Prevalence and Risk factors of Bacterial Vaginosis During Pregnancy: A Review

By

Fariha Mahmud Tithi

19326005

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Bachelors of Science in Microbiology

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing a degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Fariha Mahmud Tithi

Student ID: 19326005

Approval

The thesis titled "**Prevalence and Risk factors of Bacterial Vaginosis during pregnancy**" submitted by **Fariha Mahmud Tithi** (19326005) of Summer 2019 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Science in Microbiology on 19th December,2024.

Examining Committee:

Supervisor:

(Member)

Dr. Fahim Kabir Monjurul Haque

Associate Professor,

Department of Mathematics and Natural Sciences
(Microbiology Program),

Brac University

Program Coordinator:

(Member)

Dr. Nadia Sultana Deen

Associate Professor,

Department of Mathematics and Natural Sciences
(Microbiology Program),

Brac University

Departmental Head:

(Chair)

Dr. Md. Firoze H. Haque

Associate Professor and Chairperson

Department of Mathematics and Natural Sciences
Brac University

Abstract

Bacterial vaginosis is a polymicrobial condition marked by a reduction in *Lactobacilli* and an over proliferation of facultative and anaerobic bacteria in vaginal fluid. Despite receiving minimal attention, it has been linked to adverse pregnancy outcomes, including preterm labour and delivery, premature rupture of membranes, low birth weight, spontaneous abortion, and postpartum infections. Bacterial vaginosis (BV) is a prevalent vaginal infection defined by an imbalance in the vaginal microbiome, namely a decrease in Lactobacillus species and an increase in anaerobic bacteria. This syndrome is especially common during pregnancy, with an estimated prevalence of 10–30% among pregnant women globally, influenced by geographic, ethnic, and healthcare variables. Bacterial vaginosis, although frequently asymptomatic, can lead to vaginal discharge, malodorous emissions, and discomfort when symptoms are present. Importantly, even in asymptomatic instances, bacterial vaginosis presents considerable dangers to maternal and newborn health, such as premature labor, low birth weight, and postpartum infections. Demographic variables (e.g., age, race, socioeconomic level), behaviors (e.g., unprotected sexual activity, douching), and medical history influence its prevalence during pregnancy. Hormonal fluctuations and alterations in vaginal pH during pregnancy increase vulnerability. Diagnosis generally entails clinical evaluation utilizing Amsel criteria or Nugent scoring, while therapy predominantly consists of antibiotics such as metronidazole or clindamycin, taken judiciously due to possible pregnancy-related hazards. The recurrence of bacterial vaginosis is a problem, requiring ongoing surveillance and control in prenatal care. Future studies must concentrate on novel preventative techniques, enhanced comprehension of the vaginal microbiome-pregnancy correlation, and customized care methodologies to alleviate associated hazards. Thorough prenatal care, encompassing early BV screening, especially in high-risk groups, is essential for protecting mother and newborn health. The prevalence of bacterial vaginosis is significant. The World Health Organization guidelines advocate for the screening and treatment of symptomatic pregnant women. This recommendation should encompass all pregnant women diagnosed with HIV infection. Research is required to elucidate the biological mechanisms of bacterial vaginosis that contribute to preterm birth and low birthweight and explore antenatal interventions that may effectively disrupt these pathways.

Keywords: bacterial vaginosis, Pregnant woman, vaginal microflora, risk-factors and prevalence.

Dedication

This thesis is dedicated to my Creator and my Parents.

Acknowledgement

First and foremost, I would like to express my heartfelt gratitude to my thesis supervisor, Professor Dr. Fahim Kabir Monjurul Haque, Associate Professor of the Department of Mathematics and Natural Sciences (Microbiology Program) of Brac University, for his constant support, patience, motivation, enthusiasm, and vast knowledge throughout my thesis. Throughout the duration of this thesis, he has been a consistent source of inspiration for me. Throughout the thesis, he shared his valuable experience and time and monitored my daily progress. I feel very fortunate to have had the opportunity to learn and work under his guidance for the duration of the thesis.

I am thankful for my parents' unending love and support. From the bottom of my heart, I would like to thank all of my faculties, seniors and mates for their kind assistance, guidance and involvement.

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List of Acronyms

BV	Bacterial Vaginosis
STD	Sexually Transmitted Diseases
AGTI	Ascending Genital Tract Infection
HSV-1	Herpes simplex virus-1
HSV-2	Herpes simplex virus-2

Chapter 1

Introduction

An excessive growth of the normal vaginal flora causes Bacterial Vaginosis (BV), also known as bacterial vaginosis. Among women of reproductive age, bacterial vaginosis is the most common type of vaginal infection. An estimated 5 to 70 percent of women will encounter this infection at some point. High concentrations of anaerobic bacteria replace normal hydrogen peroxide and lactic acid-producing Lactobacillus species in the vagina, causing BV, a vaginal dysbiosis. These anaerobic bacteria include *Gardnerella vaginalis*, *Prevotella* species, *Mobiluncus* species, *A. vaginae*, and other bacteria that are associated with BV. Many studies have found that *G. vaginalis*, *Lactobacillus*, *Prevotella*, and the anaerobes *Mobiluncus* and *Bacteroides* are the germs that cause bacterial vaginosis. *Peptostreptococcus*, *Fusobacterium*, *Veillonella*, and *Eubacterium*. Various microorganisms linked to BV are *Streptococcus viridians*, *Atopobium vaginae*, and *Ureaplasma urealyticum*, which are of significant interest in microbiological studies. these microorganisms are of significant interest in microbiological studies (Kenyon et al., 2013; Redelinghuys et al., 2015).

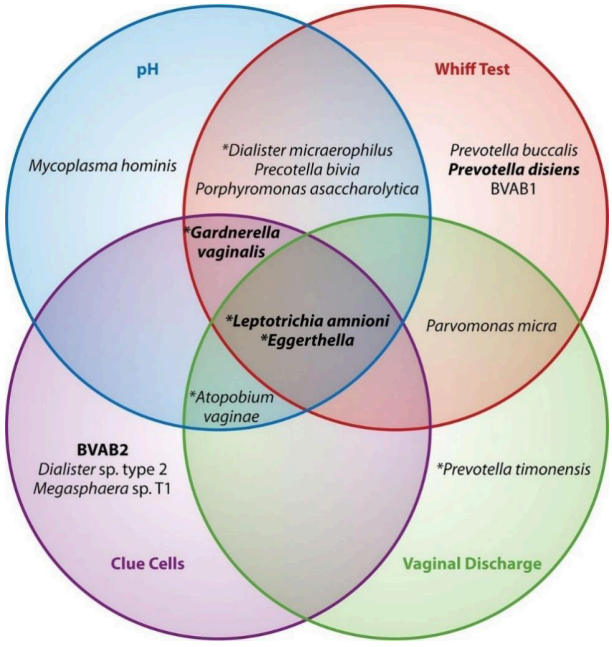

Figure 1: Relationship between Amsel's criteria and bacterial communities in women with bacterial vaginosis. (Redelinghuys et al., 2015)

Figure 1 illustrates the bacterial taxa associated with the four factors used to diagnose BV using Amsel's criteria. For example, *G. vaginalis* is associated with elevated pH, clue cells, and a positive whiff test. Asterisks represent bacteria in >75% of women with BV, and taxa in bold are associated with Amsel's criteria as a composite unit (Redelinghuys et al., 2015).

This condition, a reproductive tract disorder, causes one-third of all vaginal infections worldwide. Although it can happen at any age, it is statistically more common in reproductive-age women worldwide. Research indicates that approximately 5–10 million women seek gynecologic advice annually for vaginitis. The prevalence of bacterial vaginosis can range from 8 to 75%, with proportions significantly higher in some areas of Africa. Studies have repeatedly demonstrated that BV is a risk factor for adverse obstetric and gynecological outcomes, with pregnant women being the most frequently affected (van den Munckhof et al., 2019). These outcomes include preterm labour and delivery, premature rupture of membranes, and low birth weight, as well as spontaneous abortion, postpartum infections such as endometritis, and wound infections that occur after cesarean section (Bagnall & Rizzolo, 2017; Hay, 2000).

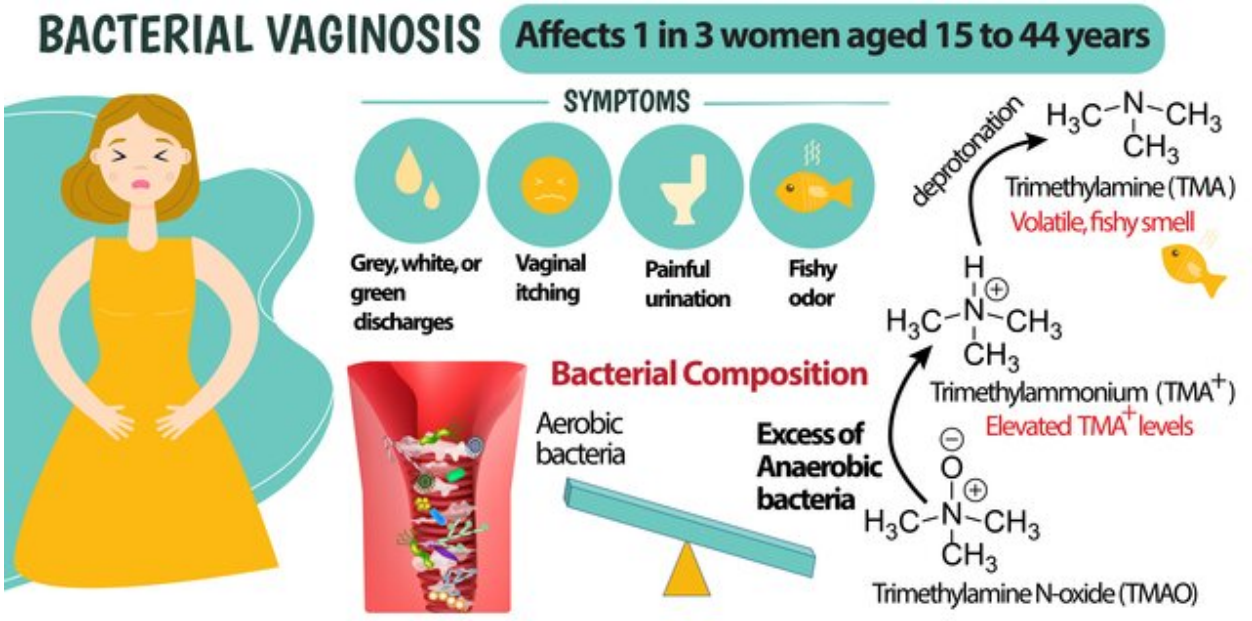

Figure 2: Symptoms of bacterial vaginosis. (Banks et al, 2023)

In terms of the factors that are associated with bacterial vaginosis, researchers have reported that marital status, residence, multiparity, a history of abortion, the frequency of douching, and having multiple sexual partners are all associated with the condition. Despite this, the understanding of the factors associated with bacterial vaginosis remains limited due to conflicting reports. Some studies found an association between bacterial vaginosis and certain socio-demographic, behavioural, and clinical characteristics of pregnant women. Another study found no association with some of these factors. There is still a significant prevalence of bacterial vaginosis among pregnant women. Early detection is essential for timely treatment and prevention of related complications and poor pregnancy outcomes. This is because most cases remain asymptomatic; early detection is essential. Consequently, the screening of pregnant women who are symptomatic or asymptomatic through the use of vaginal swabs is an essential factor. (Bagnall & Rizzolo, 2017)

There is a correlation between having multiple male sex partners, having multiple female partners, having sexual relationships with more than one person, having a new sex partner, not using condoms, not douching, and having HSV-2 seropositivity. Male circumcision reduces the risk of BV among women. Sexual contact can transmit *Gardnerella vaginalis* from one woman to another, either through direct contact with mucus membranes or through the use of shared sex toys. Despite the association of bacterial vaginosis with a wide range of health issues, it is crucial to evaluate the predisposing risk factors commonly associated with it. Consequently, the objective of the present study was to determine the risk factors associated with bacterial vaginosis and the treatment outcome in cases of BV infections. (Redelinghuys et al., 2015)

For a considerable amount of time, bacterial vaginosis has received minimal attention. In recent years, however, the association of bacterial vaginosis with ascending genital tract infection (AGTI) on the one hand and sexually transmitted diseases (STDs) on the other hand has made the infection a major global problem. Due to their increased STD risk, women with bacterial vaginosis Furthermore, studies have shown that bacterial vaginosis triggers viral replication and vaginal shedding of the HSV-1 and HSV-2 (herpes simplex virus-2) viruses, thereby contributing to their growth and dissemination. Bacterial vaginosis, in general, has become a problem for public health because it is associated with sexually transmitted infections. According to several

studies (Bagnall & Rizzolo, 2017; Bautista et al., 2016; Kenyon et al., 2013), bacterial vaginosis has become a significant public health concern.

Chapter 2

2. Research Methodology

2.1 Search Strategy

This study uses several databases, such as Google Scholar, PubMed, and ScienceDirect, to find the relevant scientific literature. During the search, several keywords were utilized, including *lactobacillus, Streptococcus viridians*, prevalence, pregnancy, risk factors, and BV. The utilization of the Boolean operators "AND," "OR," and "NOT" to ensure the search result remained specific. Original research and review articles with sufficient citations to retrieve information that is relevant to the question. (Downes et al., 2016)

2.2 Inclusion Criteria

This study looked at original literature about the prevalence of BV in pregnant women, what factors raise the risk of BV during pregnancy, and how lactobacillus and anaerobe species are spread among pregnant women. The collection also included a summary of the literature discussing the symptoms and complications of BV during pregnancy. (Kenyon et al., 2013)

2.3 Exclusion Criteria

Literature solely detailed on the prevalence of vulvovaginal candidiasis, *Candida vaginitis*, the antibiotic resistance of *Candida spp.*, and the diagnosis and treatment of BV were not considered. Furthermore, the exclusion criteria also involved papers that merely reported the prevalence of BV among women who were not pregnant or of reproductive age (Bradshaw et al., 2006; Kenyon et al., 2013)

Chapter 3

3. Prevalence of bacterial vaginosis during pregnancy.

3.1 Higher Prevalence of BV during pregnancy

There is a higher prevalence of BV during pregnancy. Numerous studies have conducted a comparative analysis between pregnant women and non-pregnant women. These studies found that pregnant women have a higher BV rate than non-pregnant women. Researchers found that the overall prevalence of BV was 20.5% (163), with comparable percentages in both fertile and pregnant women and a lower percentage (12.7%) in women who had reached menopause. We found *Gardnerella vaginalis* in 235 (29.6%) of the 793 women, 144 (88.3%) of the 163 women with BV, and 91 (14.4%) of the 630 women without BV. The study revealed the presence of *Mobiluncus* species in 8.2% (65) of the total population, 38.6% of BV-affected women, and only two (0.3%) non-BV-affected women.

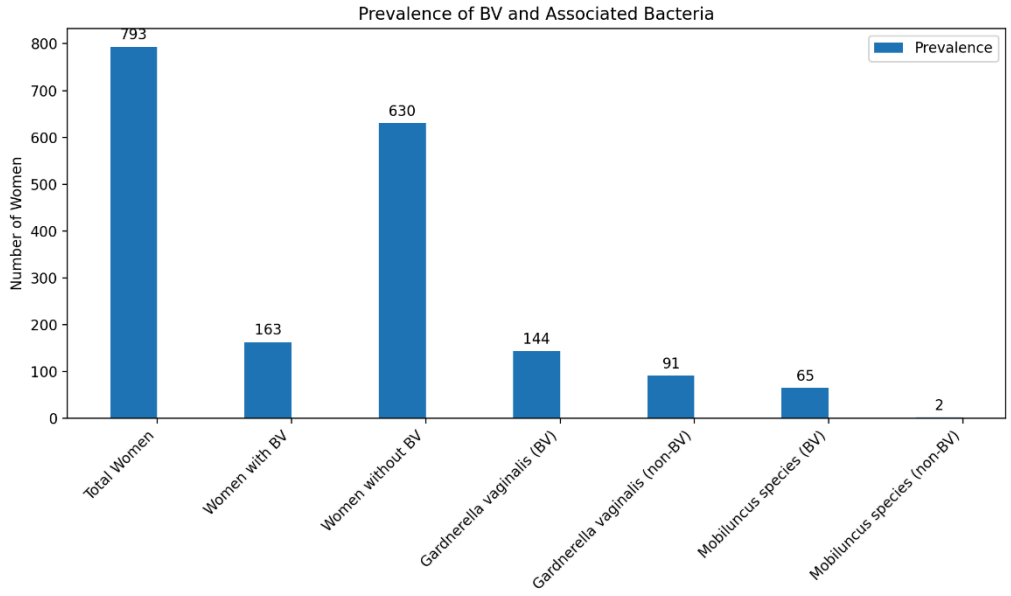

Figure 3:Prevalence of BV and Associated Bacteria

3.2 Region-wise total prevalence of BV among pregnant women

The disparity in frequency across different regions emphasizes how severe bacterial vaginosis (BV) is a global issue. African pregnant women have a higher rate of bacterial vaginosis. According to Kamga et al. (2019), in the context of BV in Africa, the cumulative incidence in Southern Africa is approximately 38.7%, with specific nations such as Zambia reporting rates as

high as 48.3%. The frequency of BV varies significantly, ranging from 7.3% in Burkina Faso to 60% in Nigeria. Socioeconomic status and healthcare access influence this variation (Kamga et al., 2019; Afolabi et al., 2016). The wide range of BV rates in East Africa, spanning from 0% in Uganda to 52% among pregnant HIV-positive women in Kenya, underscores the notable variance in this region (Farquhar et al., 2011; Marx et al., 2011). A study by Kamga et al. (2019) shows the prevalence of BV in Cameroon was 26.2%, with higher rates observed in rural areas than urban areas. Moreover, this disparity results from variations in healthcare accessibility and sanitary standards (Kamga et al., 2019). Variations in BV prevalence worldwide demonstrate the intricate interactions between social, regional, and health-related factors (Bertini, 2017).

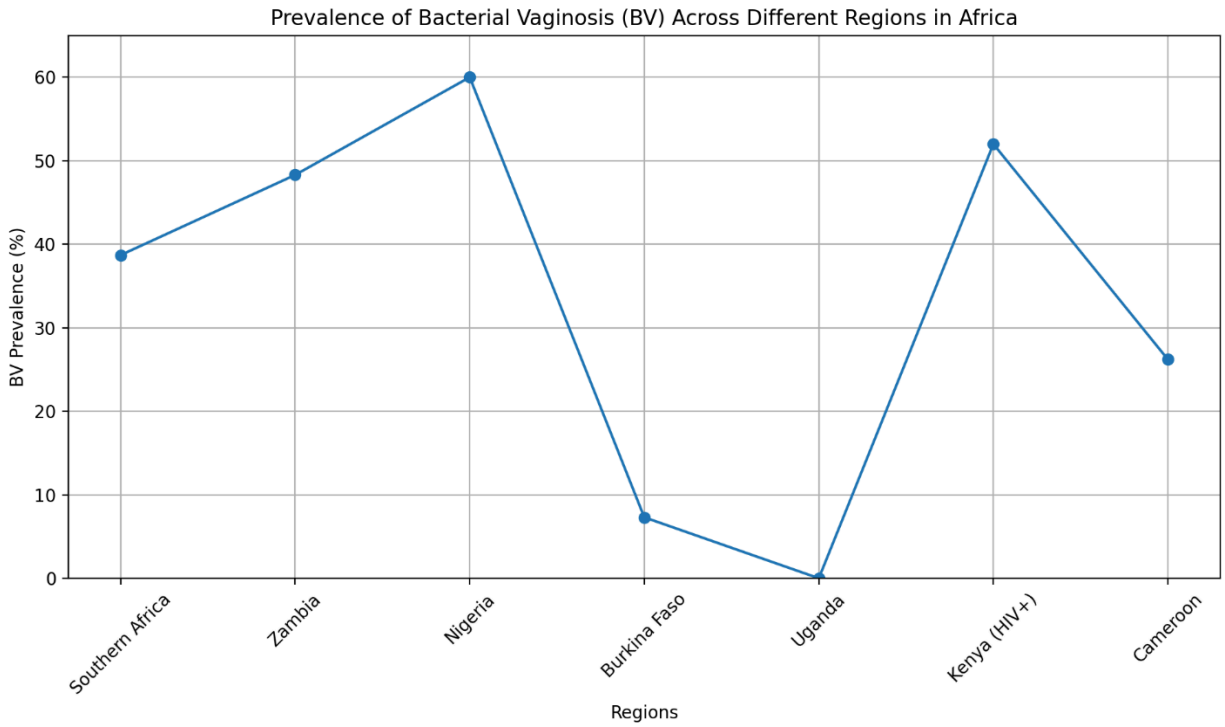

Figure 4:Prevalence of BV across different regions in Africa

3.3 The presence of symptoms determines the prevalence of BV.

Candida infections, particularly *Candida albicans*, often accompany BV in pregnant women. Research shows a wide range of Candida prevalence in pregnant women, with combined *Candida* and BV infections seen in about 9.1% of Cameroonian women (Kamga et al., 2019). Although anaerobic bacteria like *Gardnerella vaginalis* are the leading cause of BV, Candida species commonly co-occur, making diagnosis and treatment more difficult (Sobel et al., 2013).

According to the research, only 1% of women in Cameroon test positive for Trichomonas vaginalis, indicating that the incidence of co-infections with BV is still low (Kamga et al., 2019). When diagnosing and treating vaginal infections in pregnant women, it is crucial to take into account both bacterial and fungal pathogens. This is because co-infections may make clinical management of BV more difficult and result in unfavorable pregnancy outcomes (Sobel et al., 2013).

3.4 Prevalence and Diversity of Bacterial Species in Pregnant Women with Candida Infections

Pregnant women commonly detect Candida infections, particularly Candida albicans, which often coexist with BV. Studies indicate that the prevalence of Candida among pregnant women ranges widely, with mixed infections involving Candida and BV detected in approximately 9.1% of women in Cameroon (Kamga et al., 2019). Anaerobic bacteria like Gardnerella vaginalis predominantly cause BV, but the co-occurrence of Candida species often complicates diagnosis and treatment (Sobel et al., 2013). Studies have also shown that Trichomonas vaginalis co-infections with BV are still not very common. For example, only 1% of women in Cameroon tested positive for T. vaginalis (Kamga et al., 2019). The mixed infections of BV and Candida show how important it is to look at both bacterial and fungal pathogens when diagnosing and treating vaginal infections in pregnant women. This is because co-infections can make it harder to treat BV and can have bad effects on the pregnancy (Sobel et al., 2013).

3.5 Age group-wise distribution of BV

Younger pregnant women are more vulnerable to BV, and age is a major factor in its occurrence. The prevalence of BV is highest among women in their late teens and early twenties, according to several studies. According to a Cameroonian research study, women between the ages of 18 and 22 had the highest frequency, at 29.2% (Kamga et al., 2019). Sexual activity and potentially a poorly developed vaginal microbiome make this age group especially susceptible (Ibrahim et al., 2014). Studies conducted in other areas, however, have suggested that older age groups specifically, women between the ages of 30 and 40 may also have a greater incidence of BV, possibly as a result of cumulative exposure to vaginal infections over an extended period (Ranjit et al., 2018). Even if the frequency varies by age group worldwide, younger women are still

more vulnerable to BV because of their sexual behavior and lack of access to healthcare, which highlights the need for focused education and preventative measures (Mengistie et al., 2014).

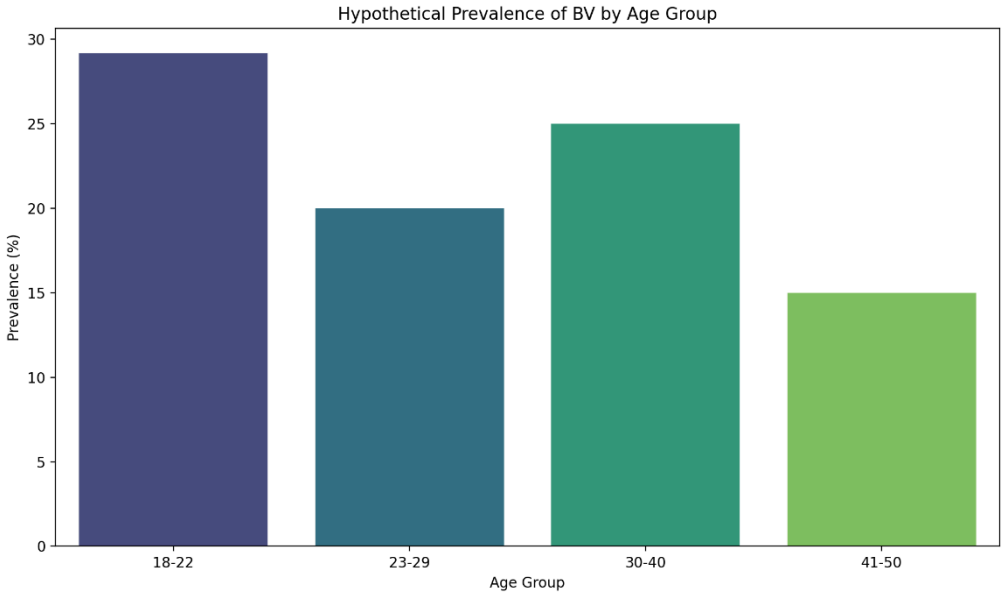

Figure 5:Prevalence of BV by age group.

3.6 Gestational period-wise distribution of BV

There are notable differences in the prevalence of BV during various stages of pregnancy. The second trimester had the most significant BV prevalence in Cameroonian research, at 31.7%, which was much higher than the first (20.7%) and third (18.0%) trimesters (Kamga et al., 2019). This finding fits with findings from around the world that show how changes in hormones during pregnancy, significantly higher levels of progesterone and estrogen, change the vaginal microbiota and make it more likely for BV to happen (Farquhar et al., 2011). Given that the second trimester is a crucial time in pregnancy, the increased incidence of BV at this time raises questions regarding possible associations with problems, including low birth weight and preterm birth (Desseauve et al., 2012). Regular monitoring at this time is therefore essential to avoiding negative results and guaranteeing the mother's and the fetus's health.

3.7 Socioeconomic level, educational status, parity-wise distribution of BV

Parity, educational attainment, and socioeconomic position are all strongly associated with the frequency of BV in expectant mothers. In Cameroon, rural women were more likely to have BV

(29.5%) than their urban counterparts (24.5%), most likely as a result of less access to healthcare and worse hygiene habits (Kamga et al., 2019). Education level also matters; women with no formal education had the most significant frequency of BV; however, this difference was not statistically significant. Parity, or the number of births, is another significant component; At the same time, the prevalence of BV was higher among primigravida women (30.9%), it was lower among multigravida women, possibly due to improved access to healthcare and hygiene education (Kamga et al., 2019; Afolabi et al., 2016). Furthermore, research demonstrated that vaginal douching posed a significant risk factor for BV. According to Kamga et al. (2019), women who engaged in douching had a significantly greater prevalence of BV (33.5%) than those who did not. To lessen the burden of BV and the hazards that go along with it, our findings emphasize the urgent need for better health education on hygiene and reproductive health, especially in rural and disadvantaged regions.

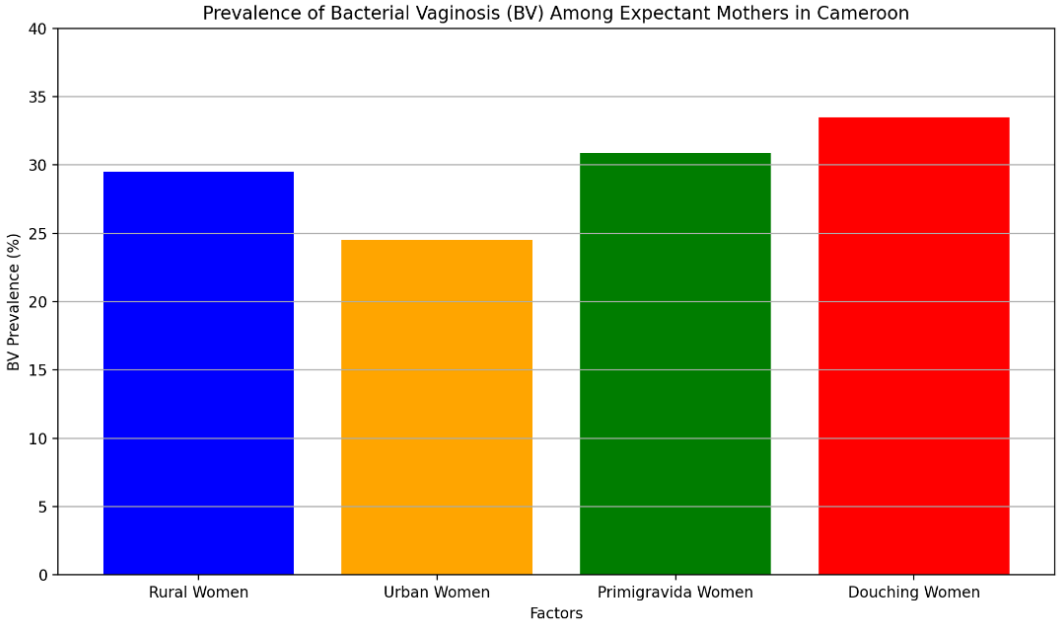

Figure 6:Prevalence of BV among expectant mothers in Cameroon.

Chapter 4

4. Risk factors for BV during pregnancy

Pregnancy causes the vagina to become more sensitive, thereby increasing the frequency of infections. Pregnancy's identification as a significant risk factor stems from this. Researchers have found a positive correlation between the number of BV cases and the role that pregnancy plays. There are several factors, including clinical, behavioral, and demographical aspects, that influence the manifestation of symptomatic BV during pregnancy. BV has been associated with several host-related factors. These include genetic predisposition, diabetes that is not under control, behavioral factors (such as the use of antibiotics or contraceptives), and conditions that have high levels of reproductive hormones during pregnancy.

4.1 Pregnancy-related factors

Different pieces of writing have talked about how a woman's body changes during pregnancy, including her immune system getting weaker, her levels of reproductive hormones going up, her vaginal pH going down, and her cell-mediated immunity getting lower. Researchers have linked these factors to an increased risk of bacterial vaginosis (BV).

4.1.1 Weakened immune system

Pregnant women are more susceptible to infections due to their compromised immune systems. Excessive stress could potentially be the cause, as previously described. During pregnancy, a woman experiences an increase in emotional stress, which leads to the suppression of the immune system. By weakening the immune system, pathogenic bacteria like *G. vaginalis*, *Prevotella* species, *Mobiluncus* species, and *A. vaginae* can grow more quickly (Esber et al., 2015; Redelinghuys et al., 2015)

4.1.2 Increased level of reproductive hormones

As the pregnancy progresses, the levels of hormones undergo a significant transformation and reach a level that is significantly higher than at any other time. Researchers discovered that an increased secretion of sex hormones, specifically progesterone and estrogen, during pregnancy is

associated with an increased risk of infection. Researchers have discovered that a high level of progesterone alters the vaginal epithelium, thereby increasing the number of bacteria present in the vagina. In addition, progesterone has the ability to inhibit the antibacterial activity of neutrophils. An increased estrogen level can disrupt the normal equilibrium of microorganisms, thereby increasing the risk of a bacterial vaginal infection. Researchers have discovered that yeast is able to more easily adhere to vaginal mucosal epithelial cells when there is a high level of estrogen present. Another thing that estrogen does is help cells grow, multiply, make hyphal structures, and make enzymes like secreted aspartyl proteinase and phospholipases, which both help colonies grow.

Furthermore, research reveals that a high level of estrogen can reduce the quantity of immunoglobulins found in vaginal secretions. Furthermore, research reveals that a high level of estrogen can diminish the capacity of epithelial cells to suppress bacterial growth, potentially leading to a higher risk of pregnancy complications. (Jespers et al., 2015; Rosca et al., 2020)

4.1.3 High amount of glycogen deposition

Both progesterone and estrogen increase vaginal glycogen. On the vaginal wall, the presence of a high level of glycogen deposition provides a beneficial source of carbon, which in turn encourages the growth and germination of a variety of bacteria. This could potentially contribute to the increased susceptibility of pregnant women to BV by creating an environment conducive to the growth of bacteria (Bradshaw, et al., 2006; Coudray & Madhivanan, 2020) support this theory.

4.1.4: Decreased pH level

Normal vaginal pH ranges from 4.0 to 4.5, providing an acidic environment that inhibits the growth of numerous vaginal pathogens. According to Yadav and Prakash (2016), any physiological change that affects both beneficial and harmful vaginal microorganisms will alter the acidity of the vagina, bringing its pH down to 5.0-6.5. This will, in turn, increase the likelihood that pathogenic organisms like *G. vaginalis* will establish themselves in the vagina. Research demonstrates that an elevated progesterone level during pregnancy can lower the pH of the vaginal environment, thereby fostering the growth of bacteria. (Bagnall & Rizzolo, 2017; Kenyon et al., 2013)

4.1.5 Decreased cell-mediated immunity

The immunologic changes that occur during pregnancy may contribute to the altered severity of infectious diseases and the increased susceptibility to contracting them. The relationship between reproductive hormones and the immune system is more complicated and involves several factors. Cell-mediated immunity plays a significant role during pregnancy in explaining altered infection responses. Evidence suggests that estrogen-rich states, like those in the final trimester of pregnancy, suppress cell-mediated immunity, according to REED (1992). Progesterone suppresses the mother's immune response and alters the balance between Th1 and Th2 responses. Researchers have discovered that high estradiol concentrations can enhance CD4+ type 2 helper T-cell (Th2) responses and humoral immunity. Estradiol levels in the maternal serum can increase by as much as 500 times as the pregnancy progresses. Additionally, during pregnancy, there is an increase in the levels of cytokines, which stimulate the recruitment or activity of phagocytic cells. During pregnancy, a recent theory presented the idea of a transition from Th1 immunity to Th2 immunity. There are several ways that Th2 cells lower the effectiveness of cell-mediated immunity. These include activating B lymphocytes, making more antibodies, and stopping the cytotoxic T lymphocyte response. Evidence suggests that pregnancy increases certain aspects of innate immunity, including phagocytic activity, defensin levels, and the numbers of monocytes, neutrophils, and dendritic cells, especially during the second and third trimesters. On the other hand, the number of CD3+ T lymphocytes in blood, including both CD4+ and CD8+ lymphocytes, decreases during pregnancy. This drop in CD4+ cells, CD8+ cells, T-cells, and natural killer cells, along with their activity, may affect the body's ability to fight bacteria while a woman is pregnant. It may also make it take longer for the microorganism causing the problem to be killed. (Mohanty et al., 2023; Svare et al., 2006)

4.1.6 Gestation period

Several studies have established a connection between the trimester of pregnancy and the susceptibility of pregnant women to contracting BV. According to Okonkwo and Umeanaeto (2011), the susceptibility of a pregnant woman to infection increases as the pregnancy progresses; consequently, the third trimester of pregnancy is when the prevalence is at its highest (Okonkwo & Umeanaeto, 2011).

Nelson et al. (2013) have demonstrated that an elevated level of estrogen and corticoids during the third trimester of pregnancy reduces the vaginal defense mechanism against opportunistic fungus (Nelson et al., 2013). Additionally, repeated vaginal and pelvic examinations, along with a reduction in hygiene status due to fatigue or the size of the pregnant mother's stomach, may contribute to the development of vaginal infections and increase the risk of bacterial vaginosis during the final trimester of pregnancy. Guzel et al. (2011) found a correlation between the prevalence and the number of weeks of gestation, aligning with previously mentioned factors (Guzel et al., 2011). On the other hand, Masri et al. (2015) discovered that pregnant women in their first and second trimesters had a higher risk of getting venous catheterization, which is a contradictory finding (Masri et al., 2015).

Multiple studies found that the third trimester of pregnancy was statistically insignificant, despite the fact that it was associated with a higher incidence of BV. In their studies, Waikhom et al. (2020) and Yadav & Prakash (2016) excluded pregnant women who had any complications, such as diabetes, a history of preterm labor, or who were taking antibiotics (Waikhom et al., 2020; Yadav & Prakash, 2016). This could potentially explain their findings of no correlation between BV and the gestational period. Therefore, the role of the gestational period, particularly the final trimester, as a risk factor for BV during pregnancy is still a contentious issue. According to (Muzny & Schwebke, 2020; Waters et al., 2008)

4.2 Clinical factors

Numerous studies have identified diabetes mellitus, HIV infection, and prior bacterial contact as potential contributors to the vaginal colonization that occurs during pregnancy.

4.2.1 Diabetes mellitus

Chronic diabetes that is not under control is a risk factor for bacterial vaginosis. Patients diagnosed with clinical diabetes mellitus significantly increase their risk of bacterial infections of the skin and vagina. When someone has diabetes mellitus, the amount of glucose in their vaginal secretions increases. This makes it easier for Candida to stick to epithelial cells, which in turn helps Candida grow and make more virulence factors. When hyperglycemia is present, neutrophils are unable to phagocytose and eliminate pathogens as effectively as they would otherwise. Additionally, hyperglycemia has the ability to stimulate the production of a protein in

bacteria, which not only makes it easier for bacteria to adhere to the host but also prevents the host from phagocytosing the bacteria. Consequently, because diabetes promotes the growth of bacteria, pregnant women with the disease may have a higher risk of contracting BV. Masri et al. (2015) found a statistically significant association between diabetes and the rate of BV during pregnancy (Masri et al., 2015).

4.2.2 HIV infection

Immunocompromised women are generally at a higher risk of bacterial infections. Previous studies have demonstrated that a loss of immune-protective mechanisms leads to increased vaginal colonization with bacteria. Bacteria strongly correlates with a diminished cell-mediated immune response in immunocompromised patients. One of the most important contributors to the development of BV is the presence of predisposing host factors, such as HIV infection and other immunosuppressive diseases. Moreover, proteinase activity plays a significant role in the pathogenesis of BV, which is why HIV-positive women are more likely to be susceptible to it. HIV-positive women exhibit increased proteinase activity. However, several studies have found that there is no statistically significant correlation between HIV infection and BV. On the other hand, HIV-positive pregnant women have a risk of BV that is more than twofold higher than the HIV-negative control group (Foessleitner et al., 2021). The fact that severely immunocompromised patients, in particular, are more likely to develop BV, could potentially explain the finding of an insignificant relationship. (Atashili et al., 2008; Esber et al., 2015).

4.2.3 Past episodes of vaginosis

Some researchers believe pregnant women with a history of candidiasis are more likely to develop BV. Possible hormonal environment and suppressed immune system cause increased susceptibility. During pregnancy, a significant number of women who suffer from chronic recurrent vaginosis become infected for the first time. Research reported that 60% of pregnant women who tested positive for vaginosis also had a history of candidiasis, and this finding was statistically significant (Kanagal, 2014). On the other hand, discovered that patients who had a history of vaginosis in the past were statistically insignificant about the occurrence of BV. As a result, it is challenging to assert that having experience with candidiasis in the past is a reliable

risk factor for developing BV. This is supported by studies conducted by Bradshaw in 2006 (Morton, et al., 2006) and (Bradshaw, et al., 2006).

4.3 Behavioral factors

Many behavioral characteristics typically associated with pregnant women may influence the rate at which bacteria grow during pregnancy. The use of antibiotics, oral contraceptives, and intrauterine devices; wearing clothing that is too tight; engaging in behaviors that involve poor personal hygiene and douching; and engaging in poor dietary habits have all been evaluated as potential risk factors for bacterial vaginosis (BV) during pregnancy in several studies.

4.3.1 Frequent use of oral contraceptives

Regular use of contraceptives, particularly hormonal contraceptives, can significantly alter the vaginal microbiome. These contraceptives can disrupt the hormonal equilibrium in the body, potentially disrupting the natural balance of beneficial bacteria in the vaginal environment. This disruption may create an environment that is favorable to the overgrowth of harmful bacteria, which will, in turn, increase the risk of bacterial vaginosis (BV). In 2019, the United Nations published a report estimating that approximately 407 million women globally utilized hormonal contraception. Twenty-seven per cent of these women used intrauterine devices, sixteen per cent used oral contraceptive pills (OCP), eight percent used injectable contraceptives, and two percent used Given the altered equilibrium between estrogen and the vaginal microbiota, it is likely that hormonal contraception could also influence changes in the CSTs present in the vagina and the colonization by Lactobacillus (Bradshaw, Morton, et al., 2006).

Research suggests that both oral contraceptives (OC) and condoms are effective in protecting against bacterial vaginosis (BV). However, we observe a less pronounced protective effect of condoms. The presence of OCs in vaginal epithelial cells may lead to an increase in glycogen levels, which in turn encourages the growth of lactobacilli, thereby inhibiting the growth of bacteria associated with BV. Condom use may be beneficial as it prevents changes in the vaginal flora that favor anaerobic bacteria associated with bacterial vaginosis. Additionally, it appears that women who have multiple sexual partners or use intrauterine devices (IUDs) are at a higher risk of contracting bacterial vaginosis. Cherpes, T. L., and Marrazzo found that the use of hormonal contraceptives affected the inflammatory response in women diagnosed with BV. Results of the study showed that women who used hormonal birth control had much lower levels

of several pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF), interferon-gamma (IFN-γ), and GM-CSF, compared to women who did not use hormonal birth control. On the other hand, hormonal contraceptives did not result in a significant reduction in the levels of IL-10, which is a cytokine that reduces inflammation. This suggests that hormonal contraceptives might alter the immune response of the genital tract by reducing pro-inflammatory molecules. This could potentially explain the commonly observed reduction in inflammatory symptoms in women with BV who are using contraceptives. (Bradshaw, et al., 2006; Seña et al., 2021)

4.3.2: Prolonged use of antibiotics

The use of antibiotics for an extended period of time can cause an imbalance in the vaginal flora by eliminating beneficial bacteria. This can lead to an overgrowth of harmful bacteria, which can then lead to the development of bacterial vaginosis (BV). Based on research findings, prolonged use of antibiotics is a significant risk factor for the development of BV (Rosca et al., 2020; Waters et al., 2008).

People who are not pregnant can receive a prescription for the antibiotic tinidazole. Pregnant women have not been the subject of any investigation. Pregnant women should avoid tinidazole. Medical professionals frequently prefer vaginal cream over pills for people who are breastfeeding, whenever possible. This is because oral medication does not achieve the same level of distribution throughout the body and breast milk as it does. Research on the role of prolonged antibiotic use in treating bacterial vaginosis (BV) during pregnancy has been extensive. This analysis sheds light on several important aspects concerning the efficacy and implications of such treatment (Coudray et al., 2020).

i. Effectiveness of Antibiotic Treatment:

Research has demonstrated that antibiotics are an effective means of eradicating BV in pregnant women. Research has demonstrated that antibiotic regimens administered orally or intravenously can achieve elimination rates ranging from 70 to 89 percent. When compared to placebo treatments, the spontaneous resolution rates that resulted from these treatments ranged from 14% to 38%.

ii. Variety of Treatment Regimens:

The treatment of BV has involved the use of a variety of antibiotic regimens. These include oral clindamycin and combinations of oral erythromycin and metronidazole. Since the results of the various studies differ, the meta-analysis does not recommend specific antibiotic combinations.

iii. Impact on Preterm Delivery Rates:

One of the most important discoveries made by the research was that prolonged use of antibiotics does not significantly reduce the rates of preterm deliveries, particularly in patients who are considered to be at low risk. Antibiotic treatment for bacterial vaginosis does not appear to result in a reduction in the number of preterm births among this population, according to the findings of subgroup analyses that have been consistent.

iv. Considerations for High-Risk Patients:

It is still unclear whether or not there are any potential benefits for high-risk patients, despite the fact that there may be some. The majority of studies found no significant reduction in the number of preterm deliveries or maternal infections attributed to antibiotic treatment.

v. Heterogeneity in Study Results:

According to the analysis, there was a significant amount of heterogeneity in the findings, particularly among the studies that involved patients with low risk and those that used vaginal antibiotic regimens. This suggests that patient characteristics and treatment methods can significantly influence the effectiveness of prolonged antibiotic use.

In conclusion, although antibiotics are a common treatment for tuberculosis (TB) during pregnancy, their use for an extended period of time does not always result in improved outcomes, particularly with regard to the rates of preterm delivery. The findings highlight the importance of conducting additional research to clarify the role that prolonged antibiotic treatment plays in the management of postpartum bacterial infections (BV) during pregnancy.

This in-depth analysis sheds light on the complexities that are associated with the use of antibiotics for the treatment of bacterial vaginosis in pregnant women, highlighting the importance of designing individualized treatment plans based on the specific circumstances of each individual patient.

4.3.3 Use of Intrauterine Device (IUD)

The insertion of an intrauterine device (IUD) has the potential to bring about changes in the vaginal microbiome, which may result in the development of bacterial vaginosis (BV). Studies have shown that intrauterine devices (IUDs) have the potential to change the environment of the vaginal area, making it more prone to the growth of bacteria. (Rosca et al., 2020)

If you have irregular vaginal bleeding during the first six months of using an intrauterine device (IUD), you may have a higher chance of getting bacterial vaginosis (BV). Researchers looked into the link between IUD use and BV. Despite the initial observation of an association, adjustments for confounding variables revealed that this relationship was not statistically significant. (P. E. Hay et al., 1994)

We highlighted the role of irregular bleeding as a potential factor because it can alter the pH of the vaginal sac or lower the concentration of lactobacillus, which can foster the growth of bacterial vaginosis (BV). Researchers also found that women who started using contraceptives with intermediate vaginal flora were more susceptible to BV. This may be because microbiota fluctuations occur prior to the onset of bacterial vaginosis.

Researchers who used a longitudinal methodology were able to gain insights into the timing and conditions under which BV may occur in individuals who use intrauterine devices (IUDs), particularly during the initial use of the device. (Rosca et al., 2020)

4.3.4 Tight and Synthetic Clothing

Wearing tight and synthetic clothing can create an environment that is warm and moist, conducive to the growth of harmful bacteria. This can result in an increased risk of bacterial vaginosis (BV). Research has demonstrated that the selection of clothing can influence the vaginal microbiome and contribute to the development of BV. (Coudray & Madhivanan, 2020)

4.3.5 Dietary Habits

Poor eating habits, such as consuming a diet that is deficient in essential nutrients, can have a detrimental effect on the immune system, making the body more vulnerable to infections, including bacterial vaginosis (BV). Several studies have demonstrated that a healthy diet is absolutely necessary in order to keep the vaginal microbiome in a state of equilibrium. Bacterial vaginosis (BV) is a common vaginal infection that affects women of reproductive age. It is

characterized by an imbalance in vaginal flora, which includes a decrease in lactobacilli and an increase in anaerobic bacteria. Several unfavorable outcomes, such as HIV acquisition, premature birth, and genital infections, are associated with the presence of BV. Despite the fact that the precise cause of BV is still unknown, some of the factors that contribute to the disease include vaginal douching, smoking, stress, and race. Research indicates that deficiencies in immune function and nutritional status, including micronutrients such as vitamin A, iron, and zinc, may have an impact on the risk of bacterial vaginosis (BV) by reducing the strength of the local immune system. Few studies have explored the relationship between dietary intake and BV, specifically focusing on energy, macronutrient, and micronutrient consumption. Dietary fat intake, particularly saturated fat, significantly increases the risk of bacterial vaginosis (BV) due to its potential to increase vaginal pH and alter vaginal microflora. It is also possible that increased fat consumption has an effect on mucosal immunity due to the role it plays in modulating the lymphoid tissue associated with the gut. On the other hand, higher intakes of folate, vitamin E, and calcium were associated with a lower risk of severe BV. This may be due to the fact that these nutrients play a role in enhancing immune functions. Despite the slight correlation between energy intake and BV, high fat consumption appears to confound the effect of energy intake. The fact that there were no significant associations with the consumption of carbohydrates or proteins draws attention to the specific role that fats play. Given the limitations of the sample in terms of socioeconomic status and dietary diversity, it is important to avoid making broad generalizations, despite the fact that the findings highlight the influence of diet on BV. The study found that a higher consumption of phytochemicals in the diet was associated with a lower risk of bacterial vaginosis (BV), even after accounting for potential confounding factors. Previous research suggests that nutrients such as vitamin D and probiotics may improve the outcomes of BV, despite the fact that there is a limited amount of research on the relationship between diet and BV. The antimicrobial properties of phytochemicals, in particular flavonoids and phenolic acids, are well-known, and they may be able to assist in providing protection against different BV pathogens. This study is the first of its kind to investigate the relationship between dietary phytochemicals and BV by employing the phytochemical index, which provides an alternative to in vitro methods that is more cost-effective. (Coudray & Madhivanan, 2020)

4.3.6 Douching habits and female hygiene products

The use of fragranced feminine hygiene products and regular douching can upset the natural balance of bacteria in the vagina, leading to the development of bacterial vaginosis (BV). Numerous studies have demonstrated that douching significantly increases the risk of bacterial vaginosis (BV).

Vaginal douching and bacterial vaginosis (BV) have a strong correlation, but the cause-and-effect relationship between the two entities remains incompletely understood. If bacterial vaginosis symptoms were to cause changes in hygienic behaviors, then one would anticipate a higher prevalence of all hygiene practices among women who have these symptoms.

Woodman et al. conducted a study in which they investigated the connection between vaginal douching and bacterial vaginosis (BV). The findings demonstrated a consistent association between the two, but they did not establish a causal relationship. In spite of the fact that douching is associated with BV in a dose-response manner, the research discovered that other hygienic behaviors, such as alterations in the type of pants worn or the utilization of menstrual protection, did not have a strong correlation with BV. Given this information, it appears that the observed connection between douching and BV is not likely the result of women douching as a result of symptoms associated with BV. (Brotman et al., 2008)

The research casts doubt on the hypothesis that women douche in response to BV symptoms. It highlights the fact that although douching is associated with a higher prevalence of BV, other hygienic practices, such as behaviors that do not involve douching, do not show similar patterns. This discovery contributes to the clarification of the potential causal factors involved in BV and highlights the necessity of conducting additional targeted research in order to comprehend the role that hygienic practices play in the existence of the condition. (Brotman et al., 2008, 2008)

Chapter 5

Virulence Factors of Bacteria that Cause Bacterial Vaginosis in Women

A study proposed a conceptual model to explain the pathogenesis of BV. The majority of *G. vaginalis* isolates obtained from BV cases exhibited a more significant number of virulence factors compared to non-BV cases. Some of these factors were sticking to vaginal epithelial cells, making biofilm, making phospholipase C and protease, making the surface hydrophobic, and hemagglutination. These factors have been documented in 2006 and again in 2020 by a group of researchers. (Bradshaw, et al., 2006); (Rosca et al., 2020).

The study collected 176 different isolates of *G. vaginalis* from 811 different women who had abnormal vaginal discharge. Nugent's established criteria divided the vaginal samples into three distinct categories. Amsel's criteria led to the formation of Group A, consisting of seventy-five women with a Nugent score ranging from four to six, a score inconsistent with BV. However, these were not considered cases of BV. These findings are supported by studies conducted by Bilardi in 2016 (Bilardi et al., 2016). Group B included 89 women with a Nugent score of seven or higher, and Amsel reported a case of BV. We determined that these were cases of BV. Group C included twelve women with a Nugent score of 0-3, which Amsel classified as non-BV cases. These women had other vaginal infections, including vulvovaginitis (n=6), cervicitis (n=4), and HIV reactive (n=2). These cases were also considered to be non-bodily fluid cases. We diagnosed 23 women with Candida in group A, 49 in group B, and eight in group C. We made all comparisons between groups A (non-BV) and B (BV), considering that group C only contains 12 women. Table I presents the factors of virulence expressed by the three groups.

Table 1: Virulence factors expressed by *Gardnerella vaginalis* isolated from the genital tract of women with and without bacterial vaginosis (BV)

Virulence factors	**Number of isolates tested**	**Number tested in each group**	**Results**	**Group A n (%)**	**Group B n (%)**	**Group C n (%)**	***P* (A vs. B)**	**Reference**
Adherence to vaginal epithelial cells	141	A – 60	Good	15 (25)	60 (83.3)	5 (55.5)	<0.001	(Rosca et al., 2020; Bradshaw et al.,2006)
		B – 72						
		C - 9	Poor	45 (75)	12 (16.6)	4 (44.4)		
Biofilm formation	133	A – 49	Moderate or good	9 (18.4)	58 (78.4)	6 (60)	<0.001	(Rosca et al., 2020; Bradshaw et al.,2006)
		B – 74						
		C - 10	Poor	40 (81.6)	16 (21.6)	4 (40)		
Phospholipase C production	145	A – 60	Positive	21 (35)	63 (82.9)	5 (55.5)	<0.001	(Bradshaw et al.,2006; Hay, 2000)
		B – 76						
		C - 9	Negative	39 (65)	13 (17.1)	4 (44.4)		
Protease production	145	A – 60	Positive	22 (36.6)	51 (67.1)	3 (33.3)	<0.01	(Bradshaw et al., 2006)
		B – 76						
		C - 9	Negative	38 (3.33)	25 (32.9)	6 (66.6)		
Surface hydrophobicity	145	A – 60	High	25 (41.7)	58 (77.3)	5 (50.0)	<0.01	(Hay, 2000; Rosca et al., 2020)
		B – 75						
		C - 10	Low	35 (58.3)	17 (22.67)	5 (50.0)		

Haemagglutination with human RBC	141	A – 59	Positive	6 (10.1)	45 (61.64)	4 (44.4)	<0.001	(Bradshaw et al., 2006)
		B – 73						
		C - 9	Negative	53 (89.8)	28 (38.35)	5 (55.5)		
Haemagglutination with sheep RBC	141	A – 59	Positive	9 (15.2)	34 (46.5)	2 (22.2)	<0.01	(Bradshaw et al., 2006; Hay, 2000)
		B – 73						
		C - 9	Negative	50 (84.7)	39 (53.4)	7 (77.7)		
Haemagglutination with chick RBC	141	A – 59	Positive	3 (5.1)	27 (36.9)	-	<0.001	(Rosca et al., 2020)
		B – 73						
		C - 9	Negative	57 (94.9)	46 (63.0)	9 (100)		

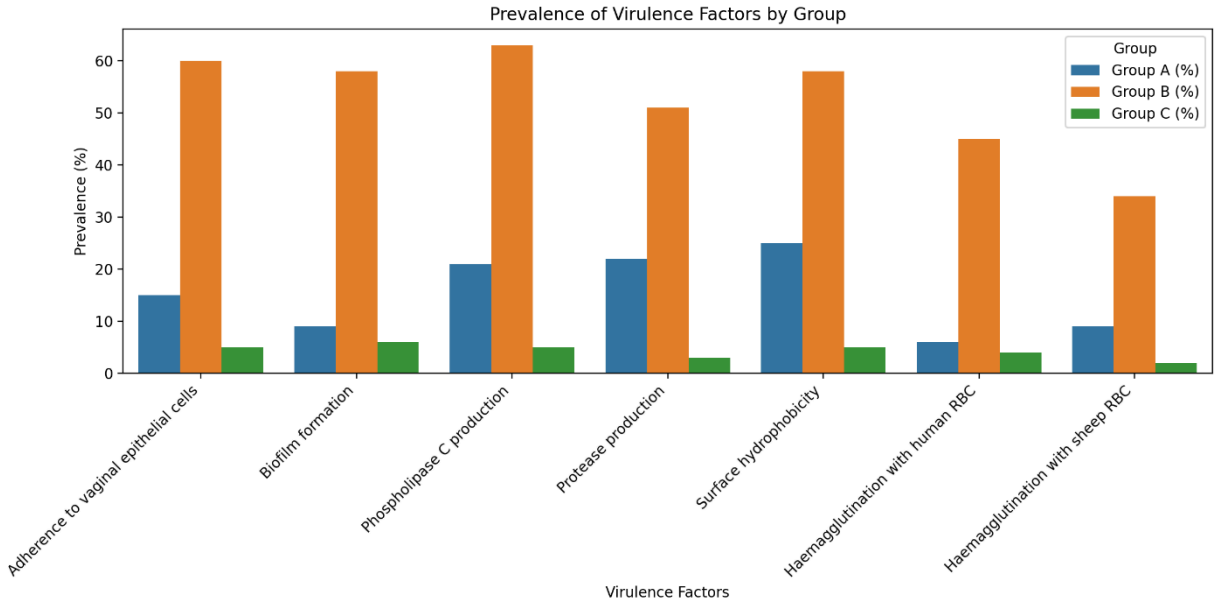

Figure 7:Prevalence of Virulence factors by groups

Some reports say that BV and non-BV isolates of *G. vaginalis* have different genotypes and different levels of virulence factors when they are grown in the lab. Previous studies have reported these differences. Compared to the isolate from a healthy vagina, the BV-associated isolate showed higher levels of adherence, aggregation, and biofilm formation. Additionally, the BV-associated isolate encoded a different variant of a bone marrow-associated protein gene. In our study, we discovered that BV-associated isolates were better at sticking to surfaces, making biofilms, hemagglutination, phospholipase production, and protease production than non-BV isolates. (P. Hay, 2000) Our research revealed that biotypes 6, 2, and 3 accounted for the majority of isolates. As shown in Table II, biotype 3 was more closely associated with non-BV cases than with BV cases. On the other hand, biotypes 6, 2, and 1 were more closely associated with BV cases (Bradshaw, Morton, et al., 2006). We found that the biotypes 6, 2, and 1 expressed the highest levels of virulence factors (Table 1). Some reports say that BV and non-BV isolates of *G. vaginalis* have different genotypes and different levels of virulence factors when they are grown in the lab. Previous studies have reported these differences. Compared to the isolate from a healthy vagina, the BV-associated isolate showed higher levels of adherence, aggregation, and biofilm formation. Additionally, the BV-associated isolate encoded a different variant of a bone marrow-associated protein gene (Muzny & Schwebke, 2020). In our study, we discovered that BV-associated isolates were better at sticking to surfaces, making biofilms, hemagglutination, phospholipase production, and protease production than non-BV isolates. (Hay, 2000) Our research revealed that biotypes 6, 2, and 3 accounted for most isolates. As shown in Table II, biotype 3 was more closely associated with non-BV cases than with BV cases. On the other hand, biotypes 6, 2, and 1 were more closely associated with BV cases (Morton, et al., 2006). Table 3 revealed that the biotypes 6, 2, and 1 expressed the highest levels of virulence factors.

Table 2: Different biotypes of *G. vaginalis* isolated from the genital tract of women with and without BV, n=143 (Nisha et al., 2019)

Biotypes	Group A	Group B	Group C	Total
	n (%)	n (%)	n (%)	
1	3 (2)	12 (8)*	1 (0.6)	16 (11.1)
2	8 (5.5)	22 (15.3)*	2 (1.3)	32 (22.3)*
3	16 (11.1)*	3 (2)	3 (2)	22 (15.3)*
4	8 (5.5)	3 (2)	1 (0.6)	12 (8.3)
5	1 (0.6)	6 (4.1)	0	7 (4.8)
6	8 (5.5)	23 (16)*	2 (1.3)	33 (23)*
7	4 (2.7)	1 (0.6)	0	5 (3.5)
8	10 (7)	6 (4.1)	0	16 (11.1)
Total				**143**

Table 3: Association of 143 biotyped isolates with virulence factors of *G. vaginalis* isolated from the genital tract of women with and without BV (Nisha et al., 2019)

Biotypes	Good adherence n (%)	Biofilm producers n (%)	Good surface hydrophobicity n (%)	Agglutination of human RBC n (%)	Phospholipase producers n (%)	Protease producers n (%)
1	11 (7.6)	7 (4.8)	13 (9)	6 (4.1)	16 (11.1)	8 (5.5)
2	20 (13.9)	17 (11.8)	19 (13.2)	10 (6.9)	32 (22.3)	21 (14.6)
3	7 (4.8)	4 (2.7)	4 (2.7)	5 (3.5)	0	7 (4.8)
4	7 (4.8)	1 (0.6)	7 (4.8)	5 (3.5)	0	5 (3.5)
5	5 (3.5)	4 (2.7)	5 (3.5)	4 (2.7)	7 (4.8)	4 (2.7)
6	17 (11.8)	19 (13.2)	20 (13.9)	18 (12.5)	33 (23)	18 (12.5)
7	1 (0.6)	1 (0.6)	0	0	0	1 (0.6)
8	8 (5.5)	4 (2.7)	10 (6.9)	3 (2)	0	6 (4.1)
P	0.084	0.033	0.003	0.139	<0.001	0.446

Phospholipase-producing biotypes 1, 2, 3, and 4 were frequently associated with cases of BV rather than non-BV, according to the findings of another study. Furthermore, the patient acquired a different biotype as a result of treatment. In a study that looked at the different biotypes of *G. vaginalis* over time, biotypes 2, 3, and 7 were found to be more frequently isolated from cases of BV, while biotype seven was found to be more isolated from cases that did not involve BV. In our research, we found that biotypes 6, 2, and 1 were associated with several different virulence factors (Svare et al., 2006).

To summarise, most *G. vaginalis* isolates obtained from cases of BV exhibited a more significant number of virulence factors than those obtained from healthy women. Compared to biotypes 6, 2, and 1, which were associated with cases of BV and expressed the highest levels of virulence factors, biotype 3 was found to be more prevalent in cases that did not involve BV (Jayaram et al., 2020).

Chapter 6

Conclusion

Bacterial vaginosis (BV) is a common disease in pregnancy, considerably affecting maternal and newborn health. The prevalence of this condition, impacting approximately 10–30% of pregnancies, highlights the necessity for thorough attention and intervention. Bacterial vaginosis (BV) is linked to a disruption in the vaginal microbiome, marked by less Lactobacillus species and an elevation of anaerobic bacteria, potentially resulting in negative pregnancy outcomes, including premature labor, low birth weight, and postpartum infections. Risk factors for bacterial vaginosis during pregnancy encompass demographic traits such as younger age, African American ethnicity, and poorer socioeconomic level, in addition to behavioral ones like unprotected sexual intercourse and douching practices. Medical disorders, a history of bacterial vaginosis or sexually transmitted infections, and hormonal fluctuations during pregnancy increase vulnerability to bacterial vaginosis. Although curable, BV often recurs, underscoring the necessity for ongoing screening and management throughout pregnancy. Diagnosis generally entails clinical criteria such as Amsel's and Nugent scores, while treatment with antibiotics like metronidazole or clindamycin is efficacious but necessitates careful administration due to possible pregnancy-related concerns, especially during the first trimester. Managing bacterial vaginosis during pregnancy necessitates a comprehensive strategy. Timely identification via regular prenatal screening, particularly in high-risk groups, is essential. Preventive interventions must prioritize education regarding modifiable risk factors, including the avoidance of douching and the adoption of safe sexual practices. Furthermore, continued study is essential to deepen comprehension of the complex interplay between the vaginal microbiome and pregnancy, formulate more effective preventative and treatment strategies, and diminish the recurrence of bacterial vaginosis. Incorporating thorough BV screening and therapy into routine prenatal care can substantially reduce the dangers linked to this illness, enhancing outcomes for both mothers and their infants. A joint endeavor among healthcare professionals, researchers, and public health efforts is crucial to mitigate the global burden of bacterial vaginosis and promote healthier pregnancies.

Chapter 7

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