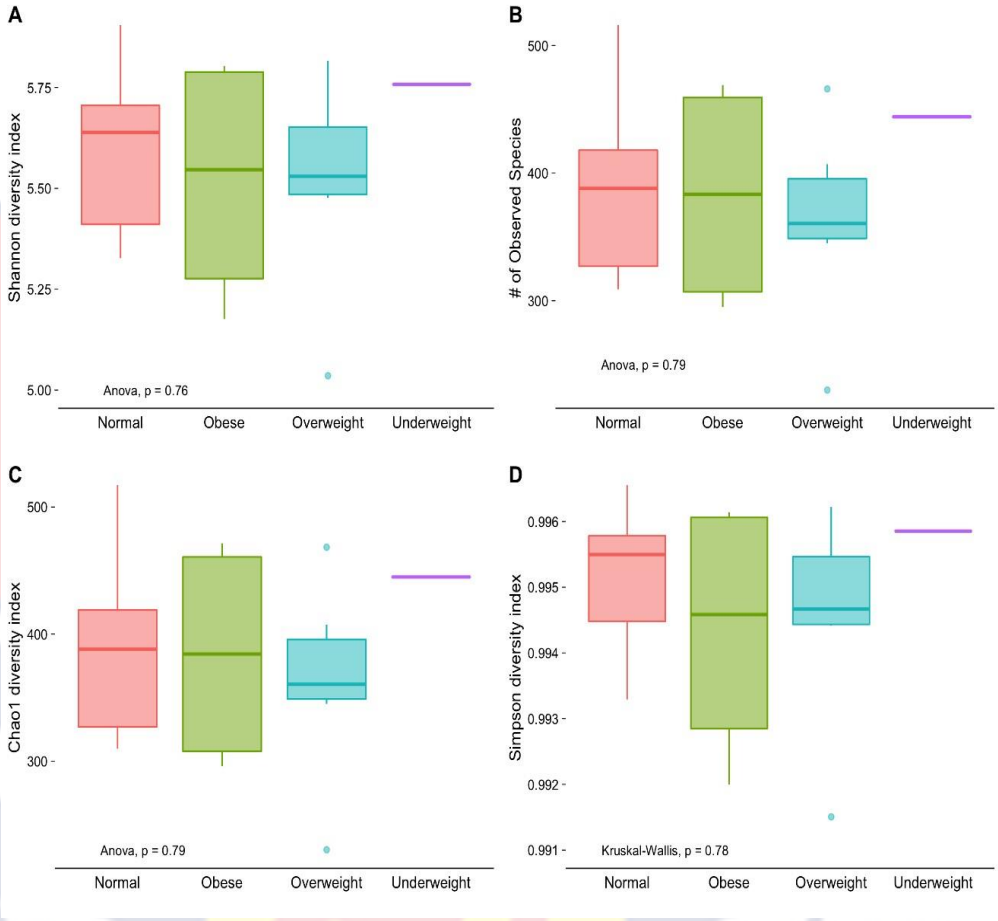

Figure 8: Box plots illustrating Alpha diversity indices ((A) Shannon diversity Index (B) Number of Observed ASVs (C) Chao1 diversity Index and (D) Simpson diversity Index) gut microbiome across categories of BMI

Beta diversity plots (Bray-Curtis dissimilarity index, Unifrac and Weighted Unifrac distances), which describes the relatedness of bacteria from the different study groups, showed no significant spatial difference was observed among the gut microbiota across BMI study groups (Fig. 4.5). The variance of the gut microbiota abundance is described by the Axis 1 and Axis 2 scores as shown in figure 9.

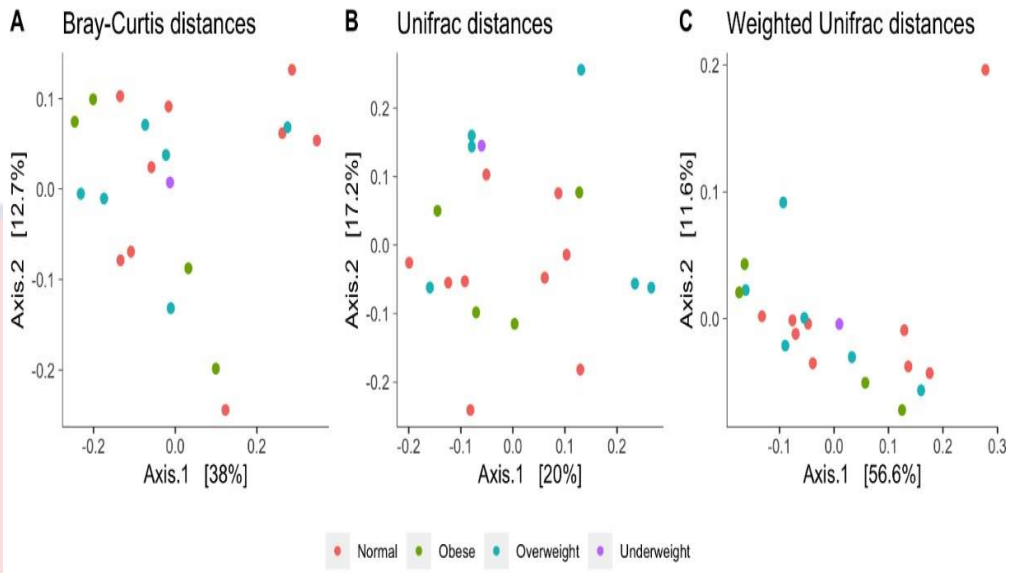

Figure 9: Beta diversity scatter plots of BMI and gut microbiome ((A) Bray-Curtis distance (B) Unifrac distances (C) Weighted Unifrac distances)

Relationship between IPTp-SP and gut microbiome

The association between IPTp-SP uptake and gut microbiome among the study participants was assessed by analyzing Alpha and Beta diversities of within various categories of IPTp-SP uptake and across the same categories respectively. IPTp-SP uptake was categorized under three groups; participants who received less than 3 doses; those who never received SP in their current pregnancy; and those who had at least 3 doses of SP as at the time of sampling.

ANOVA (ASV, Chao1, and Shannon metrics) and Kruskal-Wallis test (Simpson metric) were conducted to determine the differences across group diversities. The Simpson index showed the highest within group variation in the group of participants who had at least 3 doses of SP ($D < 0.995$). The differences across these groups were however not statistically

significant in any of the indices computed except in the Simpson diversity index which recorded a significant alpha diversity across the groups (Kruskal-Wallis test: $P = 0.03$) as shown in figure 10.

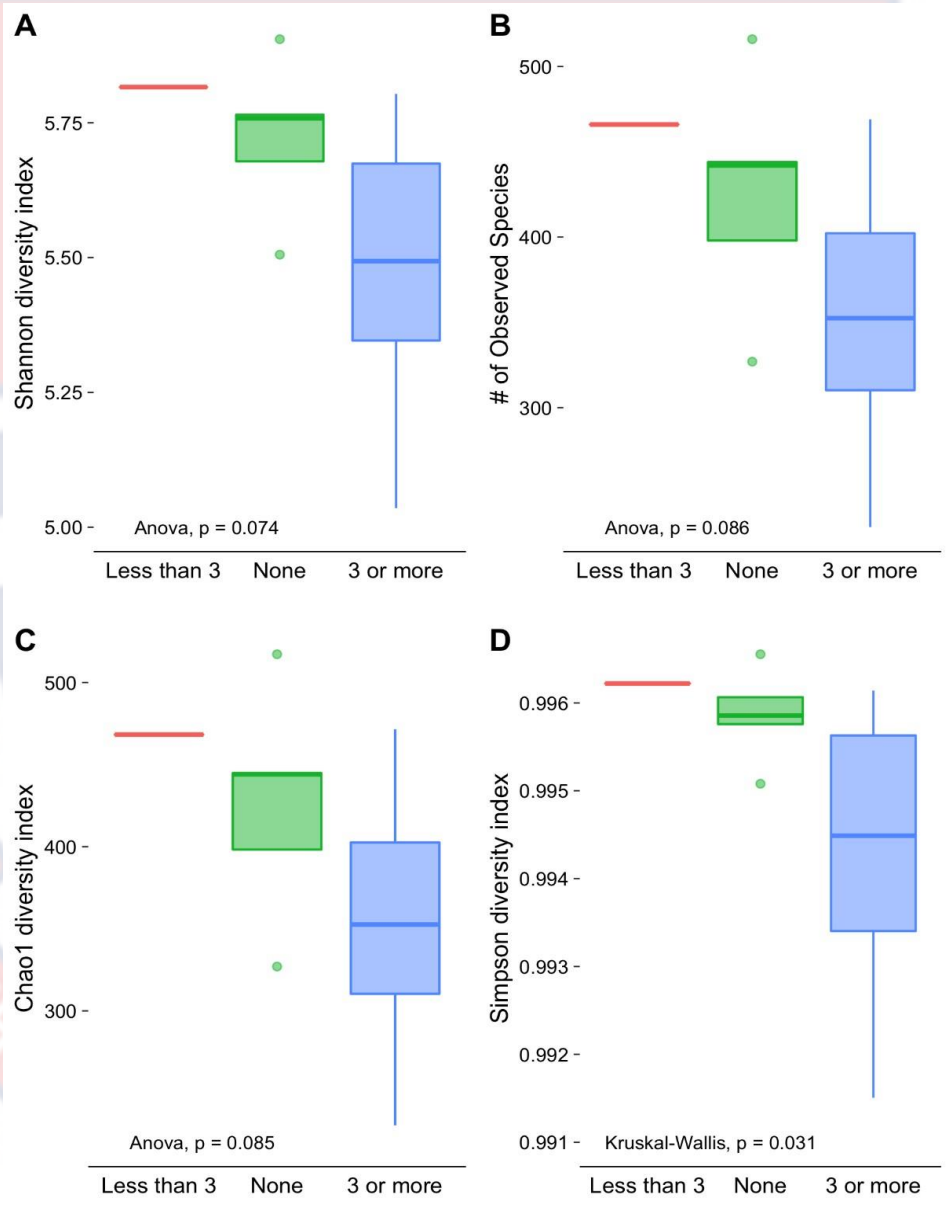

Figure 10: Box plots illustrating Alpha diversity indices ((A) Shannon diversity Index (B) Number of Observed ASVs (C) Chao1 diversity Index and

(D) Simpson diversity Index) gut microbiome across categories of SP uptake.

The beta diversity plots (Bray-Curtis, Unifrac and Weighted Unifrac distances) showed no significant spatial differences were observed among the gut microbiota of the IPTp-SP uptake categories as shown in figure 11. The score of Axis 1 and Axis 2 explains the variance of the gut microbiota abundance.

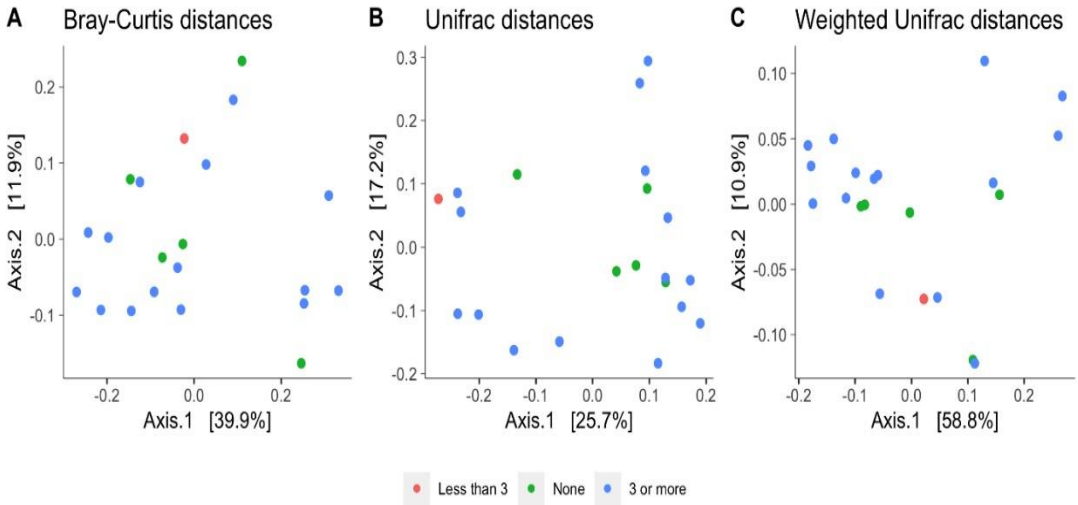

Figure 11: Beta diversity scatter plots of SP uptake and gut microbiome ((A) BrayCurtis distance (B) Unifrac distances (C) Weighted Unifrac distances).

Relationship between deworming and gut microbiome

The association between deworming and gut microbial diversity was measured by alpha and beta diversities. Deworming status was defined by YES (those who had received at least a dose of dewormer, irrespective of the type), and NO (representing those who had received no dose of dewormer during the period of the current pregnancy).

T-test (ASV, Chao1, and Shannon metrics) and Wilcoxon test (Simpson metric) were conducted to determine the differences across group diversities. We observed no significant difference in the within sample bacterial diversities across both study groups as shown in figure 12.

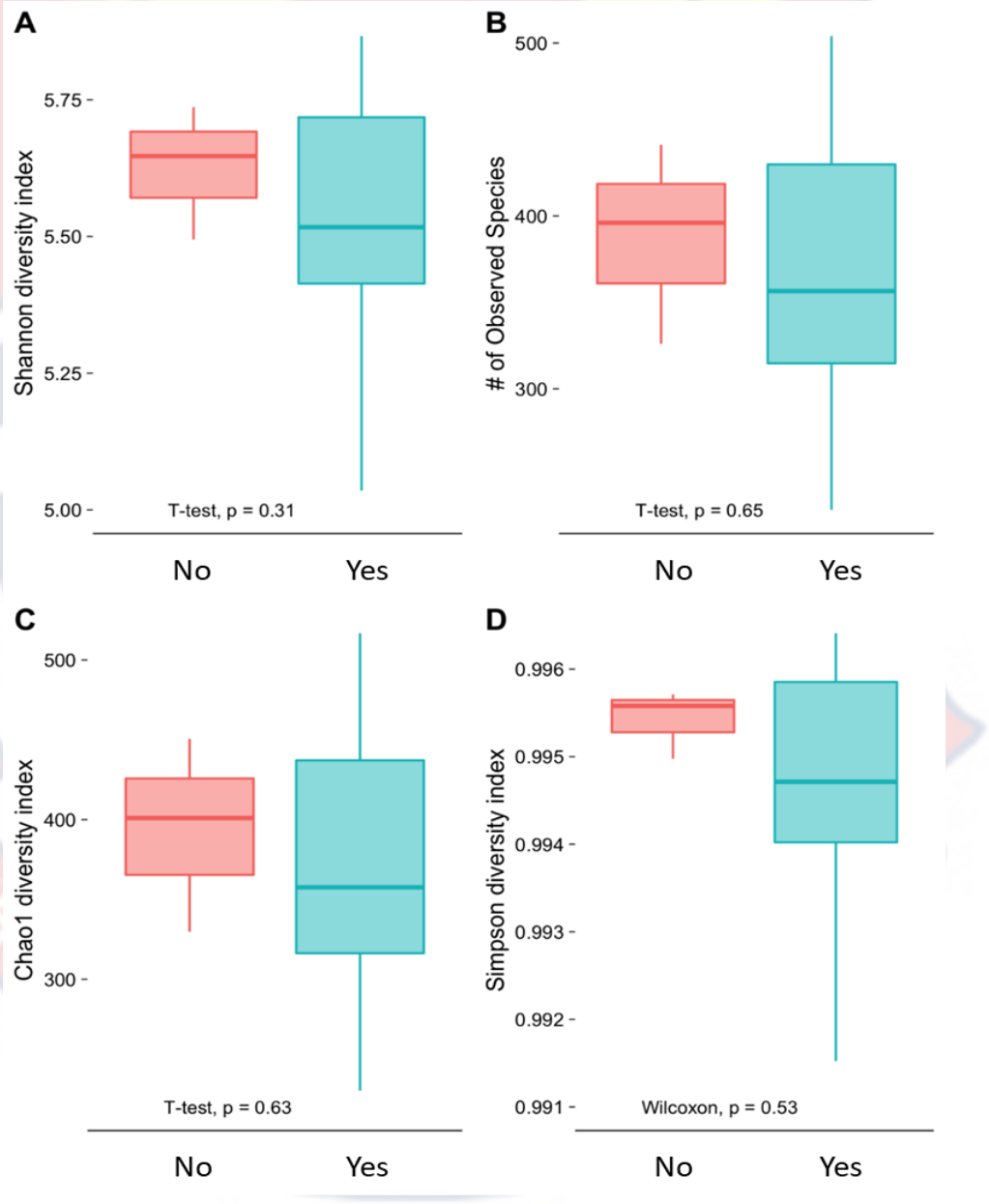

Figure 12: Box plots illustrating Alpha diversity indices ((A) Shannon diversity Index (B) Number of Observed ASVs (C) Chao1 diversity Index and

(D) Simpson diversity Index) gut microbiome across categories of deworming status.

Beta diversity which explored the relatedness of bacterial lineages from the two categories showed that bacteria across the two study groups were closely related irrespective of deworming status (Figure 13).

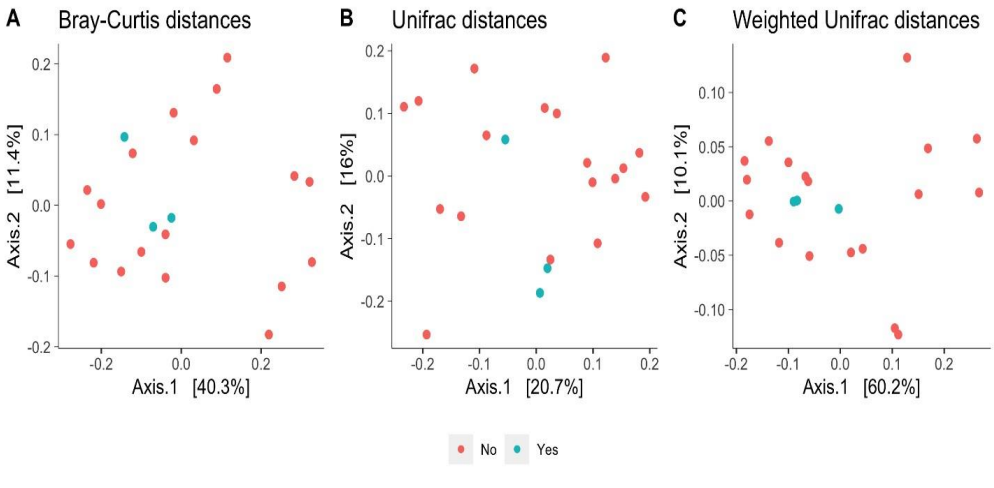

Figure 13: Beta diversity scatter plots of deworming status and gut microbiome ((A) Bray-Curtis distance (B) Unifrac distances (C) Weighted Unifrac distances)

Discussion

This study sought to employ metagenomic sequencing and bioinformatic analysis to characterize the gut normal flora of healthy third trimester pregnant women and how this bacterial structure relates with selected clinical parameters. To the best of our knowledge, this pilot study is the first in a Ghanaian population, and one of the few in sub-Saharan Africa. This section discusses metagenomic, and clinical (BMI, IPTp-SP

uptake, and deworming) data through biomedical lenses and in public health context.

The 5,283 observed OTUs belonging to 17 families, 11 phyla and 26 genera represent a very diverse population of bacteria across the participants as compared to Ruebel et al. (2021) who reported 10 families from 140 American pregnant women. Twenty-five of the captured genera descended from 5 major phyla showing increasing diversity down the phylogenetic tree. Taxa such as *Clostridiales* and more specifically, *Lachnospiraceae* were more diverse than other less represented taxa. Though, the high diversity of these *Firmicutes* did not translate into higher relative abundance, the data may point to a peculiar importance of phyla *Firmicutes* in the pregnancy of our study group. The gut microbiome structure has bacterial groups ranging from aerobes to anaerobes, from Gram positive to Gram negative bacteria, and from known normal flora to potentially pathogenic genera. Although some species were represented in less than 5% abundance, the diversity and richness of the gut microbiome, and not only the relative abundance, is important in maintaining gut integrity, aiding digestion and absorption, enhancing gut immunity, and a healthy pregnancy (Mayer et al., 2014). However, a dysbiosis in the stable bacterial structures will hold adverse effects on the gut microenvironment and beyond. The aberrant gut microbial structure reported in T3 pregnancy is thought to be associated with adiposity, insulin resistance, low-grade inflammation and hyperglycaemia (Abdullah et al., 2022). These metabolic

syndrome-like changes orchestrated by gut microbial dysbiosis may be important in the maintenance of normal pregnancy. Consequently, any conclusions about the normal or abnormal constitution or changes over pregnancy could be premature.

Till date, there is no consensus on the peculiar composition of normal gut microbiome during pregnancy. This is largely due to the vast variations across individuals and communities. This study reports a predominance of *Actinobacteria*, *Bacteroidetes, Firmicutes*, and *Proteobacteria.* In pregnancy, Black race has been associated with increased proinflammatory states (Picklesimer et al., 2008), a potential explanation for the increased abundance of the pro-inflammatory inducing *Proteobacteria* in our study. *Proteobacteria* are closely associated with inflammation (Koren et al., 2012), gastrointestinal diseases (Hollister et al., 2014) and proinflammatory cytokines such as IFN-γ, IL-2 and IL-6 and TNF-α (Abdullah et al., 2022). Taken together, the abundance of *Proteobacteria* may cause low-grade gastrointestinal mucosal surface inflammation, a state that is common in pregnancy (Edwards et al., 2017). *Firmicutes* (eg. *Lactobacillus, Clostridium*), *Actinobacteria* (eg. *Bifidobacterium*), and *Bacteroidetes* (eg. *Bacteroides fragilis*) on the other hand are associated with anti-inflammatory states (Morgan et al., 2012; Sokol et al., 2008). The predominance of bacteria phyla promoting anti-inflammatory states may be important to keep in check the proinflammatory tendencies of *Proteobacteria.* A significant imbalance of these bacterial

classes therefore may influence inflammation mediated conditions such as IBD, T2DM, enteritis, etc. *Firmicutes* are also important in the digestion of dietary fibre and complex starch producing SCFA metabolites. Probiotic *Firmicutes* (eg. *Roseburia, Eubacterium*, and *Lactobacillus*) produce SCFA and antioxidants which together maintain colon health and gut lining integrity (Riaz Rajoka et al., 2021). Thus, higher abundance of these bacteria phylum is significant for the maintenance of healthy pregnancies. However, the greater energy harvesting potential of *Firmicutes* may also induce overweight and obesity. Our findings corroborate those of Sakurai et al. (2020) among 45 Asian women, Koren et al. (2012) among 91 European women and Ruebel et al. (2021) among 140 American healthy women. This consistency in reports suggests that regardless of population and sampling time, *Actinobacteria*, *Bacteroidetes, Firmicutes*, and *Proteobacteria* are the dominant gut bacterial phyla among T3 pregnant women. This holds promise for the application of public health policies and treatments (informed by third trimester gut microbiome) developed in other countries in our setting given the similarities in bacterial structures.

Furthermore, the gut microbiome in pregnancy among our study cohort does not differ significantly from that observed among healthy adults in Ghana and Sub-Saharan Africa. Parbie et al. (2021) in a study among 55 healthy Ghanaian adults, reported highest abundance of *Firmicutes, Bacteroidetes* and *Proteobacteria* but not *Actinobacteria*. The report in Sub-Saharan Africa is no different from ours (Hansen et al., 2019; Senghor

et al., 2018; Yatsunenko et al., 2012). The 'peculiar' third trimester gut microbiome described by Koren et al. (2012) appears to be the prevalent microbial structure of the gut among Ghanaians. This may be due to the similar high-fibre, low-fat African diets consumed, irrespective of pregnancy status. The key gut microbiome signatures of our cohort suggest dietary patterns that are reflected in the gut bacteria with transition from rural to urban areas. This is consistent with the peri-urban socio-economic characteristics of our cohort.

We report a few peculiar microbial structures in some participants. The oldest sampled participant who was obese and had received five doses of SP at the time of sampling showed a unique highest relative abundance of *Clostridiaceae* family, and genus *Clostridium*, the only such record in this study. Comparatively, no other participant in the same age, weight category or SP dose level showed any such bacterial structure. This is particularly interesting given that the genus *Clostridium* contains several important intestinal pathogens such as *Clostridium pefrigenes* which can offset the health of pregnancies. However, the data collected may be limited to appreciate the source of this unique finding. Another unique observation was among two participants who were the only to show *Eubacterium* in high proportions. They showed no significantly different characteristics from all other participants nor any significant similarities in characteristics among themselves. The anaerobic non-spore forming Gram positive *Eubacteria* rods are thought to be the second most predominant gut

microflora (Schwiertz et al., 2000), with a potential to cause opportunistic infections. These two case points reflect more of a normal adult gut microflora. This is consistent with observations at the phyla level that gut microbiota of healthy pregnant women in our cohort is similar to that of healthy adult Africans (Hansen et al., 2019; Parbie et al., 2021; Senghor et al., 2018; Yatsunenko et al., 2012) is similar to that observed in third trimester of gestation.

Consistent with studies in Europe (Koren et al., 2012), America (Ruebel et al., 2021) and Asia (Sakurai et al., 2020), our study reports a high abundance of Bacteroidaceae, and *Lachnospiraceae* at the family level. *Lachnospiraceae* are involved in the synthesis of short chain fatty acids including acetate, propionate, and butyrate. These are known to be beneficial to health including their antiinflammatory and antioxidant properties (Morgan et al., 2012; Remely et al., 2014).

This study's observation of *Bacteroides* and *Faecalibacterium* as the most abundant genera across the participants draws from the high relative abundance of *Bacteroidetes* and *Ruminococcaceae* families respectively. As such, *Bacteroides* and *Faecalibacterium* were the predominant descendants of these families in the study. The reported relative abundance of *Bacteroides* and of *Faecalibacterium*, known butyrate producers with anti-inflammatory attributes that are negatively correlated with IBD (Sokol et al., 2008) and metabolic syndrome (Haro et al., 2016) may be important in toning down the inflammation associated with pregnancy. Given that pro-

inflammatory states in pregnancy have been implicated in many of the pregnancy conditions and complications (Negishi et al., 2021), the higher relative abundance of anti-inflammatory bacterial genera is beneficial in balancing metabolic syndrome in pregnancy. These findings are in synch with previously reported studies that observed high abundance of *Bacteroides, Alistipes, Faecalibacterium* and *Collinsella* (Abdullah et al., 2022). However, unlike the same study, our study did not report high abundance of *Alistipes* and *Collinsella.* Previous studies in the field have reported distinct gut microbiota across different populations (De Filippo et al., 2017; Nishijima et al., 2016; Rothenberg et al., 2019; Yatsunenko et al., 2012) These differences are largely attributable to differences in population, geographical areas, nutrition and lifestyle.

We also assessed the association between BMI and gut microbiome in our cohort. We recorded a high alpha diversity in each study group and a low beta diversity across the four study groups. We did not observe any significant correlation between gestational BMI and gut microbial diversity. We, however, report lowest within-subject diversity in the Obese and Overweight BMI categories. These findings resonate with Abdullah et al., (2022) who also reported reduced alpha diversity in obese and pre-obese BMI groups as against normal gestational BMI group. The study also observed no significant difference in Alpha diversities across the BMI categories. Again, this is in synch with Abdullah et al., (2022) who did not observe any significant difference in alpha diversities across BMI

categories. Our findings further corroborate those of Stanislawski et al., (2018) who also reported reduced alpha diversity between within overweight and obese pregnant women. It is significant to note that, normal weight pregnant women presented the most diverse bacterial compositions. Interestingly, increased bacterial diversity has been associated with better RMNCH outcomes (Smid et al., 2018). Thus, our findings suggest better pregnancy outcomes among normal weight pregnancies as compared to the relatively lower diverse bacterial groups observed in our obese and overweight participants.

Furthermore, bacteria across all three groups were closely related in lineage as we observed no significant between sample diversity between bacteria across the three main study groupings. The apparent lack of distinction between bacterial groups in the different study groups may indicate no correlation between BMI status and type of microbiome structure. This conjecture gains traction from the findings of Smid et al., (2018) who observed no correlation between maternal obesity and relative abundance of bacteria. In contrast, Collado et al., (2008) found that overweight pregnant women had significantly higher levels *of Bacteroides* and *Staphylococcus,* while Santacruz et al., (2010) detected an increase in *Staphylococcus* in overweight pregnant women and a decrease in *Bifidobacterium* (phylum *Actinobacteria*) and *Bacteroides* (phylum *Bacteroidetes*). These unique bacterial classes observed accounts for increased beta diversity as against what we report in the present study. The

similarities in bacteria across these study groups may also indicate the influence of other factors in the architecture of the gut microbiome during pregnancy other than BMI status. Diet, for example, has been widely reported to influence and modulate gut microbiome during pregnancy (David et al., 2014; De Filippis et al., 2016).

Studies in mouse models of obesity, under controlled environments and conditions, reveal robust and reproducible alterations in gut microbiome (Falony et al., 2016). Data from human studies however, are far more equivocal (Sze & Schloss, 2017). Indeed, previous studies have shown that the large inter-individual variations present in the human microbiome contributes to the much more modest divergences observed in human obesity (Falony et al., 2016; Sze & Schloss, 2017).

Sulfadoxine-pyrimethamine, a well-known antimalarial, with antibacterial properties (Gutman & Slutsker, 2017) is recommended and widely used in the prophylactic treatment of malaria among pregnant women living in malaria endemic communities. We report in this study that 3 or more doses of SP was significantly associated with relatively higher microbial diversity, richness and evenness than less than 3 or no dose of SP. This is a significant observation that signals that the number of SP doses, meant for antimalarial effects, significantly increased the microbial diversity. These findings serve as formidable scientific evidence to support the hypothesis that SP affects gut microbiome (Dingens et al., 2016). It is intriguing however, that the antibacterial properties increased the bacterial

diversity; with fewer doses achieving lesser diversification. Optimum doses of SP could be treating or preventing pathogenic infections (Capan et al., 2010) and thus allowing for increased beneficial bacteria diversification, preventing preterm delivery, and associated low birthweight. It can be inferred that 3 doses of SP other than their antiparasitic properties, also affect birth outcomes by increasing gut microbial diversity. This inference gains traction from a recent WHO review which showed that three or more doses of IPTp-SP w associated with higher mean birth weight and fewer low birth weight (LBW) births than two doses of IPTp-SP (WHO, 2012). The present study's findings provide evidence for WHO calls for increased access and uptake of optimum does of SP during pregnancy in high malaria endemic areas. This is one of the first reports of SP directly related to gut microbial diversity in pregnancy.

We also report in this study that deworming did not affect gut bacterial diversity in pregnancy. Deworming is mandatory among pregnant women in the study area and as shown in our study has a relatively wide coverage (81.8% dewormed). The dewormer of choice in all participants was Albendazole. There are limited studies exploring the role of dewormers in gut microbiomes. Dewormers, like SP, are given as antiparasitic but unlike SP have not been clearly indicated to have antibacterial properties (Venkatesan, 1998). However, they contribute to maintaining the microbial structure of the gut. Further tailored studies are

needed to explore the effects of antibacterial properties of dewormers, if any, on gut microbial structures and compositions.

Chapter Summary

This chapter presented the findings of the study and how they relate to relevant literature and their implications for health and future research. Essentially, the study observed that the major bacterial phyla in T3 pregnancy were *Actinobacteria*, *Bacteroidetes,* *Firmicutes*, and *Proteobacteria.* This is largely in sync with previously reported studies on the subject among pregnant women. Neither deworming status nor gestational BMI affected gut microbial diversities in T3 of gestation. However, the number of doses of SP taken at the time of sampling was shown to be associated gut microbial diversities. The finding that gut microbiome is dynamic between and among pregnant women and correlates with clinically important characteristics such as IPTp-SP uptake was interesting because of its implications for health and wellbeing of pregnancy and its outcomes.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

Introduction

This chapter presents the final aspects of this thesis and brings to finality the issues of this research. It first of all summarizes the key issues raised in this thesis, the key research paradigms and methodology employed to explore the study objectives and the resulting findings from the research data. The chapter also presents the conclusions drawn from the entire thesis. This conclusion draws from all the data presented in this thesis and the available literature on the subject. Finally, the chapter also presents recommendations for policy, science, research and health and wellbeing. These recommendations draw from the findings and the conclusions of this study.

Pregnancy is a critical window that affects two or more people directly – the mother and foetus(es). The health of the prospective mother and the proper development of the baby(ies) is determined by several factors, including but not limited to the gut microbiome of the pregnant woman. The various microbiome structures especially in third trimester pregnancy and their associations with various pregnancy conditions such as gestational weight gain, and the intake of antimalarial prophylactic drug sulfadoxine-pyrimethamine during pregnancy may contribute to pregnancy outcomes in ways yet unknown. This study assessed the structure of the gut microbiome in third trimester pregnancy among Ghanaian pregnant women

and how it relates with gestational BMI, deworming and SP uptake during pregnancy.

Subsequently, to answer the research questions, 22 healthy third trimester pregnant women receiving antenatal care services from two health facilities in Cape Coast were recruited, sampled and studied. The pilot study collected sociodemographic data from participants, and hospital-reported BMI, deworming statu, SP uptake, and other clinical data, and participant's collected stool sample to analyze the gut microbiome structures and their relationships with maternal clinical parameters. DNA was extracted from stool samples and 16sRNA gene amplifications done. Microbiome analysis was conducted using QIIME platform. Greengenes 99% Weighted taxonomy classifier was used in the classification of the bacterial species. Taxonomic classification was based on the Greengenes 13.8 reference database, with a similarity threshold of 97%. The open-source statistical software R (https://www.r-project.org) was used to assess the diversities (alpha and beta diversities) of bacterial structures across groupings of BMI (Underweight, Normal weight, Overweight, and Obese groups), deworming status (dewormed or not) and across categories of SP uptake (less than 3 doses, no dose, and at least 3 doses of SP). $P < 0.05$ was considered statistically significant in all analyses.

The study observed that *Actinobacteria* were the most predominant phyla, followed by *Bacteroidetes, Firmicutes*, and *Proteobacteria* respectively. We contend in this study that these represent the dominant

structures of gut bacteria in the third trimester of pregnancy among the study population. The bacterial diversities in the categories of BMI, and deworming showed no difference across the categories for either BMI or deworming status. Whilst these do not necessarily indicate a lack of difference in bacterial structures in each group, it indicates that the diversities between the groups are not different. Importantly, we also report a significant association of number of SP doses with gut microbial diversity. We subsequently inferred that gut microbiome structures and diversities may not be influenced by gestational BMI, or deworming status but by SP uptake and other potential factors such as diet, and microbiome modulating behaviours.

Summary of Findings

The study reports that the gut microbiome structure of the third trimester pregnant women in our study area mainly consisted of *Actinobacteria* which were the most predominant phyla, followed by *Bacteroidetes, Firmicutes*, and *Proteobacteria* respectively. There was also record of *Verrucomicrobia*, albeit, in lower levels. Family level analysis showed predominance of *Bacteroidaceae* followed by *Lachnospiraceae, Lactobacillaceae,* and *Prevotellaceae* respectively. Bacteria of the family *Ruminococcaceae* were also present in almost all participants but in lower levels. The study also reports *Bacteroides* as the predominant genus present. We also report *Lachnospira* and *Faecalibacterium* presence.

The study also reports no significant difference in the bacterial diversities across participants in the Normal, Underweight, Overweight, and Obese BMI categories. Subsequently, the bacterial diversities were statistically similar across the four BMI categories. Within group bacterial diversity was greatest in the Normal BMI category and less diverse in the Obese and Overweight BMI categories. These observations were however not statistically significant. We contend that other factors other than BMI may account for the widely reported gut microbiome in third trimester of gestation.

As it relates to SP uptake, the study revealed that the dose of SP received during pregnancy (as observed at the time of sampling at the time of sampling) did significantly affect the gut bacterial diversities in pregnancy. Sulfadoxinepyrimethamine, with its antibacterial properties, significantly modulated the gut microbiome in the categories that receives at least three doses as compared to the category that received no dose or less than three doses. It is worth noting that pregnant women who received at least 3 doses of Sulfadoxine-Pyrimethamine had the highest within sample richness and evenness.

Finally, the study reports that deworming did not affect microbial diversities. As measured by alpha and beta diversity metrics, gut microbial diversities remain same irrespective of deworming status (that is dewormed or not). This observation however, is true as it relates to dewormers defined

as “Albendazole” and thus does not apply for the use of other deworming chemicals.

Conclusion

The gut microbiome in the third trimester of gestation among Ghanaian women has been characterized and is typical of third trimester gut microbiomes described in Europe, Asia and the US. This holds prospect for the application and use of Microbiome-Based Therapies and related interventions developed elsewhere in our context. Three or more doses of Sulfadoxine-Pyrimethamine during pregnancy is beneficial for gut microbial diversification. Thus, IPTp-SP as an RMNCH intervention in pregnancy is encouraged for its added advantage in gut microbiomes. Furthermore, gut microbiome diversities in pregnancy are independent of BMI and deworming (using Albendazole) status. Deworming during pregnancy is healthy for gut microbiome diversities and thus is not discouraged.

Recommendation

The study encourages habits and lifestyles that promote normal weight during gestation among pregnant women. Increased bacterial diversity has been associated with better birth outcomes. The present study observed the highest within group diversity among the Normal BMI participants. Thus, maintaining normal weight during pregnancy enhances bacterial diversity and consequently improved RMNCH outcomes. Activities such as monitoring diet and eating patterns (limiting sweets and

sugars and sugary drinks, using low-fat cooking methods/foods, choosing low fat dairy products, eating appropriate portion sizes), regular exercise among others are encouraged to maintain normal weight, to assure increased bacterial diversity and

RMNCH outcomes.

The study also recommends Ministry of Health and public health stakeholders enforce and increase the reach of Sulfadoxine-pyrimethamine as IPTp intervention during pregnancy. Aside its known benefits in preventing malarial infections during pregnancy, the present study also revealed that the higher the number of doses of SP, the higher the richness and evenness of bacterial distribution, a state that promotes better RMNCH outcomes. Thus, SP may promote pregnancy outcomes by increasing bacterial diversity as well as its antimalarial functions.

The study revealed the presence of bacteria of various families and genus. Although non-pathogenic under normal conditions, dysbiosis arising from shifts in healthy lifestyle, excessive use of antibacterials, unhealthy dietary choices and patterns may expose pathogenic traits and induce infections. The study therefore recommends public health officers promote the use of prebiotics and probiotics among pregnant women to selectively promote the growth of healthy bacterial groups such as *Actinobacteria, Bacteriodetes*, and *Faecalibacterium*. Furthermore, the study recommends the reduced use of broadspectrum antibiotics that tends to destabilize gut

microbial structures, induce gut dysbiosis and the unhealthy consequences of proliferation of pathogenic bacteria.

Suggestion for Future Research

The limitations of this study present opportunities for future research to explore. First, the study is limited by its sample size which reduced the power of the study in bolding asserting the findings of this study. Future studies may explore the subject in a broader context, with larger study population to better understand the dynamics pregnancy factors as they relate to gut microbiome during pregnancy.

Future studies may also explore the effects of other pregnancy factors such as diet and the role of sociodemographic, cultural practices and economic status in gut microbiome during pregnancy. Whilst we report the effect of SP and Deworming using Albendazole, other activities that relate with the physiological, chemical and biological state of the gut may also hold potential for modulating the gut microbiome.

Furthermore, future studies may also consider exploring the relationships between gut microbiomes and vaginal microbiome as they relate to pregnancy and pregnancy outcomes. We have herein hypothesized the potential role of the gut microbiome in RMNCH outcomes, a phenomenon that has largely been associated with vaginal microbial structure. This deepens the possible interrelatedness of the two organ systems, their microbiota and birth outcomes.

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APPENDIX

UNIVERSITY OF CAPE COAST

RESEARCH INSTRUMENT

TYPE OF INSTRUMENT : QUESTIONNAIRE

TITLE OF STUDY : GUT MICROBIOME, MALARIA PREVENTION PREGNANCY AND MATERNAL HEALTH INDICES AMONG PREGNANT WOMEN IN CAPE COAST: A PILOT STUDY

DEPARTMENT : MICROBIOLOGY & IMMUNOLOGY SCHOOL OF MEDICAL SCIENCES, CoHAS, UCC.

STUDY PI : MR. EDWIN YANGNEMENGA

ETHICAL CLEARANCE : **CCTH** [CCTHERC/EC/2019/089] / **UCC** [UCCIRB/EXT/2019/26]

Study Details	Study code:	IPTp - 01

Participants Details	Participant's ID	
	Hospital ID:	
	Residential address:	
	Telephone number:	

Interview Details	Date of interview:	
	Place of interview:	
	Name of interviewer:	

	**Socio-demographics**		
No.	**Questions**	**Code**	**Response(s)**
Q1	Age in yea rs	*write here* ☞	
Q2	Marital St tus ı	1) Single 2) Married 3) Others	[ ]
Q2a		If others, please specify	……
Q3	Educatio l Status ıı	1)None 2)Primary 3)JHS/JSS/Middle 4)SHS/SSS/Secondar 5)y Tertiary	[ ]
Q4	Occupatio ı	1)Civil servant 2)Trader 3)Housewife 4)Unemployed 5)Non-disclosure 6)Others	[ ]
Q4a		If hers, please o specify	……

Q5	Monthly household income (GHC)	1) 50 - 100 2) 100-500 3) 500 - 1000 4) 1000 - 2000 5) Others	[ ]
Q5a		If others, please specify	……
Obstetric History			
Q6	No. of Pregnancies	*write here* ☞	……..
Q7	No. of births	*write here* ☞	
Social Risk factors			
Q8	Do you take alcohol?	1) Yes 2) No	[ ]
Q9	Do you smoke?	1) Yes 2) No	[ ]
Antenatal Records			
Q10	Estimated date of delivery (EDD)	*write here* ☞	
Q11	Height (cm)	*write here* ☞	[ ][ ].[ ]
12	Weight @ ANC1 (Before 12 weeks) (kg)	*write here* ☞	[ ][ ].[ ]
Q13	BMI @ ANC1 (Before 12 weeks)	*write here* ☞	[ ][ ].[ ]
Q14	Estimated desired weight @ EDD (kg)	*write here* ☞	[ ][ ].[ ]
Q15	Did you use	1)Yes	[ ]

	contraception before this pregnancy?	2) No	
Q15a		If yes, please specify	…….....
Q16	BP (mmHg) @ First Visit (12 weeks)	*write here* ☞	[ ][ ].[ ]
Q17	BP (mmHg) @ Term (36 weeks)	*write here* ☞	[ ][ ].[ ]
Q18	Weight (kg) @ Term (36 weeks)	*write here* ☞	[ ][ ].[ ]
Q19	BMI @ Term (36 weeks)	*write here* ☞	[ ][ ].[ ]
Q20	Gestational Age @ First Visit (12 weeks)	*write here* ☞	[ ]
Q21	Gestational Age @ Term (36 weeks)	*write here* ☞	[ ]
Q22	Fundal Height (cm)	*write here* ☞	[ ][ ].[ ]
Q23	Fetal Heart Rate (bpm)	*write here* ☞	[ ][ ].[ ]
Q24	Did you take Iron Folic acid (IFA)	1.Yes 2.No	[ ]
Q24a		If yes,how many did you take?	…… …
Q25	Do you take any medication/ supplements/ antibiotics?	1.Yes 2.No	[ ]
Q25a		If yes, please specify	…….....

Q26	Intermittent preventive treatment (IPTp) taken at end of 36 weeks	0 1 2 3 4 5	[ ]
Q27	Have you received any dewormin g medicine during your current pregnancy ?	1) Yes 2) No	[ ]
Q27a		If yes, please specify	…….....
Investigations			
Q28	Blood group	1. A 2. B 3. AB 4. O	[ ]
Q29	Rh typing	1. Positive 2. Negative	[ ]
Q30	Sickling status	1. Positive 2. Negative	[ ]
Q31	G6PD status	1. Normal 2. Defect	[ ]
Q32	BF for malaria	1. Positive 2. Negative	
Q32a		If positive, please	……

		specify	…
Q33	Hb* @ first visit	*write here* ☞	[ ][ ].[ ]
Q34	Repeat Hb* @ (28 weeks)	*write here* ☞	[ ][ ].[ ]
Q35	Repeat Hb* @ (36 weeks)	*write here* ☞	[ ][ ].[ ]

Food Frequency Questionnaire (Multiple choice questions)

	Food item	Code	Response
Q36	Staple/Grains/Root and tubers (Maize, Wheat, Rice, Millet, Sorghum, Cassava, Yam, Cocoyam, Plantain & Potatoes)	1) Never / less than once per month 2) 1-3 per month 3) 1-2 per week 4) 1-2 per week 5) 3-5 per week 6) Almost everyday	[ ]
Q37	Fruits & Vegetables		[ ]
Q38	Animal-source food (eg. Eggs, chicken, fish, Meat, Milk & Milk products)		[ ]
Q39	Legumes and seeds (Beans, Agushie, Sesame, Werewere & Groundnut)		[ ]
Q40	Oil and fat (Palm oil, vegetable oil, butter)		[ ]
Q41	Alcoholic beverages/		[ ]
Q42	Carbonated drinks/Sweats		[ ]
Q43	Coffee/Tea		[ ]