

Relationship with Maternal Gut Bacteria Dysbiosis and Gestational Weight Variation: A Case Study of Muhoza Health Center, Rwanda

Callixte Yadufashije, Ishimwe Gatete Grace, Lydia Mwanzia¹, Liliane Muhimpundu, Emmanuel Munyeshyaka, Joseph Mucumbitsi, Georges Bahati Sangano², Thierry Habyarimana

Department of Biomedical Laboratory Sciences, INES-Ruhengeri Institute of Applied Sciences, Musanze, Rwanda, ¹Department of Midwifery and Gender, School of Nursing, Moi University, Eldoret, Kenya, ²Department of General Nursing, University of Rwanda, Kigali, Rwanda

Abstract

Introduction: Dysbiosis is often used to describe the state where there is a disruption in the balance of organisms in the microbiota. Dysbiosis of gut microbiota has been associated with disorders within and without the gut. This study aimed to identify the microbiota composition and to determine the association between gut microbiota and gestation weight amongst women attending Muhoza health Center. **Materials and Methods:** A cross-sectional study design was used where ninety stool samples were collected amongst pregnant women attending antenatal clinic of Muhoza health center. The samples were transported to Ines microbiology laboratory for microbiological analysis. Laboratory techniques including culturing, gram stain, and biochemical tests were performed for gut microbial identification. Analysis of variance was used to test the mean bacterial difference in pregnancy trimesters, a *t*-test was performed to test for the mean bacterial difference in the gestational weight gain (GWG) and gestational weight loss groups, and Chi-square test was used to test for association with gut bacteria imbalance and the gestational weight variation. **Results:** *Lactobacillus* 23.28% was the most predominant observed in the gut. The gut bacterial variation throughout pregnancy trimesters was observed ($F=4.437904575$, $P=0.022909599$). The gut bacterial mean difference was statistically significant in the weight gain and weight loss ($t=3.45$, 95% confidence interval [CI]: 0.1487–0.5154, $P=0.005428$) in the GWG and loss. There was statistical association with gut microbiota dysbiosis and gestational weight amongst pregnant women with *Escherichia coli* ($P=0.030197$, 95% CI: 0.0741–0.4611, $x^2=7$), *Pseudomonas* ($P=0.018316$, 95% CI: 0.0941–0.4781, $x^2=8$), and *Citrobacter* ($P=0.00046$, 95% CI: 0.7855–0.9014, $x^2=15.38$). The overall association ($P=0.001869$, 95% CI: 0.9697–0.9868, $x^2=46.19$) was statistically significant. **Conclusion:** Gut bacterial alteration contributes to gestational either weight gain or loss. During antenatal care, gut bacteria should be tested to maintain the gestational weight.

Keywords: Bacteria, gestational weight, gut, microbiota

INTRODUCTION

The human gastrointestinal and genitourinary systems are surrounded by microbiota made up of trillions of microorganisms, most of which are of bacterial and viral origin. These microorganisms are considered non-pathogenic or normal 'flora' and function in tandem with the host's immune system defence to protect against pathogen colonisation and invasion.^[1] Many animals and insects are hosts to numerous microorganisms that reside in the gastrointestinal tract as well. As defined by molecular biologist Joshua Lederberg, the gut microbiota is the totality of microorganisms, bacteria, viruses, protozoa and fungi and their collective genetic material present in the gastrointestinal tract.^[2]

Dysbiosis (also called dysbacteriosis) is a term for a microbial imbalance or maladaptation on or inside the body such as an impaired microbiota. The microbiota environment, such as skin flora, gut flora or vaginal flora, can become deranged,

Address for correspondence: Dr. Callixte Yadufashije, Department of Biomedical Laboratory Sciences, INES-Ruhengeri-Institute of Applied Sciences, Ruhengeri, Rwanda. E-mail: cyadufashije@gmail.com

Submitted: 05-Aug-2021 **Revised:** 17-Sep-2021

Accepted: 23-Sep-2021 **Published:** 31-Dec-2021

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Yadufashije C, Grace IG, Mwanzia L, Muhimpundu L, Munyeshyaka E, Mucumbitsi J, *et al.* Relationship with maternal gut bacteria dysbiosis and gestational weight variation: A case study of muhoza health center, Rwanda. *Adv Hum Biol* 2022;12:27-33.

Access this article online

Quick Response Code:



Website:
www.aihbonline.com

DOI:
10.4103/aihbonline.117_21

with normally dominating species underrepresented and normally outcompeted or contained species increasing to fill the void.^[3] This derangement has been described as an unhealthy microbiota. Dysbiosis is most commonly reported as a condition in the gastrointestinal tract, associated with the overgrowth of certain microorganisms over others. The small intestine bacterial overgrowth is a disorder of excessive bacterial growth in the intestine, and unlike the colon, which is rich with bacteria, the small bowel usually has fewer than 10,000 organisms per millilitre.^[4] Dysbiosis has been associated with a change in dietary intake and other factors including antibiotic drug use.^[5]

Carding asserts that intestinal microbiota performs an essential metabolic function by acting as a source of essential nutrients and vitamins and aiding in the extraction of energy and nutrients, such as short-chain fatty acids and amino acids, from food.^[4] Food nutrient absorption during pregnancy is essential in sustaining gestational growth and development for optimal pregnancy and newborn outcomes. Although it is difficult to associate the actual impact of microbiota disparity on maternal health during pregnancy, the metabolic function of gut microbiota remains an important physiological function under study. Gestational weight gain (GWG) is defined as the amount of weight gain; a women experiences between conception and the birth of the infant. During the 20th century, recommendations for maternal weight gain in pregnancy were controversial, ranging from rigid restriction to encouragement of ample gain.^[6] GWG results from various structural and functional modifications that occur in a woman's body to meet the nutritional requirements of pregnancy. This gain includes foetal and placental growth, increase in amniotic fluid, placenta, increased blood volume, increased adipose tissue, uterine and mammary growth, all of which ranged 12–20 kgs by the end of pregnancy. GWG has been associated with a higher fat mass in childhood and greater body mass index and fat mass in later adulthood. The rate of weight gain varies throughout pregnancy, and its timing during pregnancy also has an impact on newborn birth weight.^[7]

Recently, the microbial alteration in the human gut was proposed to be the possible cause of obesity, and it was observed that the gut microbes from faecal samples harbour 3.3 million non-redundant microbial genes. However, it is still poorly understood how the dynamics and composition of the intestinal microbiota are affected by diet or other lifestyle factors. Moreover, it has been difficult to characterise the composition of the human gut microbiota due to large variations between individuals.^[8] During pregnancy, the female body undergoes hormonal, metabolic, and immunological changes to preserve the health of the mother and the offspring, and these changes alter the mother microbiota at different sites such as the gut, the vagina and the oral cavity.^[9] Normally, the gut microbiota composition undergoes dramatic alterations from the first to the third trimester with increased individual diversity β -diversity and decreased α -diversity. The alteration of gut microbiota may be associated with different intestinal complications, as well as variations in

GWG during pregnancy. The paucity of studies confirming the reason behind these variations in GWG has led to a continuing increase of overweight and obesity amongst pregnant women in developed and developing countries, where 38.9 million overweight and obese pregnant women are estimated globally.^[10]

In 2009, the Institute of Medicine guidelines reported that 73% of pregnant women faced excessive GWG. In Rwanda, overweight increased from 13% in 2000 to 16.5% in 2010. Some researchers suggest that may be gut microbiota may enable hydrolysis of indigestible polysaccharides to easily absorbable monosaccharides activating lipoprotein lipase. This leads to rapid glucose and fatty acids absorption and synthesis of liver-derived triglycerides, both phenomena which can boost weight gain.^[11] Others think that specific microbiota compositions modulate fasting-induced adipocyte factors. Whereas others say that obesity is not only caused by overnutrition but also associated with low-grade systemic inflammation triggered by an imbalance in gut microbiota that regulates inflammatory processes in the gut.^[12]

Microbiota modification before and during pregnancy may offer new directions for preventive and therapeutic applications in reducing the risk of gestational overweight. Understanding the factors associated with dysbiosis of maternal gut microbiota and GWG amongst pregnant women may provide insight into nutritional management during pregnancy.^[13] Therefore, this study sought to investigate the relationship between gut microbiota and change in weight amongst pregnant women. This study evaluated the association between the dysbiosis of maternal gut microbiota and GWG amongst pregnant women attending Muhoza health care.

MATERIALS AND METHODS

Study area

This study was conducted at Muhoza Health Center, which is located in the Muhoza sector, Musanze district, Northern Province of Rwanda.

Study design

This cross-sectional study was performed in a period of 3 months, from September 2020 to January 2021. The women participants attending the outpatient antenatal clinic were approached and requested to sign a voluntary consent form to enrol in the study.

Study population and sample size

The study population consisted of pregnant women attending Muhoza Health Center ($n = 90$). Stool samples were collected in a sterile container, and weight and height were measured. The collected samples were submitted to the clinical microbiology laboratory for analysis at INES Ruhengeri.

Inclusion criteria

Pregnant women attending Muhoza Health Center antenatal clinic in their first, second or third trimester were requested voluntarily to participate in the study.

Exclusion criteria

Participants attending the outpatient clinic for other services and who were not pregnant were excluded from the study.

Collection of stool specimens

The participants' stool samples were collected in a sterile container, and the women's weight and height were measured. The collected samples were submitted to the clinical microbiology laboratory for analysis at INES Ruhengeri. Stool samples were collected in a sterile container. Patients were instructed how the sample should be collected using standard laboratory procedures for stool sample collection.

Laboratory analysis

Macroscopic of stool

A macroscopic examination was performed by observing stool specimens with eyes. The identified abnormalities of the sample were based on colour and clarity.

Culture media preparation

Blood agar (BA), macconkey agar (MCA) and man rogosa and sharpe (MRS) agar were prepared by dissolving some grams of a given amount of dehydrated culture media to corresponding millilitres of distilled water, heated with repeated stirring and boiled to dissolve completely. Then, the prepared solution was autoclaved at 121°C for 15 min, then cooled down and distributed in the Petri dish and waited for the culture medium to be solidified.

Inoculation and incubation

The streaking method was used for inoculation of bacteria samples into prepared Petri dishes of BA, MCA and MRS agar media. The plates were aerobically incubated at 35°C–37°C for 18–24 h. Then, we examined the grown colonies.

Smear preparation and gram staining procedures

Before staining the slide, a smear was prepared. A drop of normal saline was added to the slide and aseptically transferred a colony from the Petri dish. The culture was spread with an inoculation loop to an even thin film over a circle of 1–5 cm in diameter. The culture was fixed over a gentle flame. Furthermore, the solution of crystal violet was added over the fixed culture and waited for 60 s. The stain was poured off and gently rinsed excess stain with tap water from a faucet. Then, the iodine solution was added to the smear to cover the fixed culture and waited for 60 s. The iodine solution was poured off and rinsed the slide with running water and shaking off the excess water from the slide. Moreover, a few drops of decolouriser, ethanol or acetone, was added to the slide and rinsed off with water for 5 s. The counterstain, safranin, was flooded on the slide for 40–60 s and washed off the solution with water and making the slide air-dried. Then, the stained smear was examined under the microscope on immersion oil objective.

Biochemical test

Kigler iron agar test

An inoculating straight loop was sterilised in the blue flame of the Bunsen burner and then allowed to cool. A colony of

the suspected Gram-negative microorganism from MCA and BA was picked, stabbed into the medium up to the butt of the kligler iron agar (KIA) tube and then it was streaked back and forth along the surface of the slant. Again, the neck of the KIA was flamed, capped and placed in the incubator for 18–24 h at a temperature of 37°C.

Sulfide, indole, motility and citrate tests

A grown Gram-negative bacterium was inoculated into SIM and incubated at 37°C for 24 h. Kovac's indole reagent was added, and the pink ring (red colour) formed showed the presence of indole. For the citrate test, an agar slant with a medium containing the amounts of mineral salts of sodium citrate, ammonium and bromothymol blue (pH indicator) was used to identify the positive citrate bacteria. Therefore, the positive citrate bacteria were indicated with blue colour change from green colour.

Urease test

A sterile wire loop was used to pick the growing Gram-negative colony from the culture plate then stabbed into the medium urease broth. It is incubated at 37°C for 18–24 h. Before incubation, medium was yellow in colour, then looked pink for urease positive bacteria, and there was no colour change for urease negative bacteria.

Catalase test

Two drops of 3% hydrogen peroxide were put onto a clean glass slide using a dropper, a pure colony of the organism was picked from BA using a wire loop. By placing the colony on the hydrogen peroxide on the glass slide, emulsification was done. Observation for bubble formation was done within 30 s to differentiate *Staphylococcus* and *Streptococcus* species.

Coagulase test

Fresh plasma from human blood was used to identify coagulase-positive bacteria (*Staphylococcus aureus*). A loopful of the test organism was put into the fresh plasma in the sterile test tube, which made a complete suspension. Incubation of the suspension was done at a temperature of 37°C, then examination for clot formation was made for coagulase positive.

Statistical analysis

We analysed the gut microbial difference in three pregnancy trimesters, and analysis of variance was used to test for the mean difference. The variation of gut microbiota was analysed to its difference in weight gain or weight loss, and a *t*-test was used to test for mean difference amongst the two situations. A Chi-square test was used to test for association with the gut microbiota imbalance and gestational weight. Figures and tables were used to present the results of this study.

Ethical considerations

We obtained approval from the head of Muhoza Health Center (MH/AC2020/113). Ethical clearance from INES-Ruhengeri-Institute of applied sciences was given (INES/BLS236/2020). The written informed consent was signed by

each participant before collecting data. All patients' data gathered in this study were handled confidentially by the researcher. Furthermore, patients' coding was used to identify patients from whom the data were obtained.

RESULTS

Age characteristic of patients

Table 1 indicates the demographic characteristics of patients with their frequencies and percentages. The most dominant age was between 28 and 32 years (37.8%), followed by the range of age between 23 and 27 years (22.2%) and age between 33 and 37 years (17.8%), and the least age ranges were between 17–22 and 38–42 years (both at 11.1%).

Body mass index in different trimesters

Table 2 indicates the distribution characteristics BMI of patients with their frequencies and percentages regarding trimesters. It indicates 60% of women from the 1st trimester is overweight, 73.3% and 86.6 from 2nd to 3rd, respectively, to trimesters.

The microorganisms identified from the gut amongst pregnant women

Figure 1 indicates the microorganisms isolated from stool sample collected among pregnant women at Muhoza Health Center. Isolated microorganisms were *S. aureus* (19.17%), *Escherichia coli* (12.32%), *Streptococcus spp* (8.21%), *Lactobacilli spp* (23.38%), *Salmonella spp* (5.47%), *Bacilli spp* (4.1%), *Pseudomonas spp* (6.84%), *Enterobacter spp* (4.1%), *Citrobacter spp* 1.36%, *Actinobacter spp* (2.73%), *Yersinia* (4.1%), and *Staphylococcus epidermidis* (8.21%).

Variation of gut microbial community in pregnancy trimesters

The variation of gut bacteria was investigated in different trimesters. The mean difference of the isolated bacteria ($F=4.437904575$, $P=0.022909599$) was statistically significant. Some bacteria were high in some trimesters while low in others and sometimes absent in others [Table 3].

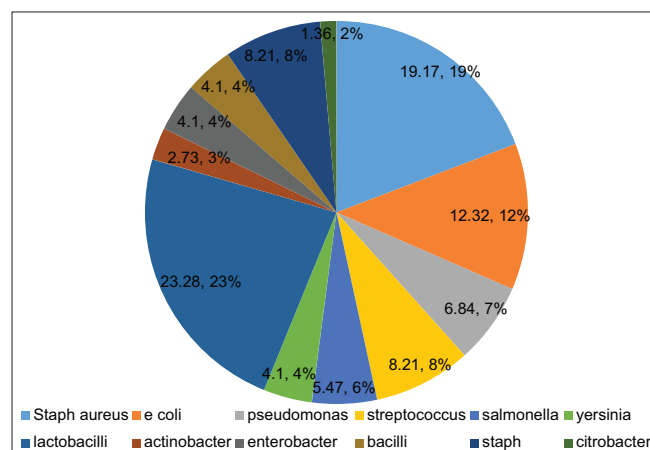


Figure 1: Bacterial identification.

Comparison of gut microbiota in gestation weight

Table 4 indicates the comparison between gut microbiota in gestational weight amongst the pregnant women participants. The mean difference of the gut bacterial alteration in GWG and loss was statistically significant ($t=3.45$, 95% confidence interval [CI]: 0.1487–0.5154, $P=0.005428$).

Association of gut microbiota and gestational weight

We compared the total weight gain of the pregnant woman at the time of study and the dominant gut colonisation. Table 5 indicates the association between gut bacteria dysbiosis and gestational weight. Gestational weight was partially associated with *E. coli* ($P=0.030197$, 95% CI: 0.0741–0.4611, $x^2=7$), *Citrobacter* ($P=0.00046$, 95% CI: 0.7855–0.9014, $x^2=15.38$) and *Pseudomonas* ($P=0.018316$, 95% CI: 0.0941–0.4781, $x^2=8$). The overall association with gut microbiota imbalance ($P=0.001869$, 95% CI: 0.9697–0.9868, $x^2=46.19$) was statistically significant.

DISCUSSION

Gut bacteria play a huge role in metabolism, and this is not limited only to pregnant women. The predominant gut microorganisms were observed as *S. aureus* with 19.17%, *Lactobacilli* with 23.38%. *Lactobacillus* are the most significant bacteria of the human gut and ensures good intestinal health. It not only breaks down some molecules such as glucose but also prevents some pathogenic and opportunistic pathogens in the gut.^[14] The rise in oestrogen and progesterone during pregnancy alter the gut function and microbiome composition and increase the vulnerability to pathogens.^[15] The gut microbiota progressively changes with each trimester of pregnancy. The composition which is most commonly dominated is *lactobacilli* amongst pregnant women [Figure 1]. In the recent clinical trial, supplementation with specific strains of probiotics,

Table 1: Demographic characteristics of patients

Age	Frequency (%)
17-22	10 (11.11)
23-27	20 (22.22)
28-32	34 (37.77)
33-37	16 (17.77)
38-42	10 (11.11)
Total	90 (100)

Table 2: Distribution characteristics of body mass index

BMI	Frequency (%)		
	Trimester 1	Trimester 2	Trimester 3
17-21	3 (20)	0	0
22-27	9 (60)	11 (73.3)	13 (86.6)
28-33	2 (13.3)	2 (13.3)	2 (13.3)
34-39	1 (6.6)	1 (6.6)	0
40-45	0	1 (6.6)	0
Total	15	15	15

BMI: Body mass index

Table 3: Variation of gut bacteria in pregnancy trimester

Microorganisms	Term one	Term two	Term three
<i>Salmonella</i> spp	2	2	8
<i>Lactobacilli</i> spp	14	12	34
<i>Enterobacter</i> spp	2	2	6
<i>S. aureus</i>	8	4	12
<i>S. epidermidis</i>	2	6	28
<i>Streptococcus</i> spp	8	0	12
<i>Yersinia</i> spp	0	0	6
<i>Pseudomonas</i> spp	0	6	10
<i>Actinobacter</i> spp	0	4	10
<i>Bacilli</i> spp	0	4	6
<i>E. coli</i>	6	8	20
<i>Citrobacter</i>	0	2	2
Total	42	50	154

ANOVA

Source of variance	SS	MS	F	P	df	F crit
Rows	16,590.5641	1382.547009	5.107771006	0.000341469	12	2.18338
Columns	2402.461538	1201.230769	4.437904575	0.022909599	2	3.402826
Error	6496.205128	270.6752137			24	

S. aureus: *Staphylococcus aureus*, *S. epidermidis*: *Staphylococcus epidermidis*, *E. coli*: *Escherichia coli*, SS: Sum of squares, MS: Sum squares

Table 4: Comparison of gut microbiota in gestational weight

Microorganism	GWG	GWL	Difference	MD	(Difference – MD)2	t-test	95%	P
<i>S. aureus</i>	12	0	12		6.25			
<i>E. coli</i>	18	0	18		72.25			
<i>Pseudomonas</i>	6	4	2		56.25			
<i>Streptococcus</i>	10	2	8		2.25			
<i>Salmonella</i>	8	0	8		2.25			
<i>Yersinia</i>	6	0	6		12.25			
<i>Lactobacilli</i>	28	4	24		210.25			
<i>Actinobacter</i>	10	0	10		0.25			
<i>Enterobacter</i>	4	2	2		56.25			
<i>Bacilli</i>	6	0	6		12.25			
<i>S. epidermidis</i>	24	4	20		110.25			
<i>Citrobacter</i>	0	2	-2		132.25			
Total	132	18	114	9.5	673	3.45	0.1487-0.5154	0.005428

GWG: Gestational weight gain, GWL: Gestational weight loss, MD: Mean difference, *S. aureus*: *Staphylococcus aureus*, *S. epidermidis*: *Staphylococcus epidermidis*, *E. coli*: *Escherichia coli*

such as *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus*, combined with dietary counselling, has been found to improve glucose metabolism in healthy pregnant and lactating women. During the course of normal pregnancy, gut microbiota has been reported to remain relatively stable or change dramatically, with an increase in *Proteobacteria* and *Actinobacteria*, a decline in butyrate-producing bacteria, a reduction in bacterial richness and within-subject (α) diversity and higher between subject (β) at the end of pregnancy.^[16] The findings of the study carried out in China reported the gestational age associated with variation in the gut microbiota. The microbiota covariates are concentrated in basic host properties (e.g., age and residency status), suggesting that

individual heterogeneity is the major force shaping the gut microbiome during pregnancy.^[17]

Moreover, the findings reported on the identification of microbial and functional markers that are associated with age, pre-pregnancy body mass index, residency status and pre-pregnancy and gestational diseases. The gut microbiota during pregnancy is also different between women with high or low GWG. Four genera, (*Ruminococcus*), *Collinsella*, *Megamonas* and unclassified *Erysipelotrichaceae*, increased continuously with gestational age, whereas *Ruminococcus*, *Dialister* and unclassified *Lachnospiraceae* decreased continuously. Parallely, *Streptococcus*, *Megasphaera*,

Table 5: Association with gut bacteria derangement and Gestation weight

Microorganisms	Gestation weight gain	Unchanged gestation weight	Gestation weight loss	χ^2	95% CI	P
<i>S. aureus</i>	12 (10.2)	0 (0.3)	0 (1.4)	2.9	-0.0274-0.3716	0.367879
<i>E. coli</i>	18 (17.14)	2 (0.51)	0 (2.33)	7	0.0741-0.4611	0.030197
<i>Pseudomonas</i>	6 (8.6)	0 (0.25)	4 (1.16)	8	0.0941-0.4781	0.018316
<i>Streptococcus</i>	10 (10.28)	0 (0.31)	2 (1.4)	0.56	-0.1273-0.8785	0.75578
<i>Salmonella</i>	8 (6.8)	0 (0.2)	0 (0.93)	1.32	-0.0857-0.5379	0.516851
<i>Yersinia</i>	6 (5.14)	0 (0.15)	0 (0.7)	0.99	-0.3321-0.7449	0.609571
<i>Lactobacilli</i>	28 (29)	2 (0.88)	4 (3.9)	3.72	0.1749-0.5349	0.155673
<i>Actinobacter</i>	10 (8.5)	0 (0.25)	0 (1.16)	1.64	-0.1543-0.8181	0.440432
<i>Enterobacter</i>	4 (5)	0 (0.15)	2 (0.7)	2.81	0.0849-0.4663	0.24537
<i>Bacilli</i>	6 (5)	0 (0.15)	0 (0.7)	0.99	-0.3321-0.7449	0.609571
<i>Staph</i>	24 (24)	0 (0.72)	4 (3.2)	0.88	-0.3614-0.7297	0.644036
<i>Citrobacter</i>	0 (1.17)	0 (0.05)	2 (0.23)	15.38	0.7855-0.9014	0.00046
Total	132	4	18	46.19	0.9697-0.9868	0.001869

S. aureus: *Staphylococcus aureus*, *S. epidermidis*: *Staphylococcus epidermidis*, *E. coli*: *Escherichia coli*, CI: Confidence interval

unclassified-*Clostridiales* and *Bacteroides* seemed to be the most common taxa in mid-trimester. *Streptococcus* and *Megasphaera* were enriched at 21–28 weeks of pregnancy, unclassified-*Clostridiales* was enriched at 17–24 weeks and *Bacteroides* was reduced at 21–28 weeks.^[18] Dissimilar to this study in the first trimester from 0 to 13 weeks, *E. coli* and *Lactobacilli* are the most dominant bacteria in this group, where in the second trimester 14–27 weeks, *E. coli*, *lactobacilli* and *staphylococcus* are mostly high in this trimester, while *Lactobacilli* and *Enterobacter* are the highest in the third trimester from 28 to 36 weeks of gestation age. The comparison with gut microbiota dysbiosis and gestational weight was analysed. There was a significant difference in gut microbial community composition in gestational weight increase and decrease ($t = 3.45$, $P = 0.004$) [Table 4]. The current study analysed the association between gut microbiota alteration and gestational weight. There was a significant association between gut microbial community composition and gestational weight [Table 5]. The negative linear relationship between the maternal GWG and gut microbiota was reported. *Bacteroides* dominated the faecal microbiota profile ($P = 0.01$). In unadjusted multinomial models, higher GWG was associated with a decrease of a *Bacteroides*-dominant microbiota. Specifically, mothers who had a higher GWG were less likely to have a *Bacteroides*-dominant profile than an *Enterobacter*-dominant profile, corresponding to an unadjusted relative risk ratio of 0.83 (95% CI: 0.71–0.96; $P = 0.01$) per 1 kg increase in weight. Although this relative risk was attenuated slightly, the associations remained significant in adjusted models (all $P < 0.05$). The dynamic equilibrium of the gut microbiome is altered by environmental conditions and external interferences (e.g., antibiotics, hormone therapy and nutrition). These alterations can result in microbial imbalances or dysbiosis in the gastrointestinal tract.^[19] Normally commensal bacterial communities present in the

gut can act as opportunistic pathogens in immunosuppressed individuals. The condition of alteration may favour their competitiveness. Thus, changes in the gut microbiota can lead to intervals of increased susceptibility that negatively impact the ability of the community to resist pathogen colonisation^[20]

Figure 1 and Table 3 represent the spontaneous increase of gut microbiota changes during the first, second and third terms of pregnancy, respectively. Furthermore, the result observed in the study [Table 5] in trimesters means that the more the terms of pregnancy increase, the more microbiota changes, which lead them to gestational weight increase or decrease. The association with gut microbiota alteration and trimesters was analysed; there was a significant association with gut microbial community composition and trimesters. However, *Enterobacter* ($\chi^2 = 7.94$, $P = 0.01$), *S. epidermidis* ($\chi^2 = 6.38$, $P = 0.04$), *S. aureus* ($\chi^2 = 5.84$, $P = 0.05$) and the overall ($\chi^2 = 40.697$, $P = 0.008952$) were statistically significant.

According to Yang *et al.*, 2020, associations with host factors of the gut microbiota during pregnancy and strengthens the understanding of microbe-host interactions. The results from this study offer new materials and prospects for gut microbiome research in clinical and diagnostic fields. Alterations in the gut microbiota across pregnant stages have been of special concern. The results from this study offer new materials and prospects for gut microbiome research in clinical and diagnostic fields. Alterations in the gut microbiota across pregnant stages have been of special concern. The current study is in agreement with a previous longitudinal study that also demonstrated a relatively stable microbiota throughout gestation, while in challenge to another finding that the gut microbiota is dramatically altered during pregnancy. The results observed in this study also indicate that the higher the trimester of pregnancy, the more the gut microbiota becomes variant.

CONCLUSION

Gut bacteria variation contributes to the GWG amongst pregnant women. The gut bacterial variation was observed throughout pregnancy trimesters and was found independent for each trimester. This study recommends that stool testing for microbial analysis is a routine test, particularly for women underweight of stunted or excessive increase in weight during pregnancy. The study recommends that nutritional assessment and counselling during pregnancy consider the gut environment for optimum nutritional absorption during pregnancy.

Data sharing

All data are available and obtained through the corresponding author.

Acknowledgements

We acknowledge Muhoza health centre for samples availability and INES NRuhengeri for providing laboratory equipment for microbiological analysis.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, *et al.* Structure, function and diversity of the healthy human microbiome. *Nature* 2012;7402:207.
2. Hanning I, Diaz-Sanchez S. The functionality of the gastrointestinal microbiome in non-human animals. *Microbiome* 2015;3:51.
3. Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. *Gut* 2004;53:1-4.
4. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. *Microb Ecol Health Dis* 2015;26:26191.
5. Wang J, Zheng J, Shi W, Du N, Xu X, Zhang Y, *et al.* Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. *Gut* 2018;67:1614-25.
6. Abrams BA. Pregnancy weight gain: Still controversial. *Am J Clin Nutr* 2000;71:1233-41.
7. Zheng Z, Bennett WL, Mueller NT, Appel LJ, Wang X. Gestational weight gain and pregnancy complications in a high-risk, racially and ethnically diverse population. *J Womens Health (Larchmt)* 2019;28:375-83.
8. Million M, Lagier JC, Yahav D, Paul M. Gut bacterial microbiota and obesity. *Clin Microbiol Infect* 2013;4:305-13. doi: 10.1111/1469-0691.12172.
9. Nuriel-Ohayon M, Neuman H, Koren O. Microbial changes during pregnancy, birth, and infancy. *Front Microbiol* 2016;7:1031.
10. Chen C, Xu X, Yan Y. Estimated global overweight and obesity burden in pregnant women based on panel data model. *PLoS One* 2018;13:e0202183.
11. Brandtzaeg P, Isolauri E, Prescott SL. Microbial-host interaction: Tolerance versus allergy. In: Nestlé Nutr Inst Workshop Ser Pediatr Program. Vol. 64. Basel: Nestec Ltd., Vevey/S: Karger AG, 2009, 63-74. doi: 10.1159/000235783.
12. Johnson J, Clifton RG, Roberts JM, Myatt L, Hauth JC, Spong CY, Peaceman AM. Pregnancy outcomes with weight gain above or below the 2009 Institute of Medicine guidelines. *Obstet Gynecol* 2013;121:969. doi: 10.1097/AOG.0b013e31828aea03.
13. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr* 2008;88:894-9.
14. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, *et al.* Expert consensus document. The international scientific association for probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014;11:506-14.
15. Edwards SM, Cunningham SA, Dunlop AL, Corwin EJ. The maternal gut microbiome during pregnancy. *MCN Am J Matern Child Nurs* 2017;42:310-7.
16. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, *et al.* Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012;150:470-80.
17. Yang H, Guo R, Li S, Liang F, Tian C, Zhao X, *et al.* Systematic analysis of gut microbiota in pregnant women and its correlations with individual heterogeneity. *NPJ Biofilms Microbiomes* 2020;6:32.
18. Stanislawski MA, Dabelea D, Wagner BD, Sontag MK, Lozupone CA, Eggesbø M. Pre-pregnancy weight, gestational weight gain, and the gut microbiota of mothers and their infants. *Microbiome* 2017;5:113.
19. Robinson A, Fiechtner L, Roche B, Ajami NJ, Petrosino JF, Camargo CA Jr., *et al.* Association of maternal gestational weight gain with the infant fecal microbiota. *J Pediatr Gastroenterol Nutr* 2017;65:509-15.
20. Huang B, Fettweis JM, Brooks JP, Jefferson KK, Buck GA. The changing landscape of the vaginal microbiome. *Clin Lab Med* 2014;34:747-61.