Bacterial identification of the vaginal micro biota and presence of pathogens causing bacterial vaginosis during pregnancy in Bangladeshi women

By

Md Mahmud Hasan

18326032

Mashyat Sultana

18126076

Tasnova Tabassum Khan

18326019

A thesis submitted to the Department of Mathematics and Natural Science

in partial fulfilment of the requirements for the degree of Bachelor of Science in Microbiology

Department of Mathematics and Natural Science

BRAC University

February 2022

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing 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/We have acknowledged all main sources of help.

Student's Full Name & Signature:

Md Mahmud Hasan

Mashyat Sultana

Tasnova Tabassum Khan

Approval

The thesis titled "Bacterial identification of the vaginal micro biota and presence of pathogens causing bacterial vaginosis during pregnancy in Bangladeshi women" submitted by Md Mahmud Hasan (18326032), Mashyat Sultana (18126076), Tasnova Tabassum Khan (18326019) of Spring, 2018 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Science in Microbiology on 8th February 2022.

Examining Committee:

Supervisor:

1. Dr. Fahim Kabir Monjurul Haque
   Assistant professor, Microbiology program,
   Department of Mathematics and Natural Sciences. Brac University
2. Dr. Munira Ferdousi
   Professor and head of Department of Obstetrics and Gynecology,
   Shaheed Suhrawardy Medical College and Hospital

Co-supervisor:

1. Akash Ahmed
   Assistant professor, Microbiology program,
   Department of Mathematics and Natural Sciences. Brac University
2. Dr. Jobaida Sultana
   Associate Professor of Department of Obstetrics and Gynecology,
   Shaheed Suhrawardy Medical College and Hospital

Departmental Head:

(Chair) Prof. A. F. M Yusuf Haider

Chairperson, Mathematics and Natural Science. Brac University

Ethics Statement

This study has been conducted with vaginal swab samples of pregnant women from the Department of Obstetrics and Gynecology, Shaheed Suhrawardy Medical College and Hospital and the consent was taken in agreement to use the samples for thesis purpose.

Abstract/ Executive Summary

Bacterial vaginosis is a one-of-a-kind upheaval of the complex vaginal bacterial flora with the absence of *lactobacilli* and the expansion of *Gardnerella vaginalis, Atopobium vaginae, prevotella bivia*, and resident anaerobic vaginal bacteria. Vaginosis is linked to preterm labor, early delivery, and preterm premature rupture of the membranes, amniotic fluid infection, postpartum endometritis, and post cesarean wound infections, as well as spontaneous abortion during pregnancy, according to recent studies. According to a recent study, 10-41 percent of women get bacterial vaginosis, which has been linked to maternal and fetal morbidity. Sociodemographic variables, previous health history, previous UTI and BV record, vaginal discharge, and weight have all been linked to variations in bacterial vaginosis prevalence rates. In this study, vaginal swab samples were taken from 50 patients and cultured it on HBT, Blood agar and MRS. The majority of the patients in the study (n=50) were young adults between the ages of 25 and 30, who had the highest average colony count in both HBT and Blood agar. In addition, patients from metropolitan regions had a higher risk of contracting bacterial vaginosis. Furthermore, 11 out of 50 patients had a prior UTI, and these patients had a higher average colony count of 135.54 and 28.63 for HBT and Blood agar, respectively, than those who did not have a prior UTI, but a very low average colony count of 37 for *Lactobacillus* species, which is lower than 52.70 for patients without a prior UTI, indicating that patients with a prior UTI have a higher risk of bacterial vaginosis. In addition, the majority of patients with vaginal discharge have higher average colony growth in HBT and Blood agar but lower average colony growth on MRS than patients without vaginal discharge, according to our findings. In conclusion, patients with higher *Gardnerella vaginalis and Atopobium vaginae* culture growth have lower growth for lactobacillus spp in our entire study is an indicator of bacterial vaginosis is consistent.

Acknowledgment

We would like to start with thanking the Almighty for keeping us in good health and giving us the strength to push through and finish writing this paper which was the most challenging part of our undergrad life.

Our heartfelt gratitude goes to Professor A F M Yusuf Haider, Chairperson, MNS Department, and MNS Department for maintaining the department regulations and delivering proper education to every student including myself.

We would like to convey my deepest gratitude to Fahim Haque, Ph.D. Assistant Professor, Mathematics and Natural Sciences, BRAC University and Akash Ahmed Assistant Professor, Mathematics and Natural Sciences, BRAC University for checking up our work progress from time to time and keeping us on track. We would like to convey our sincere gratitude to Dr. Munira Ferdousi Professor and head of Department of Obstetrics and Gynecology, Shaheed Suhrawardy Medical College and Hospital and

Dr. Jobaida Sultana, Associate Professor of Department of Obstetrics and Gynecology, Shaheed Suhrawardy Medical College and Hospital for taking us under their wings and allowing us to use their resources to complete our research.

We would also like to thank, Md Mahmudul Hasan, Lab Officer and Mrs. Asma Binte Afzal, Senior Lab Officer, BRAC University Lab for mentoring us throughout this time. My heartiest gratitude goes to Ms. Nadira for helping us in lab.

We are also thankful to Samia Rashid, Antara ridha, Anindita Farzanafor for their company during our period in the lab.

Finally, we would like to talk about our parents for whom gratitude is not the right word to express my feelings. From paying the tuition to making sure I was healthy and strong during my time at BRAC University and in ShsMC, they did every possible thing they could. It would not have been possible for us to finish this degree without their contribution.

Md Mahmud Hasan

18326032

Mashyat Sultana

18126076

Tasnova Tabassum Khan

18326019

Table of Contents

List of Figures

List of Acronyms

BV……. Bacterial Vaginosis

HBT……... Human Blood Tween

BA……. Blood Agar

MRS…….. De Man, Rogosa and Sharpe agar

BHI…… Brain Heart Infusion

DNA…….. Deoxyribonucleic acid

RNA…….. Ribonucleic acid

et al……'et alia' meaning 'and others'

Gardnerella vaginalis

Atopobium Vaginae

Prevotella bivia

Lactobacillus species

RT-PCR……Reverse transcriptase-polymerase chain reaction.

MRC…… Medical Research Council

ERC……..Ethical Review Committee

P-value……..Estimated probability

-Introduction-

1.0: Introduction

Bacterial vaginosis is the most common cause of vaginal discharge in women of reproductive age (BV). Although Increased vaginal discharge is usually related with pregnancy, which is generally non-pathological, however abnormal vaginal discharge is related with bacterial vaginosis, candidiasis, and trichomoniasis. (Ibrahim et al. 2014)

In recent decades, Bacterial vaginosis has become a global health concern as a result of its negative effects on pregnancy and the postpartum period (Ibrahim et al. 2014)**.** Before 1950 it was considered as non-specific vaginitis. Even today, though BV has become a well-known medical condition, mystery remains as it does not follow as it is characterized by an overgrowth of anaerobic bacteria and mycoplasmas, which include *Gardnerella vaginalis*, *Mobiluncus*, *Bacteroides spp*, *Mycoplasma hominis*, *Atopobium vaginae*, *Prevotella bivia* that replace the normal vaginal *Lactobacilli*, and does not follow Koch's postulate that a single pathogen is responsible for a specific disease(Leitich et al. 2003) It has made serious concern as recent studies show that vaginosis is related to preterm labor, early delivery, preterm premature rupture of the membranes, amniotic fluid infection, postpartum endometritis and postcesarean wound infections, spontaneous abortion during pregnancy. From a recent study it has seen that 10-41 percent women develop bacterial vaginosis and it has a correlation with maternal and fetal morbidity. (McGregor 2000)

Even after considering for other significant risk factors, the relative risk of preterm birth in women with BV was determined to be >2-fold in a large research (Goldenberg et al. 1998) A study shows that BV in the first trimester of pregnancy may be a greater risk factor for preterm birth than BV later in the pregnancy. (Hay et al. 1994),(Kurki et al. 1992)

1.1: Background of the study: Bangladesh is a developing country and most of its population are not privileged enough to avail health services properly. In contrast to this, most of the people are not educated and aware of many diseases that they are carrying. Specially diseases related to reproductive system. Developing countries like Bangladesh it is a taboo to talk about diseases related to reproductive system. Thus, they are acquiring such diseases and facing complications without knowing the root cause. Bacterial vaginosis is one of the diseases related to reproductive system. During the months of May to December 2000, a cross-sectional research was done among

pregnant women attending an urban maternity and childcare delivery facility in Dhaka, Bangladesh, reported that among 284 pregnant women, 17.7 % had bacterial vaginosis. (Begum et al. 2003) This disease should be taken under consideration and needs proper research as it is related to preterm birth and increased susceptibility to the human immunodeficiency virus (HIV) and sexually transmitted infections (STI). (Eschenbach et al. 1988)**.**

As bacterial vaginosis can have serious impacts on pregnant women so it is high time to screen their vaginal flora and how it is related to bacterial vaginosis and preterm delivery which will definitely contribute in order to establish effective treatment for this. Thus, complications related to bacterial vaginosis can be reduced.

1.2: Objective of the study

1.2.1: General objectives:

The goal of our research project is to identify the different species of *lactobacillus* usually present in pregnant women. However, we will mainly focus on to detect the *Gardnerella vaginalis, Atopobium vaginae* and *prevotella* species are of particular importance in the etiology of bacterial vaginosis which can lead to preterm birth and other complications during child delivery.

1.2.2: Specific objectives

Percentage of pregnant women in Bangladesh suffering from bacterial vaginosis during pregnancy.

To identify the bacterial species which mainly causing the bacterial vaginosis during pregnancy in Bangladeshi women.

To find out the species of *Lactobacillus* present in vaginal swab and also their amount of presence, as these species maintain a healthy vaginal state in pregnant women.

To find out socio-demographic characteristics of the respondent.

To find out the relationship between socio-economic characteristics and bacterial vaginosis in Bangladeshi pregnant women.

1.3: literature review

1.3.1: Bacterial vaginosis:

Bacterial vaginosis (BV) is defined by a change in the vaginal flora from the generally dominating lactobacillus to a mixed flora that produces sialidase enzymes, such as *Gardnerella vaginalis Mobiluncus, Prevotella, Bacteroides* and Mycoplasma species. Bacterial vaginosis was once thought to be a completely harmless illness. However, current research has linked bacterial vaginosis to a number of obstetric and gynecological illnesses and disorders, including spontaneous miscarriage, preterm labor, premature membrane rupture, placental infection, wound infection, and pelvic inflammatory disease (PID) (Guerra et al. 2006)

1.3.2: Organisms related to bacterial vaginosis:

The ecology of the normal vaginal microbiota is affected by BV, which is characterized by a reduction in the prevalence and concentration of Lactobacillus species and an increase in the prevalence and concentration of numerous pathogenic bacteria, primarily anaerobes. (Machado and Cerca 2015)

These pathogens are mostly *Gardnerella vaginalis*, *Atopobium vaginae*, *Prevotella bivia*.

1.3.2.1: *Lactobacilli*:

Lactobacillus (genus Lactobacillus) is a gram-positive, non-spore-forming bacterium belonging to the *Lactobacillaceae* family. *Lactobacillus*, like other genera in the family, is distinguished by its capacity to produce lactic acid as a by-product of glucose metabolism. *Lactobacillus* species are utilized in the commercial production of sour milks, cheeses, and yogurt, as well as in the production of fermented vegetables (pickles and sauerkraut), drinks (wine and juices), sourdough breads, and certain sausages.

Lactobacillus is a nonmotile bacteria that can exist in both aerobic and anaerobic conditions. The type species of the genus, *L. delbrueckii*, is 0.5 to 0.8 micrometer wide by 2 to 9 m long and can

be found alone or in small chains. *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus casei*, and *Lactobacillus sanfranciscensis* are some more well-known *Lactobacillus* species.

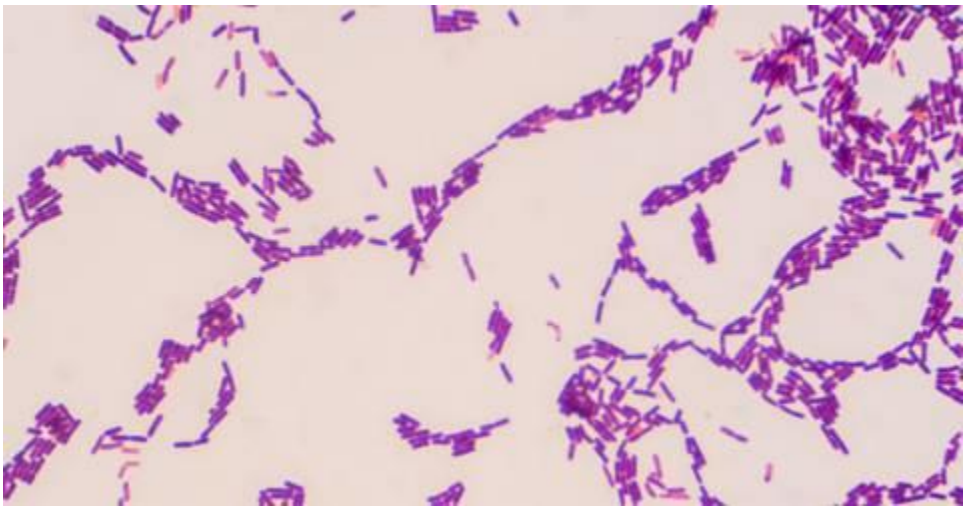


Fig 1.1: *Lactobacillus* after gram staining

Various *Lactobacillus* organisms create different amounts of lactic acid. Several species, including *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus plantarum*, have homofermentative glucose metabolism because lactic acid is the major output, accounting for at least 85 percent of end metabolic products. Other species, including *L. brevis* and *L. fermentum*, have heterofermentative glucose metabolism, with lactic acid accounting for roughly half of metabolic byproducts and ethanol, acetic acid, and carbon dioxide accounting for the other half. Other heterofermentative *Lactobacillus* species are poor in their glucose metabolism and must rely on other organic molecules for energy, such as galactose, malate, or fructose. (Britannica et al. 2018) *Lactobacillus* is found in the gastrointestinal systems of both animals and humans, as well as the mouth and vagina. Moreover, *Lactobacillus* species contribute to a healthy human vagina, and they perform an important role in preventing women from genital infection. In a healthy vagina, facultative lactobacilli predominate. *Lactobacilli* are assumed to be important for keeping the vaginal pH acidic by metabolizing the glucose produced by glycogen metabolism (Stewart-Tull 1964).Some organisms may be directly inhibited by low pH. The oxidation-reduction potential rise 60 mV for every 1.0-U fall in pH. (Holmes et al. 1985) As a result, by maintaining a greater Eh and a less reduced environment, low pH may also restrict anaerobic microbes. A lack of *Lactobacilli* can disrupt the vaginal microbial balance, leading to the bacterial vaginosis (BV)

syndrome(Spiegel et al. 1980) In a recent investigation, the species of vaginal lactobacilli were identified (Eschenbach et al. 1989). *Lactobacilli* that produce hydrogen peroxide were found in 27 (96 percent) of 28 healthy women and 4 (6 percent) of 67 women with BV. *Lactobacillus acidophilus, L. jensenii, and L. catenaformis,* which produce hydrogen peroxide, were discovered alone or in combination with other *Lactobacillus sp*. in 16 (76%) of 21 healthy women and 3 (5%) of 66 women with BV whose *Lactobacilli* were identified to the species level.

Hence, bacterial vaginosis is defined by a total loss of *lactobacilli* and a simultaneous rise in Gram-variable and Gram-negative rods, the most common of which are Gardnerella vaginalis, Bacteroides, Prevotella, and Mobiluncus species(Spiegel et al. 1980). Preterm delivery (PTD) and low birth weight are linked to the existence of an aberrant vaginal microbiota in early pregnancy.

With that said, Women who have a normal vaginal microbiota in the first trimester have a 75% reduced chance of giving birth before 35 weeks of pregnancy than women who have an abnormal vaginal microbiota (Donders et al. 2009)**.** In the first trimester, however, the lack of *Lactobacilli* and the development of BV or aerobic vaginitis were linked to an increased risk of PTD between 25 and 35 weeks (Donders et al. 2009) Ensuring the natural, healthy balance of the *Lactobacillus* microbiota in the vagina is especially crucial during pregnancy(Reid and Bocking 2003)**.** Since vaginal infection is a major cause of premature birth (Goldenberg et al. 1998). *Lactobacilli* produce lactic acid, hydrogen peroxide, and bacteriocins such acidolin, lactacin B, and lactocin 160, which defend against genital pathogens.

1.3.2.2: *Gardnerella vaginalis:*

Gardnerella vaginalis is hard to classify in terms of microbiologic classification and clinical significance. It is relatively unique to maintain its own genus based on 16S rRNA sequence analysis, yet it is quite related to Bifidobacterium species, which are anaerobic gram-positive rods. (VAN ESBROECK et al. 1996) Since, it has a thin cell wall it cannot retain crystal violet/iodin complex, as a result it is hard to determine whether it is gram positive or gram negative. The latest study indicates that *G. vaginalis* has a gram-positive ancestor. Normally Gardnerella vaginalis is found in human vagina. The optimal temperature for its growth is 350°C, which is facilitated by

carbon dioxide. It is indole, nitrate, and urease negative. On its surface, Pili have been identified. (Boustouller, Johnson, and Taylor-Robinson 1987)

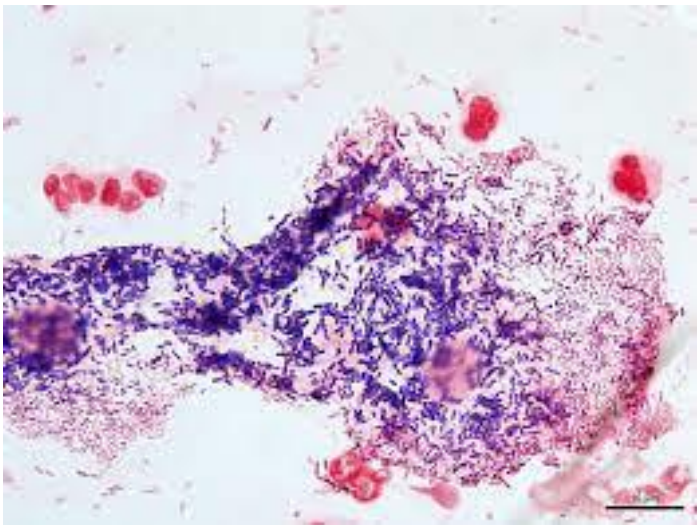


Fig 1.2: *Gardnerella vaginalis* after gram staining

Its presence in human vagina does not indicates that the particular individual has developed bacterial vaginosis. It can be present inside vagina with the normal flora of vagina. However, sometimes overgrowth of this organism alters the pH level of vagina which reduces lactobacilli and replaced healthy flora with pathogenic flora, thus contributes to bacterial vaginosis. Not all *Gardnerella vaginalis* has the capability to cause bacterial vaginosis, instead the strains of G. vaginalis that produce cytolysin and can form biofilm are more prone to cause bacterial vaginosis(Verstraelen and Swidsinski 2013) In mid of 1950's it was believed that this organism is a sole reason behind bacterial vaginosis. However, recent studies has shown that BV symptoms are linked to a variety of bacterial species, but that no single species appears to be present in all situations(Srinivasan et al. 2012) Dr. Sullivan reported that recently the concept of bacterial vaginosis has changed which means, in recent years it is believed that not only *Gardnerella* is capable of causing bacterial vaginosis, instead mixture of other bacteria *Atopobium*, *Mobiluncus*, and *Prevotella* (among others) along with Gardnerella vaginalis contribute to the development of BV.

1.3.2.3: *Atopobium vaginae:*

Atopobium vaginae, a gram-positive bacterium which is elliptical and rod-shaped cocci and is a part of the genus *Atopobium*. Within the family Coriobacteriaceae lies the genus Atopobium. Moreover, three formal species *Lactobacillus minutus, Lactobacillus rimae* and *Streptococcus parvulus* have been reclassified as genus *Atopobium.* (Lamont et al. 2011) *Atopobium vaginae* has been reportedly mentioned in many studies about its association with the bacterial vaginosis of reproductive age women.

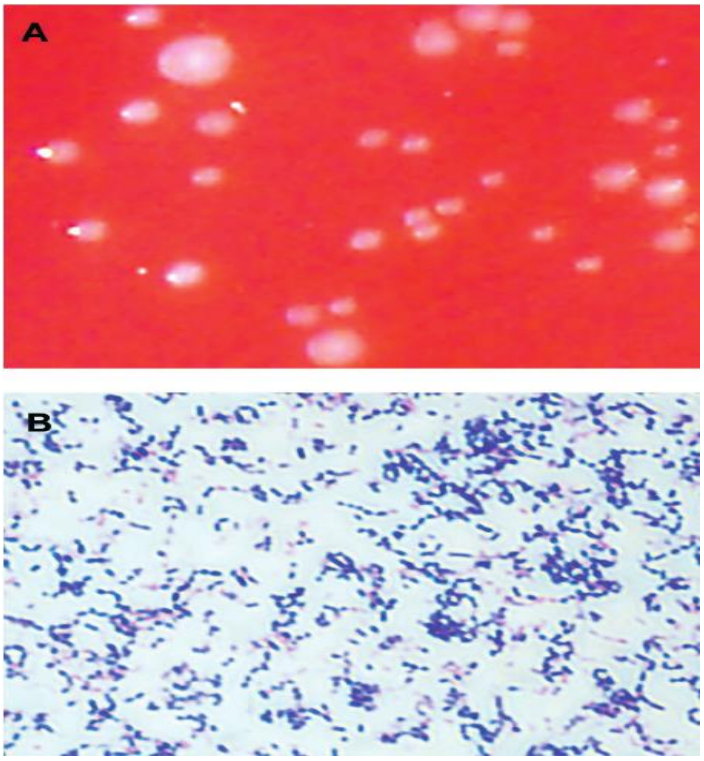


Figure 1.3: Greyish white colonies of *A. vaginae* after 48h of culture

B- *A. vaginae* after gram staining. Rod-shaped gram-positive bacteria visible under the microscope.

In normal vaginal flora, the presence of *A. vaginae* is seen along with *Gardenella vaginalis* whose percentage ranges from 8 to 25%. (Fredricks, Fiedler, and Marrazzo 2005) In addition to this, this presence is much more prominent in patients with bacterial vaginosis. *A.vaginae* is seen to have associated with vaginal discharge, high pH and clue cells.((De Backer et al. 2007) It was moreover

depicted that high vaginal loads of A. vaginae in combination with G. vaginalis are related with late unsuccessful labor and prematurity.

Though a number of microorganisms have been associated with the bacterial vaginosis in women, *A.vaginae* is one of the recently identifies species which is more specific in bacterial vaginosis. In one of the recent study, cross-sectional data reported that *A. vaginae* is present in 0%–20% of women with normal vaginal flora and in 50%–78% of women with bacterial vaginosis. (Verstraelen et al. 2004) *A. vaginae* not only shows up to be more particular for BV, but its copresence with *G. vaginalis* was related with essentially higher rates of repetitive BV (83%) and irregular vaginal flora (89%) than those seen in women with only *G. vaginalis* (38% and 49%, separately).

1.3.2.4: *Prevotella bivia:*

Prevotella species are anaerobic Gram-negative microscopic organisms of the Bacteroidetes phylum, which too incorporates the clinically critical genera Bacteroides and Porphyromonas. (Larsen 2017) *Prevotella* forms circular, convex gray colonies and on gram stain, it shows short gram negative rods and mostly presumed by coccobacilli forms. (Garrett and Onderdonk 2015)

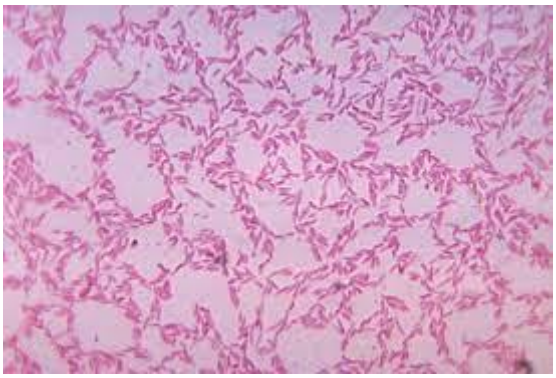


Figure 1.4: *P.bivia* after gram staining.

Though *Prevotella* strains are classically considered commensal microscopic organisms due to their broad presence within the sound human body and their uncommon involvement in diseases, only a few strains reported in endogenous infection .(Brook 1998)Moreover, *Prevotella bivia*

specifically alters physicochemical barrier-related proteins and metabolites like-mucins, sialic acid, polyamines, and this alteration correlates with bacterial vaginosis symptoms in women .(Łaniewski and Herbst-Kralovetz 2021) *P.bivia* seems to have symbolic relationship with *Gardenerella vaginosis* and they grow together in 59.1% of women with bacterial vaginosis compared to 3.9% of women without bacterial vaginosis. (Thorsen et al. 1998) In addition, when a blend of facultative anaerobes and *P. bivia* were developed together, the pathogenicity of *P. bivia* expanded and showed prominent symptoms of bacterial vaginosis.

-Materials and Methods-

2.1 Study design

A prospective study was conducted in collaboration with the Department of Obstetrics and Gynecology of Shaheed Suhrawardy Medical College and Hospital. A Government hospital had been chosen so that we can readily recruit patients from a variety of socioeconomic backgrounds, which is a part of our quantitative research. The research was a mixed cross-sectional study to assess the organisms causing bacterial vaginosis among pregnant women in both rural and urban areas. Thus, Quantitative (Questionnaires) and qualitative (laboratory work) methods were used in this research. A predefined protocol was used to select patients, their information was collected using a questionnaire form, which included information about patient's socio-demographic variables like- name, age, address, contact number, monthly income, patient's previous health history, recent physical history etc. For this research purpose, we chose patients from the antenatal section who were running 35 to 39 weeks of pregnancy and also excluded unmarried women as our main goal was to identify bacterial vaginosis from pregnant women.

2.2 Laboratory Procedure

In the lab, we used six different types of media, that includes: (1) Amies transport media for transporting samples from the hospital to the BRACU lab. (Barry, Fay, and Sauer 1972). (2) HBT (Human Blood Tween) media (Totten et al. 1982)which is a selective differential Human Blood Bilayer Media for Isolation of *Gardnerella vaginalis*. (3) Blood Agar and (4) MRS media for culture, (5) BHI (Brain Heart Infusion) media for enrichment purpose and (6) T1N1 media for stocking the bacteria. All of those media were made in our university's lab.

2.3.1 Culture Media Preparation

a. HBT (Human Blood Tween) Media

HBT (Human Blood Tween) Bilayer Medium is a selective and differential medium used in the primary isolation and presumptive identification of *Gardnerella vaginalis* (Totten et al. 1982) from clinical specimens. We used three different antibiotics in this media, which include: Colistin, Amphotericin B and Nalidixic acid. Colistin and Nalidixic acid inhibit most gram-negative

organisms and Amphotericin B is active against yeasts and filamentous fungi. Furthermore, a thin layer of the medium with human blood enhances the detection of the characteristic diffuse beta hemolysis of *Gardnerella vaginalis*.(Casari et al. 2010)

Materials

- Casein
- Peptic digest of animal tissue
- Yeast extract
- Beef extract
- Peptone
- Corn starch
- NaCl
- Agar
- Tween 80
- Colistin
- Amphotericin B
- Nalidixic acid
- Human blood
- Glass beaker
- Foil paper
- Laboratory microbalance

Procedure

- At first we took a conical flask and put all the ingredients in the flask without colistin, amphotericin B, Human Blood and tween 80.
- Then all the powders were mixed with distilled water and for better mixing, we heated the media for a few minutes.
- Then autoclave was done of the media

- After autoclave we mixed colistin, amphotericin B, and tween 80 with the proper amount by adding distilled water as well as human blood.
- Then the media was poured into medium size Petri dish plate.
- Usually, HBT media has two-layer. The first layer was filled without human blood and the second layer was filled with human blood.
- All the procedure was done under laminar flow and when the media was solidified it was wrapped with plastic paper and stored in a 4 degree refrigerator.

b. Blood Agar

Blood Agar is used to grow a wide range of pathogens, particularly the fastiddious ones. For our research, mainly *Atopobium vaginae* was observed in blood agar, which gave white and off-white colonies in most media. Majority of the bacteria were medium in size. (Prevalence *Atopobium vaginae* in vaginal samples of symptomatic non-pregnant women)

Materials

- Blood agar powder
- Distilled water
- Human blood
- Conical flask

Procedure

- The powder form of blood agar media was mixed with distilled water and then it was heated for mixing appropriatly.
- Later, autoclave was done.
- After the autoclave, human blood was mixed with the media, during this time media should be below 50 degree celsius and then it was poured into Petri dish plates.
- When the media solidified, it was wrapped with plastic paper and stored in a 4 degree refrigerator.

c. MRS Agar

MRS Agar is a medium used for the cultivation of *Lactobacillus* species. The addition of magnesium, manganese and acetate, together with polysorbate, provides an improved medium for the growth of lactobacilli.

Materials

- MRS powder
- Agar
- Distilled water
- Conical flask

Procedure

- MRS powder and agar were mixed with distilled water and heated for a few minutes for proper mixing.
- Then autoclave was done and the media was poured into Petri dish plates.
- When the media solidified, it was wrapped with plastic paper and stored in a 4 degree celsius refrigerator.

2.3.2 Transport Media Preparation

a. Amies Transport media

Amies transport medium is a widely used and effective liquid medium for the transportation of swab specimens to the laboratory. As we collected specimens from patients with a sterile cotton swab and our desired bacteria were strict anaerobe, so Amies transport media was chosen.

Materials

- Sodium Chloride

- Potassium Chloride
- $CaCl_2$
- $MgCl_2$
- Mono Potassium Phosphate
- Disodium Phosphate
- Sodium Thioglycolate
- Charcoal

Procedure

- All the ingredients were at first mixed in distilled water according to their appropriate proportion.
- The media was heated for a few minutes until it turned clear.
- The media was autoclaved at 37 degree celsius & 15 psi pressure.
- After that, 2 ml media was taken by micropipette and placed into the glass vial.
- Lastly, the liquid transport media was stored in a 4 degree celsius refrigerator.

2.3.3 Enrichment media preparations

a. BHI (Brain heart infusion) media

Brain heart infusion is a nutrient-rich medium for enriching the growth of the vaginal micro-organisms. (RN Okwoli et al 2002) (Laboratory diagnosis of *Gardnerella vaginalis*) We used BHI media for the enriched bacterial colony.

Materials

- BHI agar powder
- 10% Human blood serum
- Distilled water

Procedure

- Firstly, BHI powder was mixed with distilled water according to appropriate amount.
- For better mixing the media was heated and then it was autoclaved.
- After the autoclave process, 10% human blood serum was mixed with the media and then it was poured into Petri dish plate.
- When the media solidified, it was wrapped with plastic paper and stored in a 4 degree refrigerator.

2.3.4 Stock media preparation

a. T1N1 media

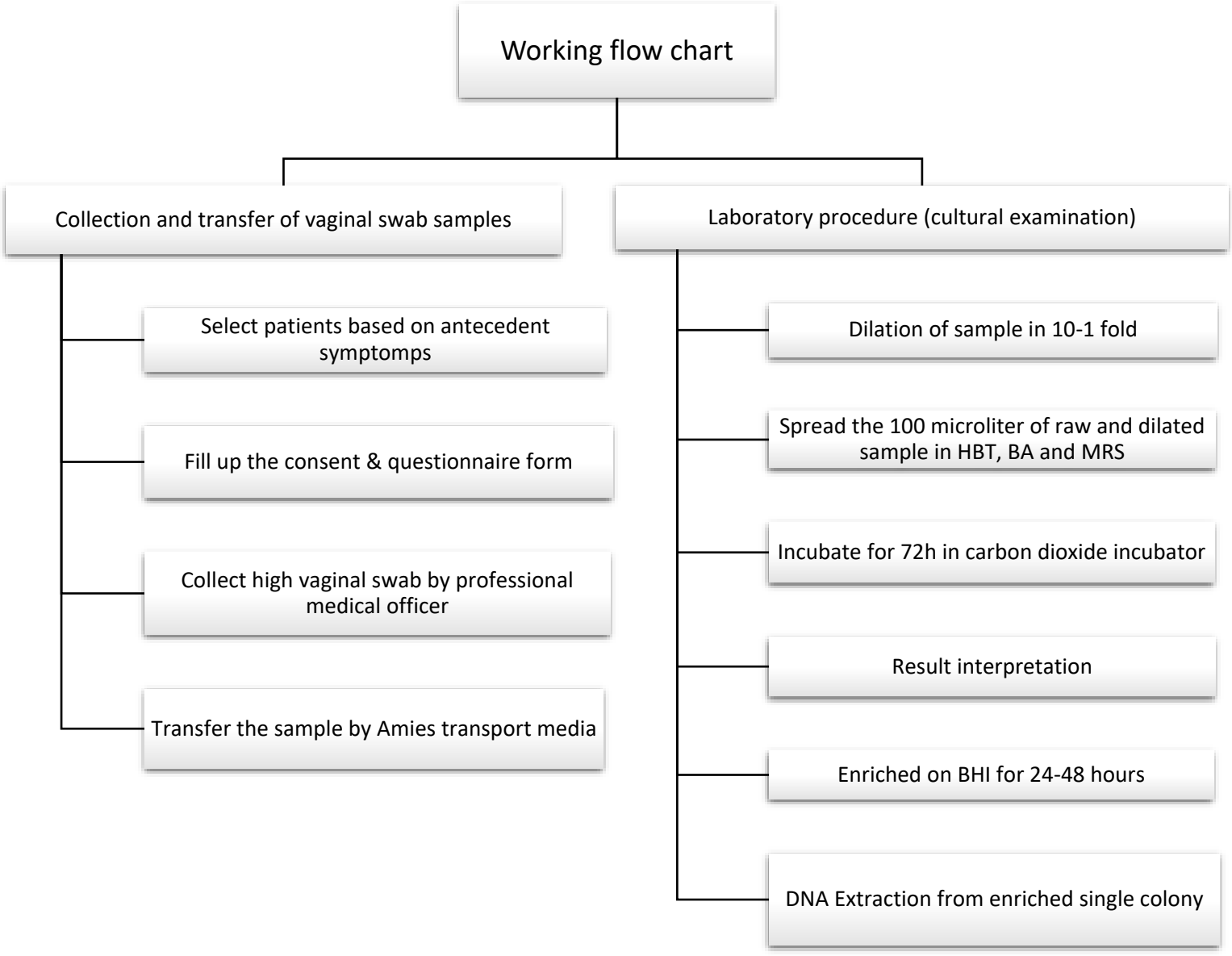
Materials

- Tryptophan
- NaCl
- Agar
- Distilled water

Procedure

- Firstly, tryptophane, NaCl, and agar were mixed with distilled water.
- Then the media was heated and later it was autoclaved.
- Next, the media was poured into vial and stored in 4 degree refrigerator.

2.3.5 Working Flow Chart



2.3.6 Sample Collection

We spoke with a senior doctor in the Gynecology section first, and then she assigned a doctor for collecting samples. Then we took care of the patients and collected information from them via a questionnaire. A sterile cotton swab was used to take vaginal swab from the patients. The cotton swab was dipped into the amies transport media and then placed in the ice box.

2.3.7 Dilution of sample

The collected sample was diluted into 10^{-1} fold for decreasing heavy growth of microbes and then mixed well by the vortex. For dilution we used 900 µl normal saline and 100 µl raw sample. All the diluted samples were transferred into Eppendorf and later they were marked as P-1, P-2.

2.3.8 Sample plating

Both 100 µl of raw and diluted samples were taken by using 100µl micropipette and we spreaded on MRS, Blood agar and HBT media plate.

2.3.9 Sample incubation

All MRS, Blood Agar, HBT plates were incubated into a CO2 incubator at 37℃ for 94 hours. As our desired microbes were strict anaerobes, that's why we used a CO2 incubator.

2.3.10 Result interpretation

After the incubation, bacterial colony was counted and the resuts were logged accordingly.

2.3.11 Enrichment of isolated bacterial colony

Single colonies were taken from HBT, BA and MRS and then enriched in BHI (Brain Heart Infusion). Using a sterile loop, a single colony was collected and streaked across the plate in three quadrants. For 48 hours, the cells were incubated in a CO2 incubator at 37°C.

2.3.12 Stock in T1N1 media

From enriched media single colony of bacteria was picked by using sterile niddle and later it was stabbed into T1N1 media. Next, immersion oil was added into the media for further stocking.

2.3.13 DNA extraction

- For DNA extraction 500 µl of distilled water was taken using a 1000µl micropipette and then it was poured into Eppendorf.
- From enriched media, a single colony of bacteria was picked using a sterile loop and then it was dissolved into Eppendorf.
- Next, vortex was done for proper mixing and the eppendorf cap sealed with paraffin.
- After that, a 11-minute heat shock at 95°C was performed.
- Then cold shock was performed for 12 minutes
- In the next step, centrifugation was done at 13,000 rpm for 7 minutes.
- Then the supernatant was transferred into another Eppendorf and lastly it was stocked it at -20℃.

-Result-

3.0 Result: Patients' questionnaire data of our project is attached below. The chart includes info about, patients age, duration of pregnancy, number of conceive, patient health history, area, pre-existing UTI record, previous BV record, vaginal discharge, color of vaginal discharge, abdominal pain, height and weight.

Patient's questionnaire data:

Patent no.	Age	Duration of pre	Number of con	Patient health h	Area	Previous UTI re	Previous BV re	Vaginal dischar	Color of VD	Abdominal pain	Height	Weight (kg)
1	26	39	2	No	Urban	No	No	Yes	White	No	5'2"	52
2	19	37	2	No	Urban	No	No	Yes	White	No	5'4"	60
3	22	35	1	No	Rural	No	No	No	-	No	5'1"	53
4	28	36	2	Hepatitis B, Hy	Urban	Yes (Ongoing)	No	Yes	White	No	4'11"	49
5	19	37	1	Hbst	Rural	Yes (Ongoing)	No	Yes	White	Yes	5'1"	54
6	18	39	1	No	Urban	No	No	Yes	White, pinkish	Yes	4'6"	50
7	19	36	1	Hypertension, H	Urban	Yes (Ongoing)	No	Yes	White	No	4'11"	75
8	19	37	1	Gastric	Urban	No	No	Yes	White	No	5'1"	59
9	30	37	2	High BP, Difficu	Urban	Yes (Ongoing)	Yes	Yes	White	Yes	5'	70
10	24	32	2	No	Urban	No	No	No	-	No	5'1"	83
11	25	39	1	No	Urban	Yes (Ongoing)	No	Yes	White	Yes	4'3"	53
12	29	36	2	Diabetes	Urban	No	No	Yes	White	Yes	5'	61
13	21	36	1	No	Urban	No	Yes	Yes	White	No	4'11"	49
14	35	36	3	No	Urban	No	No	No	-	No	4'11"	55
15	25	36	3	Asthma	Urban	Yes (Ongoing)	Yes	Yes	White	No	5'3"	65
16	20	36	1	No	Urban	No	No	Yes	White	Yes	5'3"	60
17	20	36	2	No	Urban	No	No	Yes	White	No	4'11"	63
18	27	35	5	No	Urban	No	No	Yes	White	No	5'3"	60
19	21	36	1	No	Urban	Yes (Ongoing)	No	Yes	White	Yes	5'3"	54
20	25	38	2	No	Rural	No	No	Yes	White	No	5'4"	59
21	23	34	1	No	Rural	No	Yes	Yes	White	No	5'4"	62
22	22	36	3	No	Urban	No	No	Yes	White	Yes	4'10"	50
23	25	32	2	Diabetes	Urban	No	No	Yes	White	Yes	5'1"	56
24	30	39	1	No	Urban	No	No	Yes	White	Yes	5'	62
25	19	39	1	No	Urban	Yes (Ongoing)	No	Yes	White	Yes	5'	63
26	24	36	2	Diabetes	Urban	No	No	Yes	White	No	5'2"	75
27	30	31	1	High Bp	Rural	No	Yes	Yes	White	No	5'1"	66
28	22	36	2	No	Urban	No	No	Yes	White	Yes	5'1"	60
29	26	36	1	No	Urban	Yes (Ongoing)	No	Yes	White	Yes	5'3"	60
30	25	36	2	No	Urban	No	No	No	-	Yes	5'2"	65
31	35	36	3	No	Rural	No	No	No	-	No	5'	63
32	22	36	2	No	Urban	No	No	No	-	No	5'	60
33	20	36	1	No	Urban	No	No	Yes	White	Yes	5'1"	55
34	22	35	1	No	Urban	No	No	Yes	White	Yes	5'1"	70
35	20	34	1	No	Urban	No	No	Yes	White	No	5'1"	65
36	25	38	3	No	Urban	No	No	No	-	No	5'4"	85
37	31	37	2	No	Urban	Yes (Ongoing)	No	No	-	No	5'	66
38	28	35	2	No	Urban	No	Yes	Yes	White	Yes	4'6"	60
39	33	36	4	HBA+	Rural	No	No	Yes	White	No	5'1"	57
40	32	40	1	Diabetes	Urban	No	No	Yes	White	No	5'	62
41	26	32	1	SLE patient	Urban	No	No	Yes	White	Yes	5'	51
42	33	32	2	No	Urban	No	No	Yes	White	Yes	5'3"	70
43	27	37	2	No	Urban	No	No	Yes	White	Yes	5'1"	58
44	19	32	1	No	Urban	No	No	Yes	White	Yes	5'5"	61
45	25	35	1	No	Urban	No	No	Yes	White	Yes	5'	58
46	24	36	1	No	Urban	No	No	Yes	White	No	5'8	60
47	27	36	2	No	Urban	No	No	Yes	White	Yes	4'9"	60
48	20	32	1	No	Urban	Yes (Ongoing)	No	Yes	White	No	5'3"	57
49	26	37	1	High BP, diabete	Rural	No	No	Yes	White	No	5'3"	71
50	29	33	1	Diabetes	Rural	No	No	Yes	White	Yes	5'1"	59

AGE

3.1 Demography

In the studied cohort (n=50), majority of the patients were young adults ranging from the age of 25-30 years (34%). The second major group comprised of people who were less than 25 years old (32%) and only 6% women ranged from 35-40 Years.

Table 3.1 Patient's age demography (n=50)

Age Range	Number of patients	Percentage
15-20	7	14%
20-25	16	32%
25-30	17	34%
30-35	7	14%
35-40	3	6%

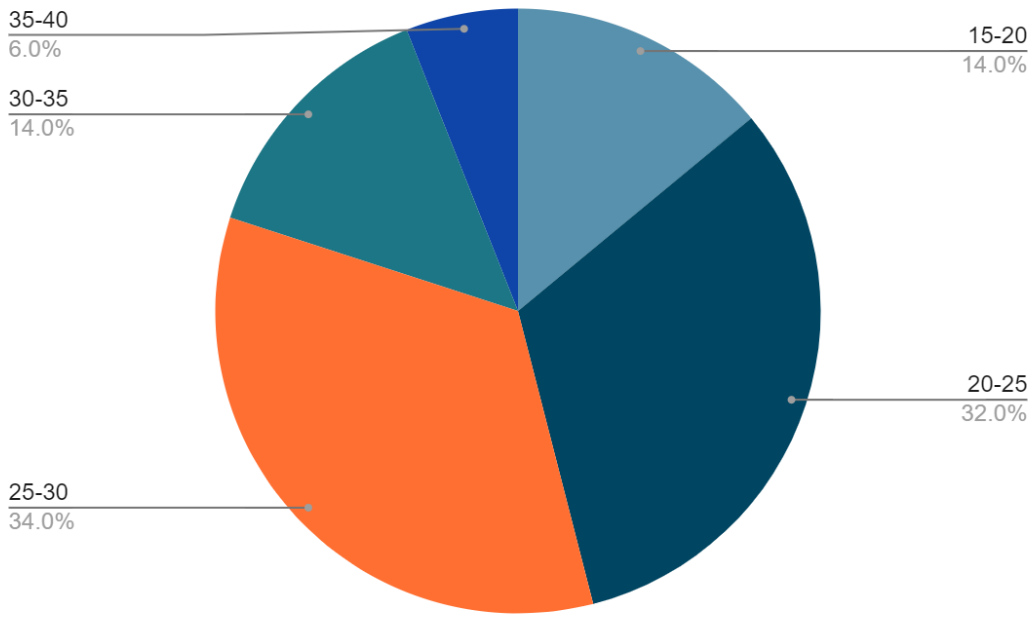


Fig 3.1: Age distribution of bacterial vaginosis patients during pregnancy, in Bangladesh

Duration of pregnancy

3.2 Demography of duration of pregnancy

According to the researched cohort (n=50), majority of the patient's duration of pregnancy ranging from 35-40 weeks (78%) and only 22% women's duration of pregnancy range from 30-35 weeks.

Duration of pregnancy Range	Number of patients	Percentage
30-35	11	22%
35-40	39	78%

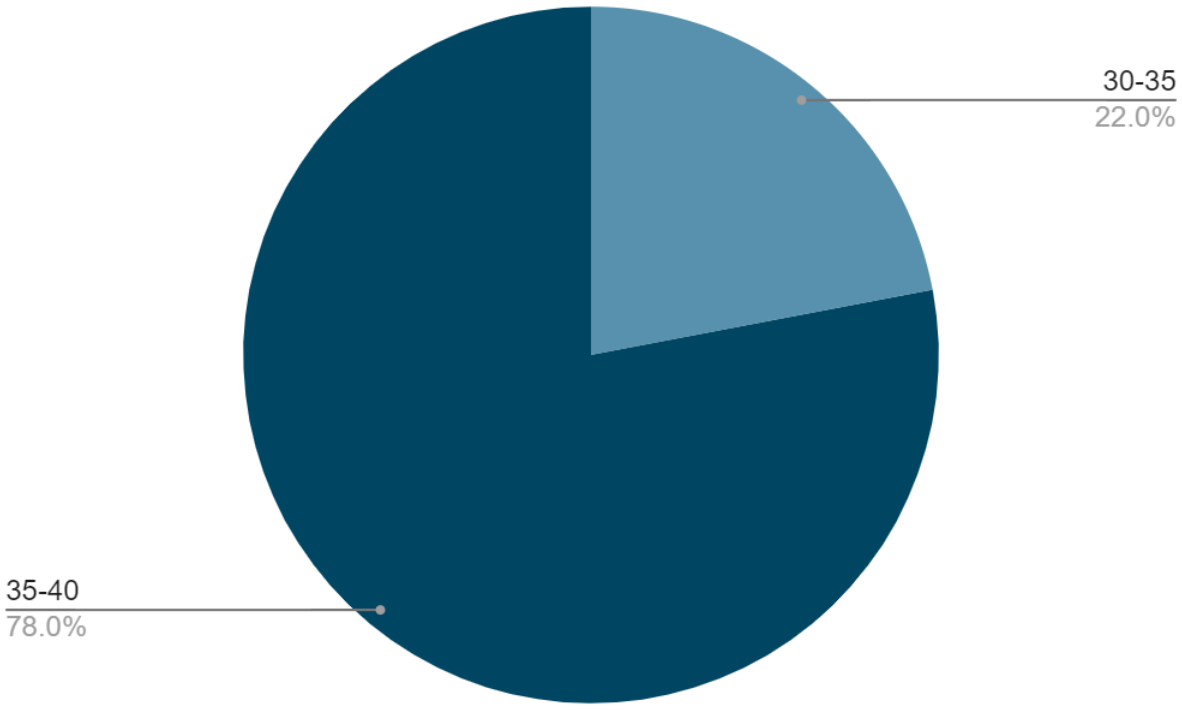


Fig 3.2 Demography of duration of pregnancy

Number of conceive

3.3 Analysis about Number of conceive

From the study, about 50% of the patients who are pregnant with their first child and only 4% of the women are pregnant with 4-5th child.

Range of conceive	Number of patients	Percentage
1-2	25	50%
2-3	18	36%
3-4	5	10%
4-5	2	4%

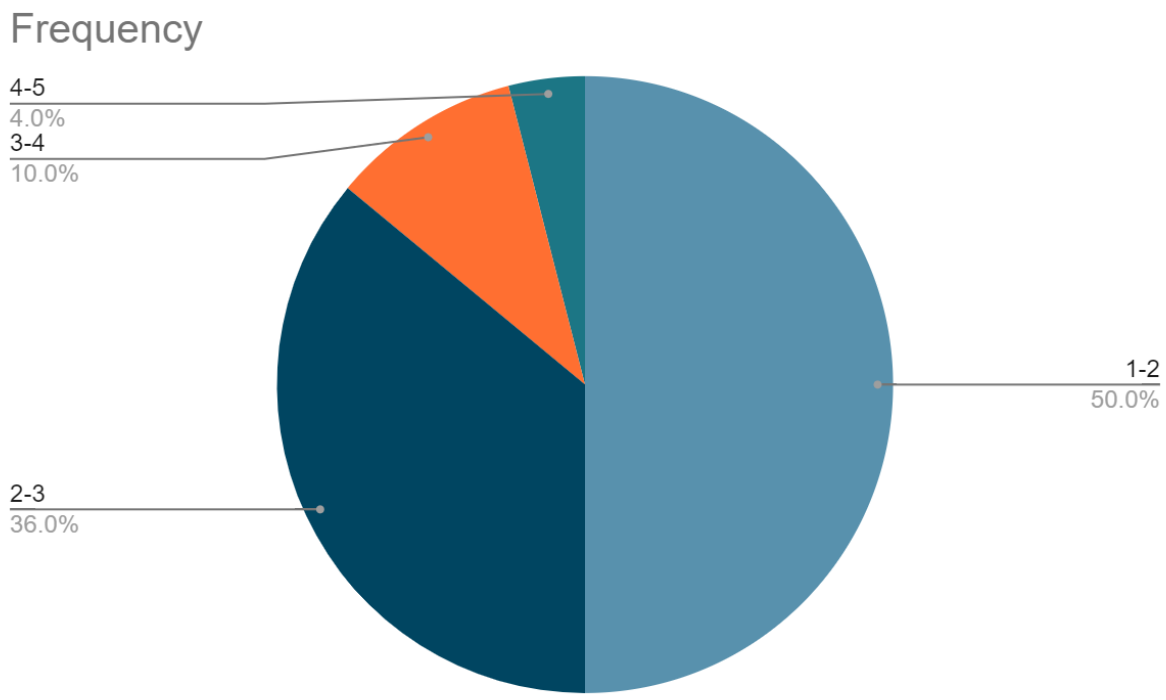


Fig 3.2 Demography on range of conceive.

Pre-existing health issues

3.4 Demography on pre-existing health issues

In the studied cohort (n=50), majority of the patients (70%) do not have any pre-existing health issues, whereas only 30% women have, for example: some patients were found to have diabetes, hypertension, gastric, high BP, hypothyroidism, asthma etc. diseases.

Pre-existing health issues	Number of patients	Percentage
Yes	15	30%
No	35	70%

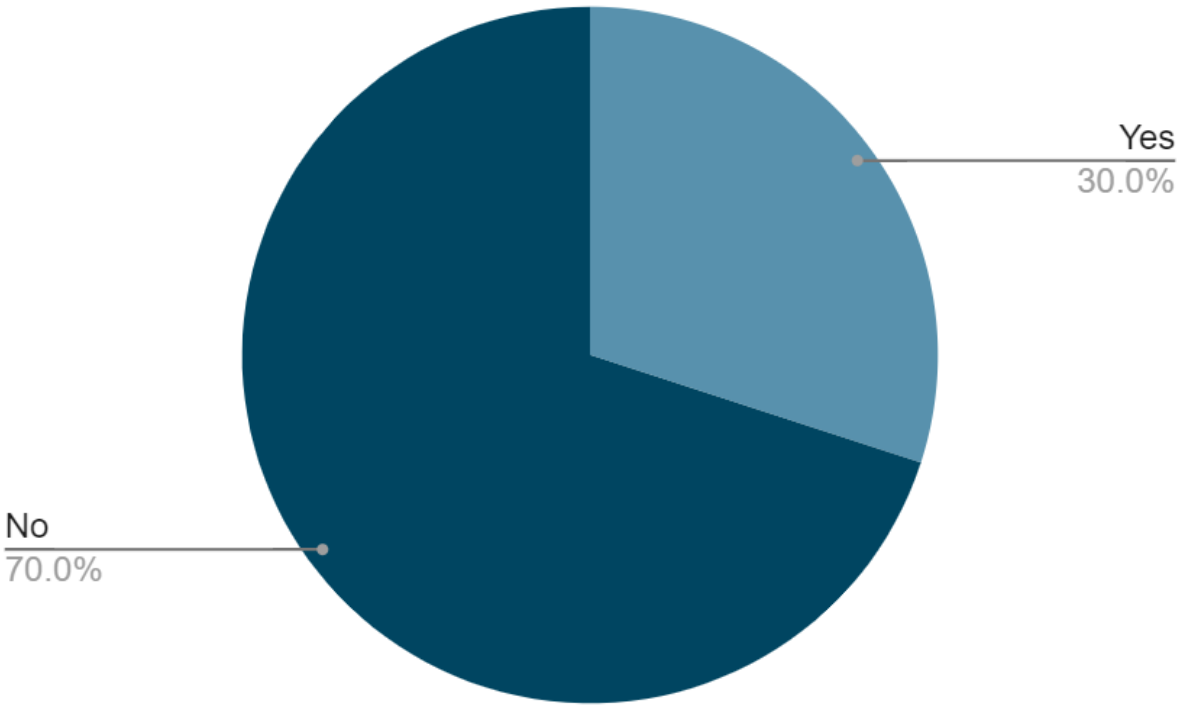


Fig 3.4 Demography on patients' pre-existing health issues:

Location

3.5 Frequency distribution on patients' location

In our study, most of the patients are from urban areas which is 82% and only 18% women are from rural areas.

location	Number of patients	Percentage
Urban	41	82%
Rural	9	18%

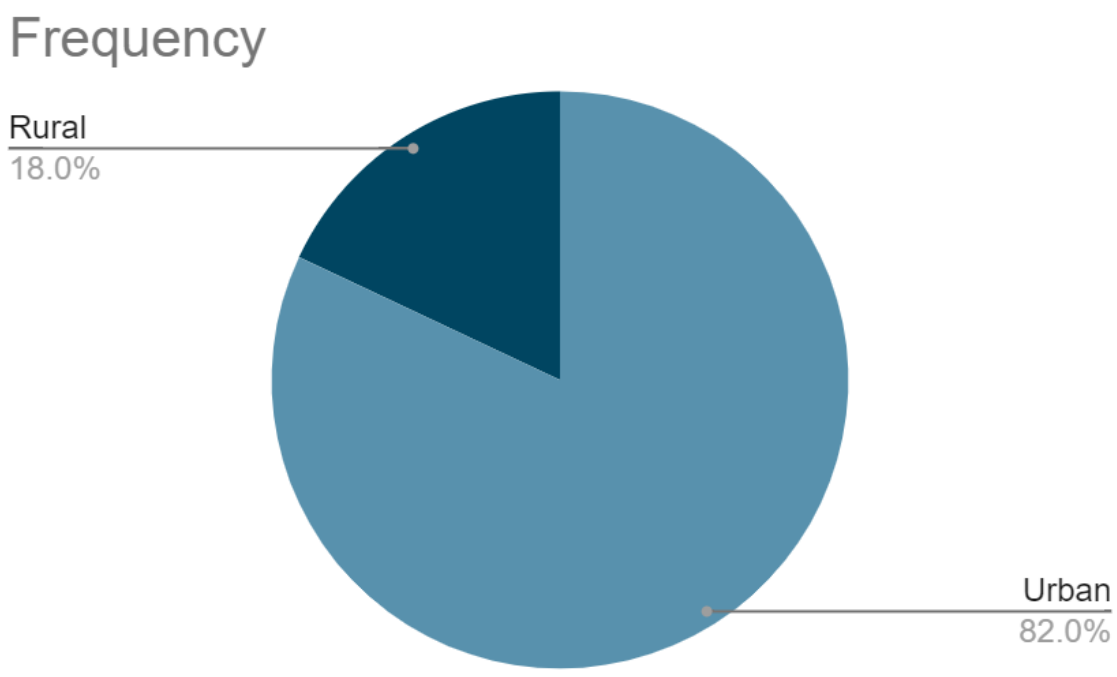


Fig 3.5 Demography on patients' location

Pre-existing UTI

3.6 Studies on pre-existing UTI

According to our study, 39 among 50 patients do not have any pre-existing UTI and only 11 women have pre-existing UTI.

Pre-existing UTI	Number of patients'	Percentage
Yes (ongoing)	11	22%
No	39	78%

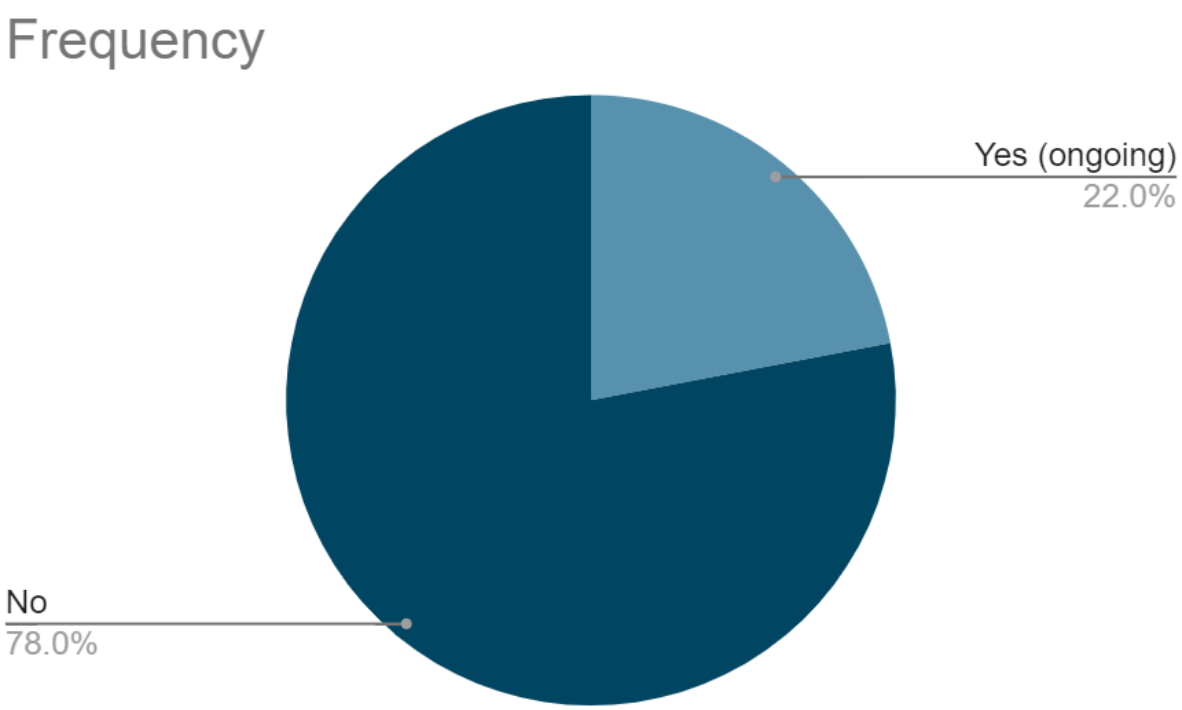


Fig: 3.6 Demography on pre-existing UTI

Previous BV record

3.7 Analysis on previous Bacterial vaginosis record

From our researched data, 44 among 50 patients do not have any previous BV record and only 6 women have previous BV record.

Previous BV record	Number of patients	Percentage
Yes	6	12%
No	44	88%

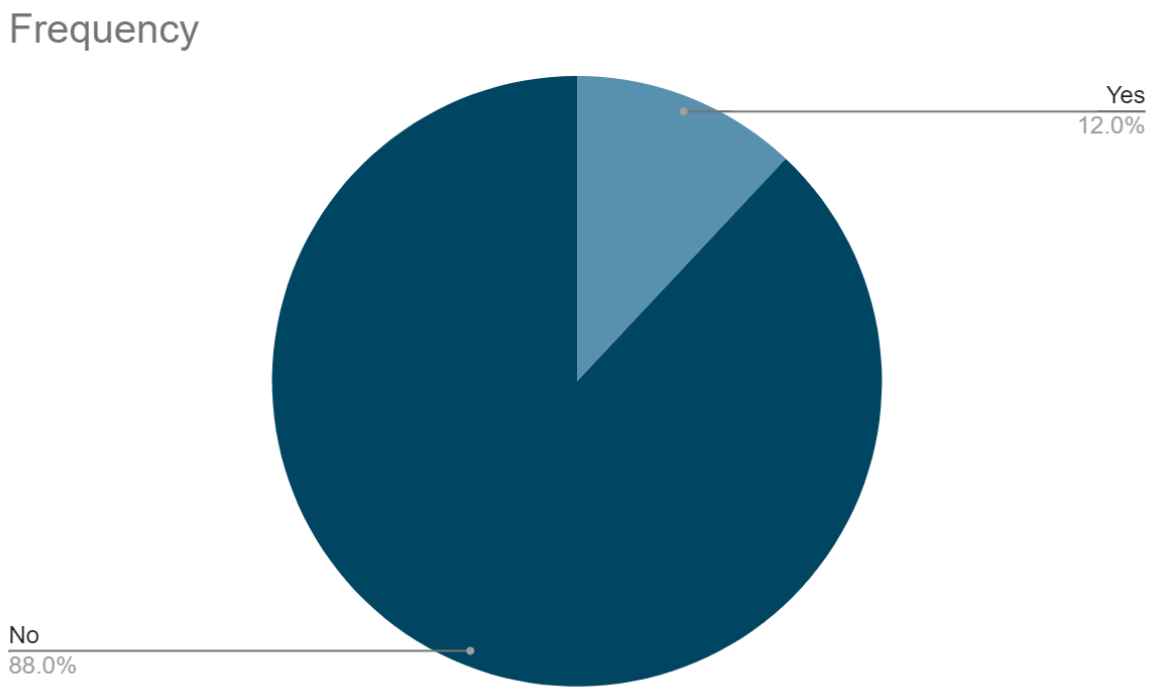


Fig 3.7 Demography on previous Bacterial vaginosis record

Vaginal discharge record

3.8 Demography on vaginal discharge record:

According to our study, around 84% patients have vaginal discharge, which is mainly white in color and 16% women do not have any vaginal discharge.

Vaginal discharge	Number of patients	Percentage
Yes	42	84%
No	8	16%

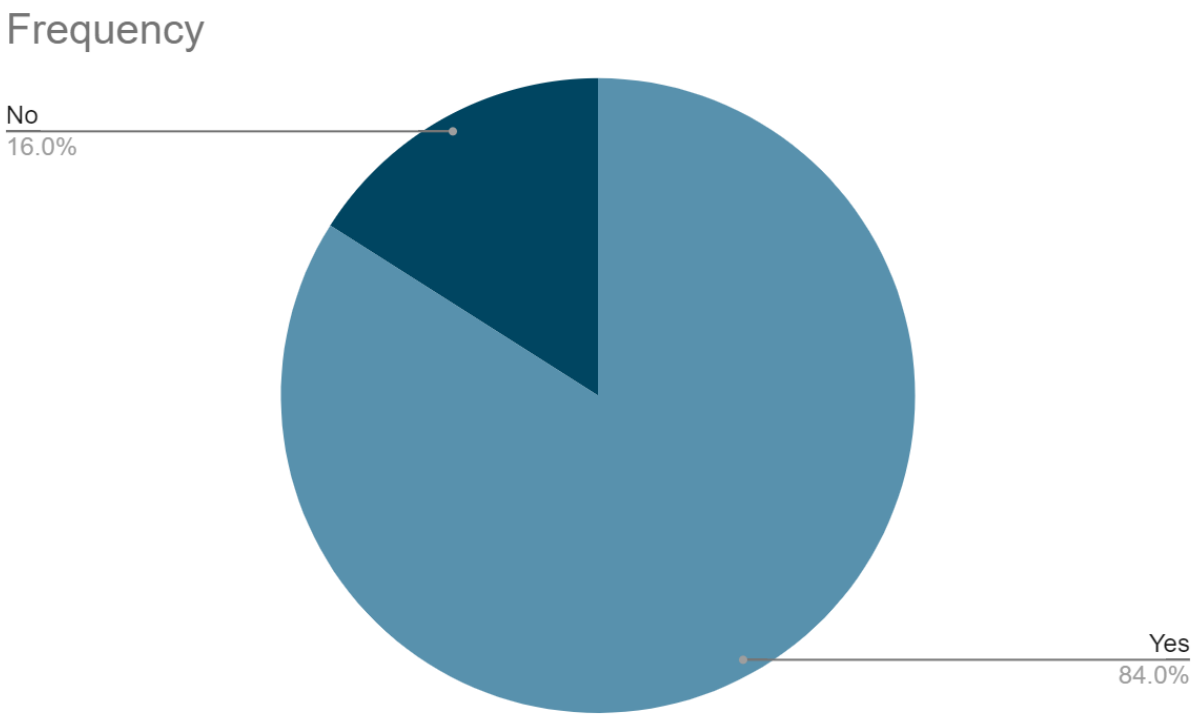


Fig 3.8 Demography on vaginal discharge record

Weight

3.9 Demography on patient's weight

In the studied cohort (n=50), majority of the patients have weight between 55-65 kg, which is 56%. And very few patients (8%) who have weight between 75-85 kg.

Weight Range	Number of patients	Percentage
45-55	9	18%
55-65	28	56%
65-75	9	18%
75-85	4	8%

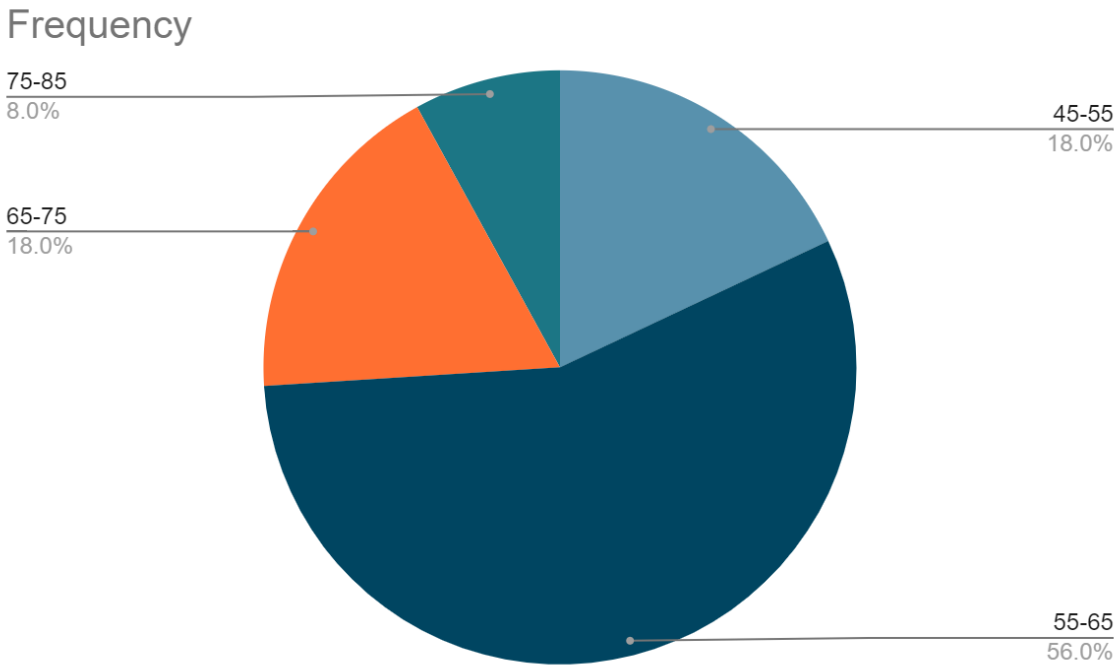


Fig 3.9 Demography on patient's weight

Patient's culture & morphology data

Patient no	HBT				Blood agar				MRS			Enrichmer	NA Isolatio	T1N1
	RAW	Morphology	Dilution	Morphology	RAW	Morphology	Dilution	Morphology	RAW	Morphology	Dilution	Morphology			
1	29	White small col	x	-	0	-	x	-	20	White small co	x	-	✅	✅	✅
2	0	-	x	-	87	White small col	x	-	37	White small co	x	-	✅	✅	✅
3	17	White small col	x	-	0	-	x	-	24	White small co	x	-	✅	✅	✅
4	0	-	x	-	37	White small col	x	-	33	White small co	x	-	✅	✅	✅
5	0	-	x	-	23	White, off-white	x	-	40	White small co	x	-	✅	✅	✅
6	0	-	x	-	110	White, off-white	x	-	11	White small co	x	-	✅	✅	✅
7	0	-	x	-	95	White small col	x	-	5	White small co	x	-	✅	✅	✅
8	0	-	x	-	60	White small col	x	-	28	White small co	x	-	✅	✅	✅
9	0	-	x	-	85	White, off-white	x	-	39	White small co	x	-	✅	✅	✅
10	240	White & off-whi	x	-	172	White colony	x	-	33	White small co	x	-	✅	✅	✅
11	48	White, off-white	x	-	22	Mixed white col	x	-	21	White small co	x	-	✅	✅	✅
12	1016	White small col	x	-	104	White small col	x	-	26	White small co	x	-	✅	✅	✅
13	37	White, off-white	x	-	0	-	x	-	38	White small co	x	-	✅	✅	✅
14	27	White small col	x	-	134	White small col	x	-	55	White small co	x	-	✅	✅	✅
15	492	White small col	x	-	0	-	x	-	60	White small co	x	-	✅	✅	✅
16	90	Off-white, medi	x	-	140	Off-white, small	x	-	37	White small co	x	-	✅	✅	✅
17	67	White & off-whi	x	-	22	Off-white, medi	x	-	44	White small co	x	-	✅	✅	✅
18	230	Off-white, small	x	-	27	Off-white, Large	x	-	29	White small co	x	-	✅	✅	✅
19	270	Off-white, small	x	-	23	Off-white, small	0	-	1	White small co	19	White small co	✅	✅	✅
20	0	-	0	-	200	White & off-whi	1	Off-white colo	35	White small co	17	White small co	✅	✅	✅
21	1	Off-white colon	0	-	10	White & off-whi	0	-	27	White small co	13	White small co	✅	✅	✅
22	0	-	0	-	0	-	0	-	31	White small co	15	White small co	✅	✅	✅
23	44	White, medium	0	-	77	White & off-whi	1	Off-white colo	6	White small co	0	-	✅	✅	✅
24	7	Off-white colon	0	-	30	White & off-whi	12	White & off-wh	367	White small co	0	-	✅	✅	✅
25	377	White & off-whi	92	White & off-w	30	Diffuse Off-whit	10	White & off-wh	1	White small co	0	-	✅	✅	✅
26	70	White & off-whi	32	White & off-w	80	White & off-whi	15	White & off-wh	0	-	0	-	✅	✅	✅
27	30	White & off-whi	0	-	323	White & off-whi	0	-	0	-	0	-	✅	✅	✅
28	9	White & off-whi	0	-	150	White & off-whi	0	-	50	White small co	21	White small co	✅	✅	✅
29	4	White & off-whi	0	-	0	-	0	-	367	White small co	55	White small co	✅	✅	✅
30	9	White & off-whi	0	-	4	White colony	0	-	1	White small co	0	-	✅	✅	✅
31	100	White & off-whi	37	White & off-w	60	Brown colony	309	Off-white colo	0	-	0	-	✅	✅	✅
32	70	White & off-whi	31	White & off-w	0	-	0	-	50	White small co	40	White small co	✅	✅	✅
33	0	-	0	-	300	Smuged Off-wh	70	Single Off-whi	0	-	0	-	✅	✅	✅
34	120	White & off-whi	150	White & off-w	11	White heavy co	93	Off-white colo	0	-	0	-	✅	✅	✅
35	400	White & off-whi	200	White & off-w	36	Heavy mixed co	300	Single Off-whi	3	White small co	0	-	✅	✅	✅
36	8	Off-white small	70	Off-white sma	240	Big sticky, smal	60	Big sticky, sma	360	White small co	35	Big white colo	✅	✅	✅
37	300	Off-white small	200	Off-white sma	0	-	1	Off-white colo	0	-	0	-	✅	✅	✅
38	150	Off-white small	65	Off-white sma	35	Big Off-white co	250	Off-white smal	0	-	0	-	✅	✅	✅
39	121	Off-white small	81	Off-white sma	100	Off-white small	53	Off-white smal	100	White small co	32	White small co	✅	✅	✅
40	50	Off-white small	20	Off-white sma	0	-	0	-	84	White small co	2	White small co	✅	✅	✅
41	323	Off-white small	35	Off-white big	321	Off-white small	30	Off-white diffu	30	White small co	6	White small co	✅	✅	✅
42	327	Off-white small	298	Off-white sma	357	Off-white small	257	Off-white smal	123	White small co	87	White small co	✅	✅	✅
43	200	Off-white diffus	15	Off-white diffu	0	-	25	Off-white singl	4	White small co	0	-	✅	✅	✅
44	321	White & Off-wh	44	Off-white big	9	Off-white colon	8	White small co	170	White small co	47	White small co	✅	✅	✅
45	47	White big colon	29	Off-white larg	43	White big colon	37	Off-white smal	9	White small co	0	-	✅	✅	✅

HBT:

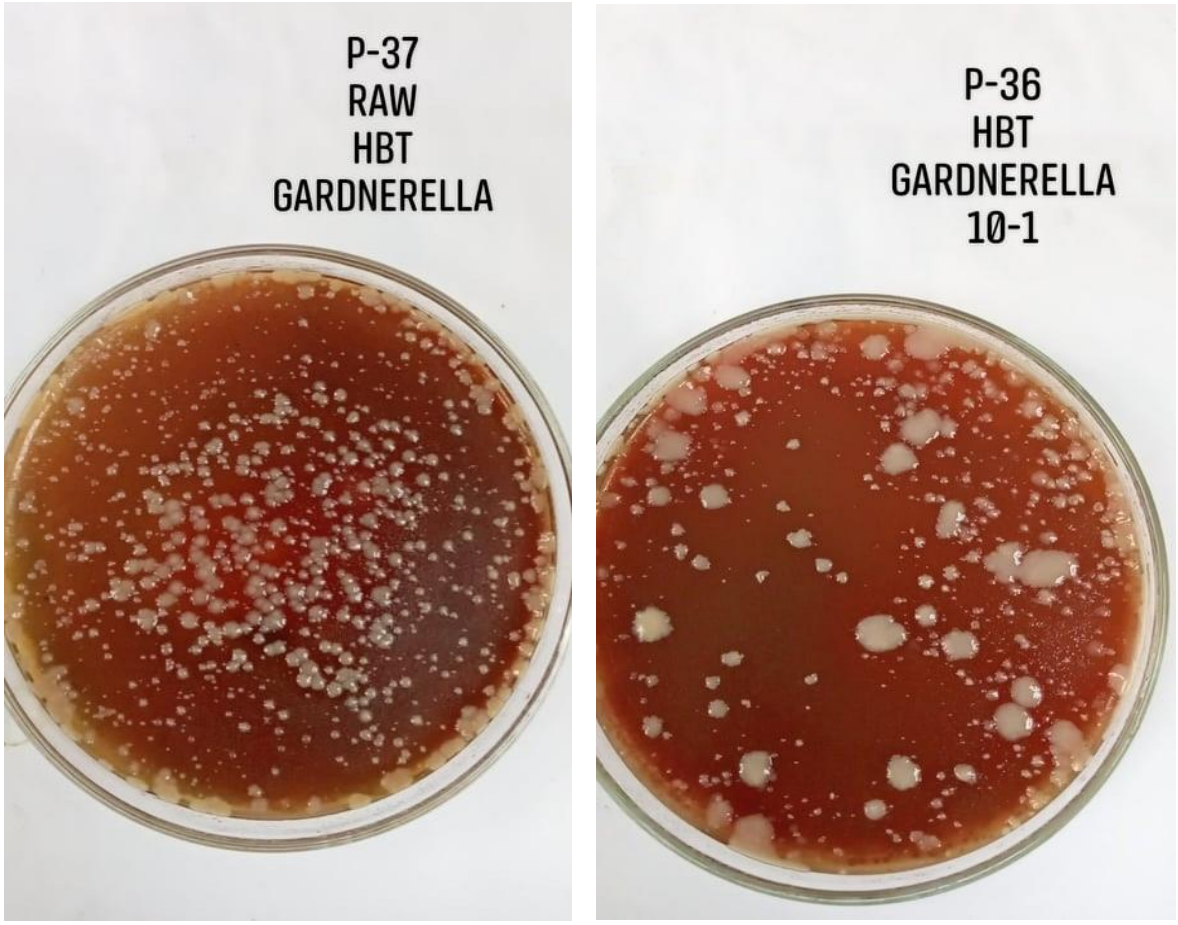


Fig 3.10 HBT plate for *Gardnerella vaginalis*

Raw:

***Gardnerella* culture Range**	**Number of patients**	**Percentage**
0-100	29	64.4%
100-200	4	8.9%
200-300	4	8.9%
300-400<	8	17.8%

3.10 cultural colony feature of *Gardnerella vaginalis*

After culturing on HBT for detection of ***Gardnerella vaginalis,*** growth was observed in most of the plate. However, the CFU number varied from patient to patient. Most (64.4%) of the patients have CFU between 0-100 and heavy growth of the bacteria was seen in 17.8% patients.

Colony morphology:

Most of the colonies observed were white & off-white, their size was small-large. Also, some medium and very small sized colonies were seen.

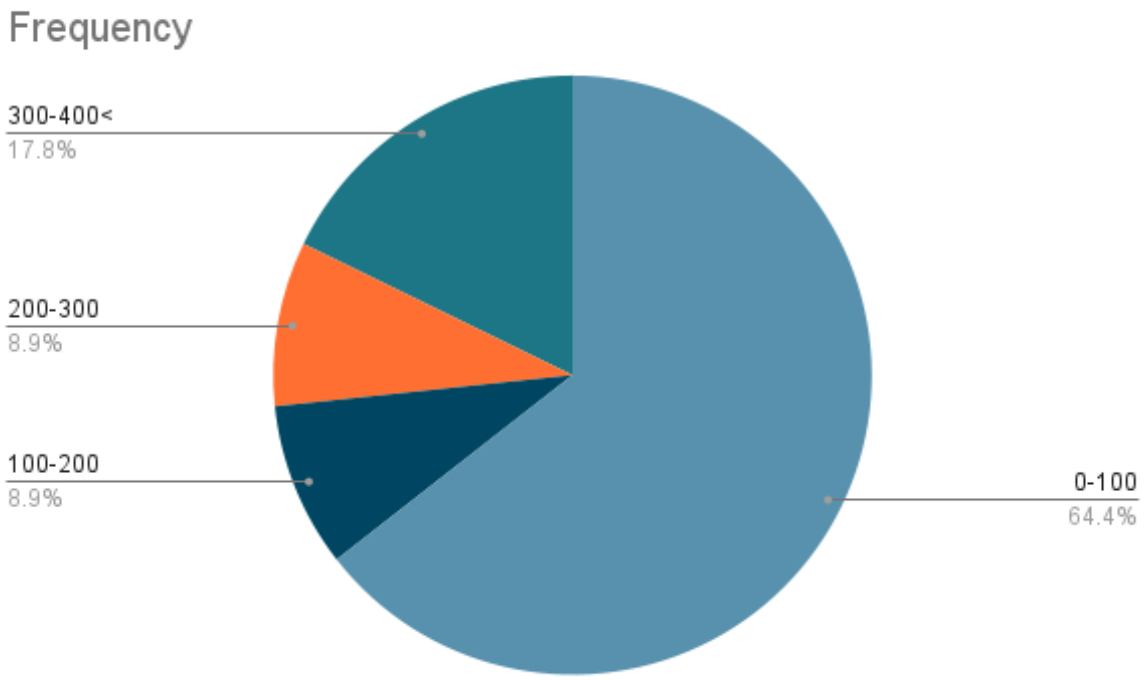


Fig 3.10 Growth frequency of *Gardnerella vaginalis*

Blood Agar:

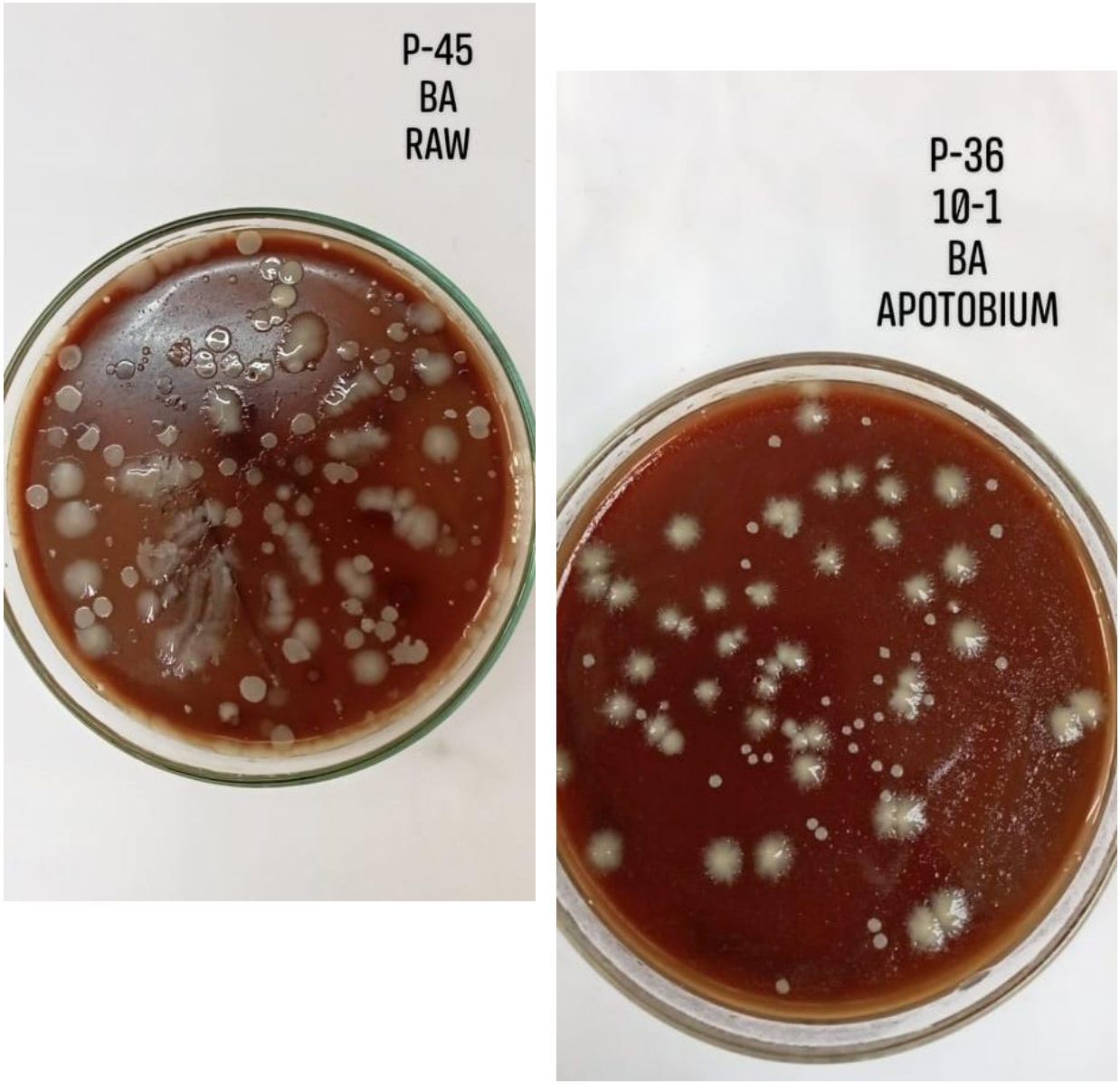


Fig 3.11 BA plate for *Atopobium vaginae*

Raw:

***Atopobium* culture Range**	**Number of patients**	**Percentage**
0-100	32	71.1%
100-200	7	15.6%
200-300	2	4.4%
300-400	4	8.9%

3.11 cultural colony feature of *Atopobium vaginae*

After culturing on Blood agar for detecting ***Atopobium vaginae,*** growth was observed in most of the plate. However, the CFU number varied from patient to patient. Most (71.1%) of the patients have CFU between 0-100 and heavy growth of the bacteria was seen in 8.9% patients.

Colony morphology:

Most of the colonies observed were white & off-white, small-large. But, in case of some patients diffused and smudged colonies were notably seen. Furthermore, in some plates brown colonies were found and also heavy growth of mixed big sticky colonies were seen.

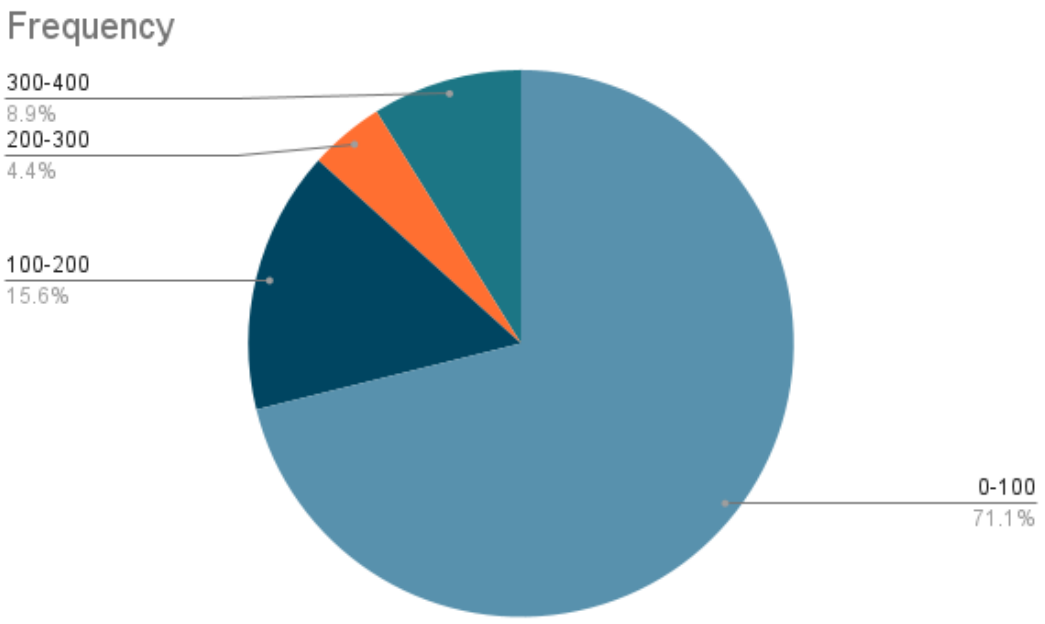


Fig 3.11 Growth frequency of *Atopobium vaginae*

MRS:

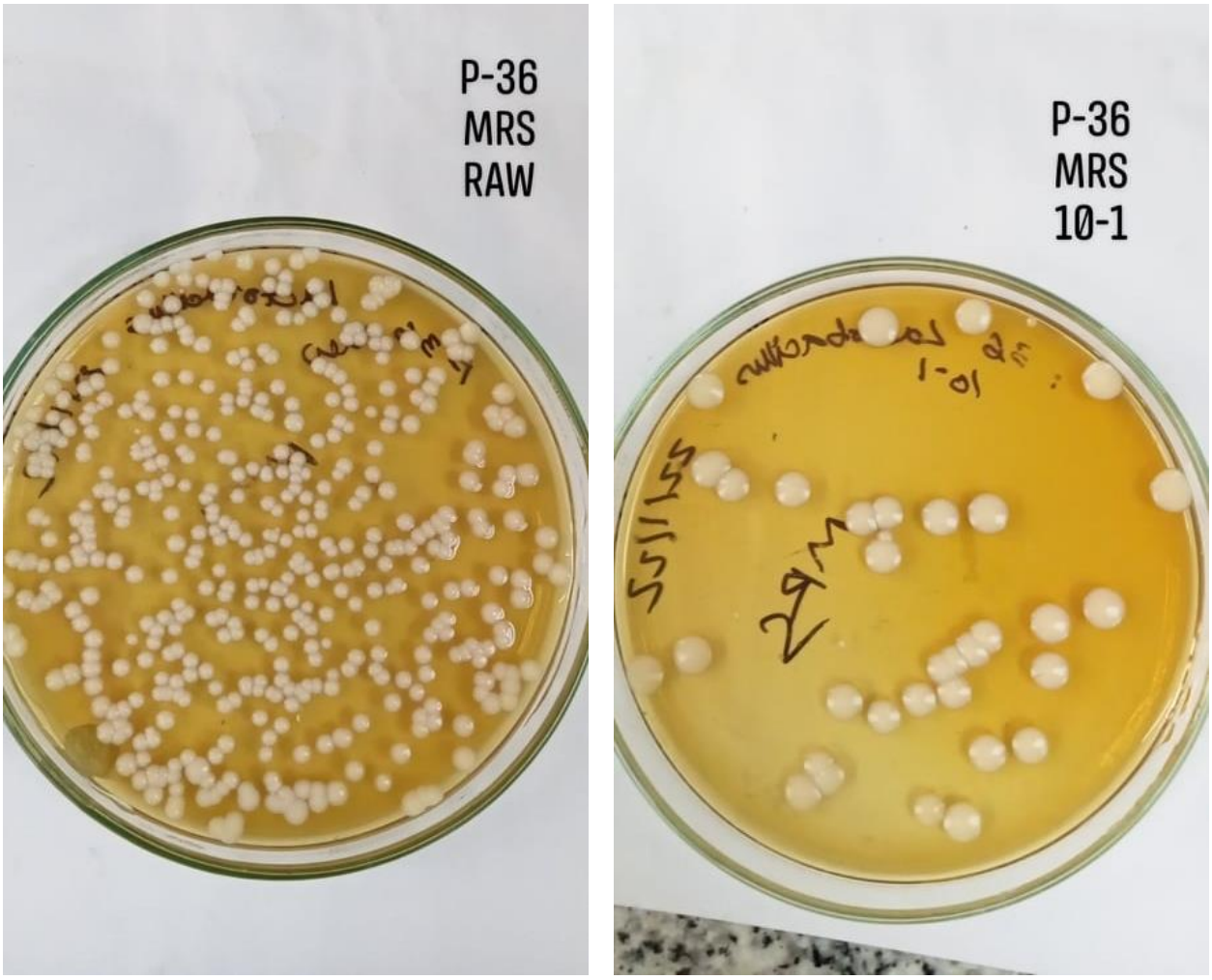


Fig 3.12 MRS plate for *Lactobacillus species*

Raw:

Lactobacillus culture Range	**Number of patients**	**Percentage**
0-100	39	86.7%
100-200	3	6.7%
200-300	0	0%
300-400	3	6.7%

3.12 cultural colony feature of *Lactobacillus species*

After culturing on MRS for detection of ***Lactobacillus species,*** growth was observed in most plates. However, the CFU number varied from patient to patient. Most (86.7%) of the patients have CFU between 0-100 and heavy growth of the bacteria was seen in 6.7% patients.

Colony morphology:

Majority of the colonies observed were white and small. But some big colonies were also seen.

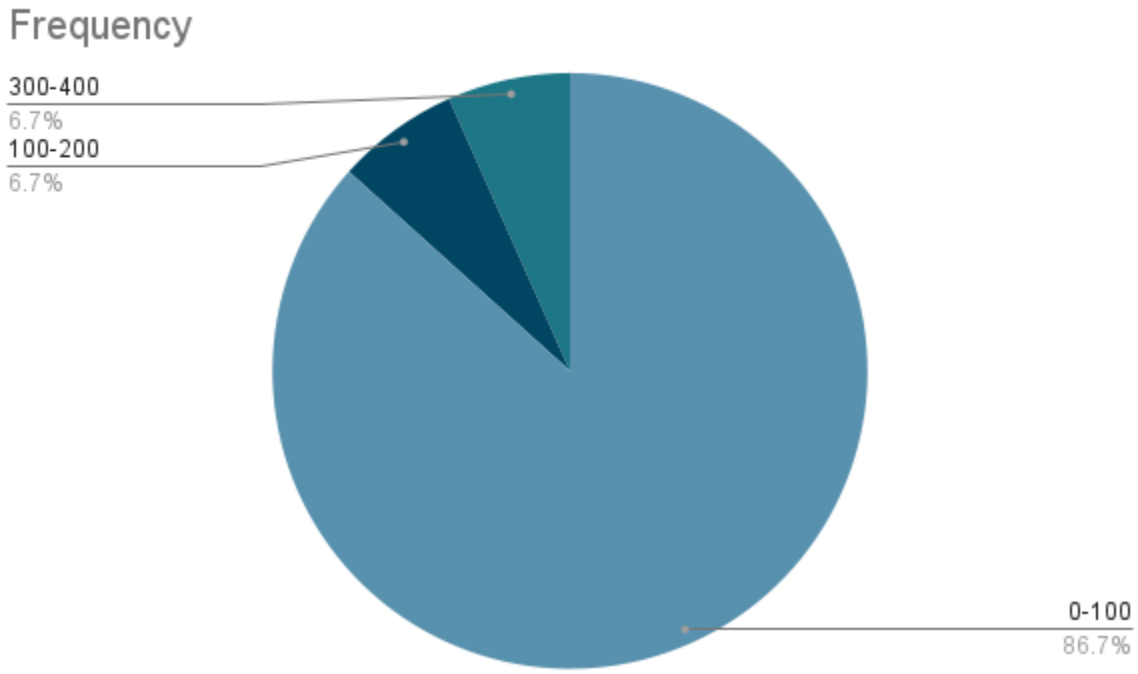


Fig 3.12 Growth frequency of *Lactobacillus species*

3.13 Co-relationship between patients age and colony count of *Gardnerella vaginalis*

Here, according to the co-relationship between patients age and colony count of *Gardnerella vaginalis*, it can be seen that, average colony count in HBT is highest in case of patients aging between 25-30y.

Table 3.13 Patient's age and colony count of Gardnerella vaginalis

Age Range	Number of patients	Average colony count in HBT
15-20	7	99.71
20-25	16	85.06
25-30	17	152.94
30-35	7	119.2
35-40	3	59.00

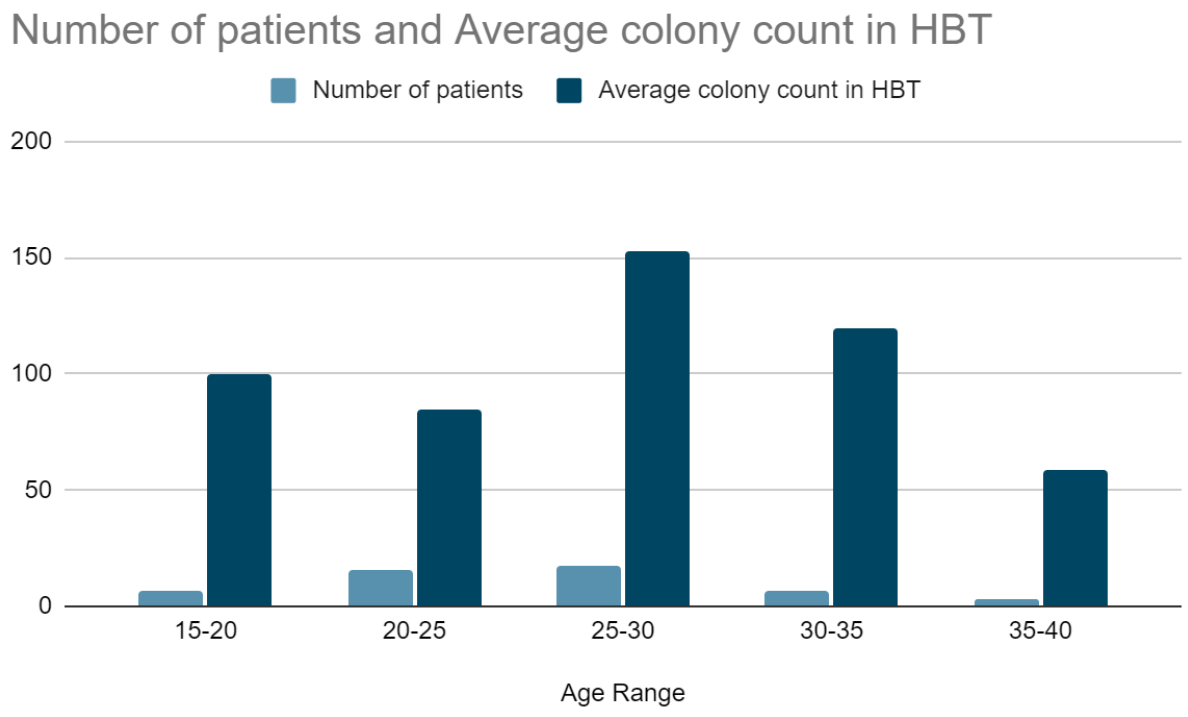


Fig: 3.13 Patient's age and colony count of Gardnerella vaginalis

3.14 Co-relationship between patients age and colony count of *Atopobium vaginae*

According to the co-relationship between patients age and colony count of *Atopobium vaginae*, it is observed that, average colony count in Blood agar is highest in case of patients aging between 30-35 years.

Table 3.14 Patient's age and colony count of *Atopobium vaginae*

Age Range	Number of patients	Average colony count in BA
15-20	7	59.14
20-25	16	59.00
25-30	17	65.29
30-35	7	142.14
35-40	3	64.66

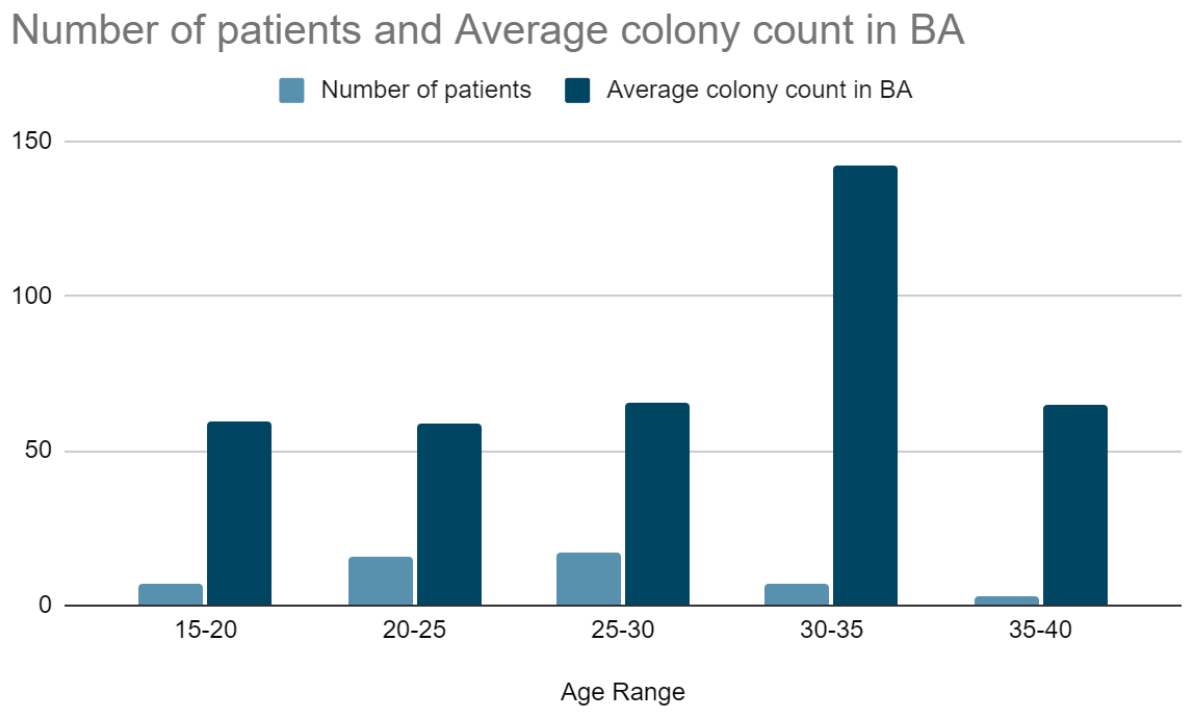


Fig: 3.14 Patient's age and colony count of *Atopobium vaginae*

3.15 Co-relationship between patients age and colony count of *Lactobacillus species*

Corresponding to the acquired data between patients age and colony count of *Lactobacillus species*, it is observed that, average colony count in MRS is highest in case of patients aging between 30-35 years.

Table 3.15 Patient's age and colony count of *Lactobacillus* species

Age Range	Number of patients	Average colony count in MRS
15-20	7	41.71
20-25	16	21.12
25-30	17	25.29
30-35	7	101.85
35-40	3	18.33

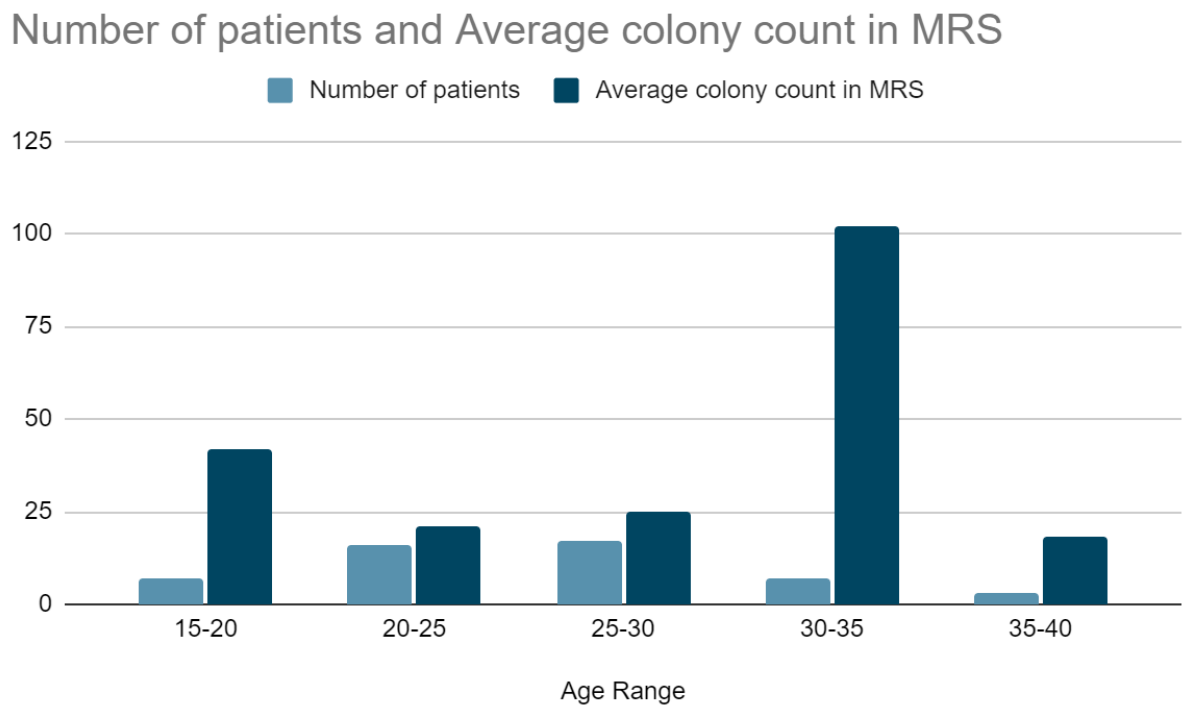


Fig: 3.15 Patient's age and colony count of *Lactobacillus* species

3.16 Co-relationship between pre-existing UTI patients & colony count of *Gardnerella vaginalis*

According to the co-relationship data between pre-existing UTI patients & colony count of *Gardnerella vaginalis*, it is observed that, average colony count in HBT is highest in case of patients who have pre-existing and ongoing UTI.

Table 3.16 pre-existing UTI patients & colony count of *Gardnerella vaginalis*

Pre-existing UTI	Number of patients'	Average colony count in HBT
Yes (ongoing)	11	135.54
No	39	106.54

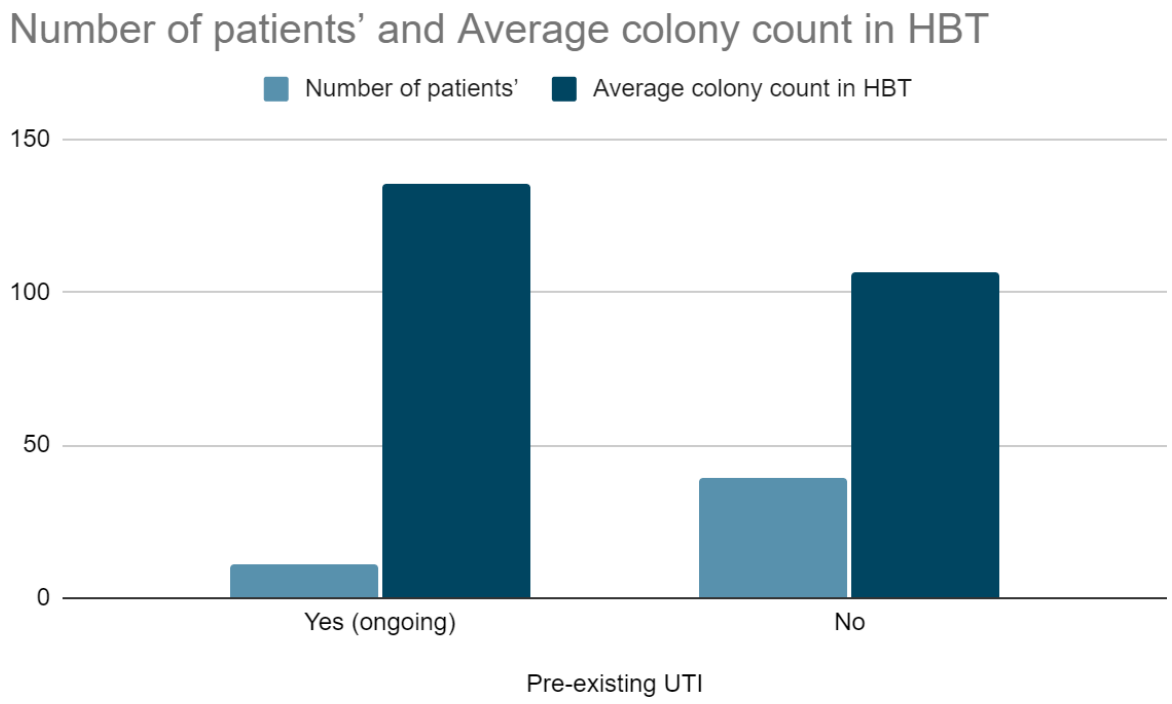


Fig: 3.16 pre-existing UTI patients & colony count of *Gardnerella vaginalis*

3.17 Co-relationship between pre-existing UTI patients and colony count of *Atopobium vaginae*

According to co-relationship between pre-existing UTI patients & colony count of *Atopobium vaginae*, it can be said that average colony count in BA is highest in case of patients who did not have any pre-existing UTI.

Table 3.17 pre-existing UTI patients & colony count of *Atopobium vaginae*

Pre-existing UTI	Number of patients'	Average colony count in BA
Yes (ongoing)	11	28.63
No	39	83.12

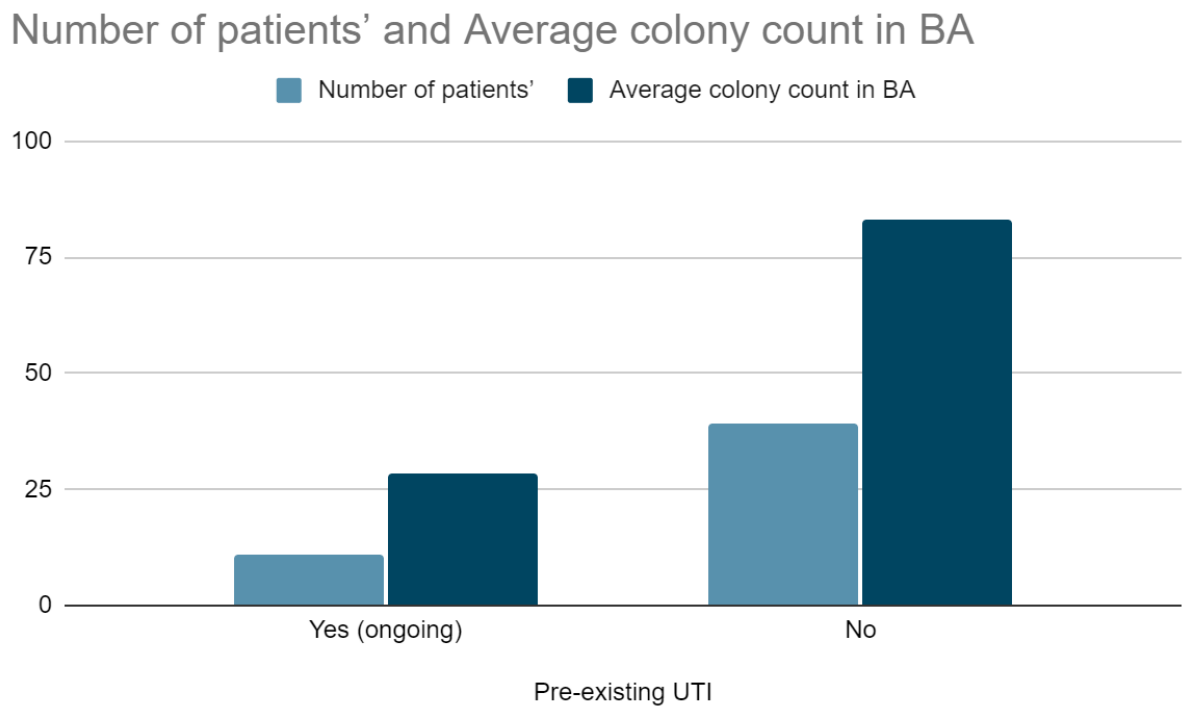


Fig: 3.17 pre-existing UTI patients & colony count of *Atopobium vaginae*

3.18 Co-relationship between pre-existing UTI patients and colony count of *Lactobacillus species*

According to co-relationship between pre-existing UTI patients & colony count of *Lactobacillus species*, it can be said that average colony count in MRS is highest in case of patients who did not have any pre-existing UTI.

Table 3.18 pre-existing UTI patients & colony count of *Lactobacillus species*

Pre-existing UTI	Number of patients'	Average colony count in MRS
Yes (ongoing)	11	37
No	39	52.70

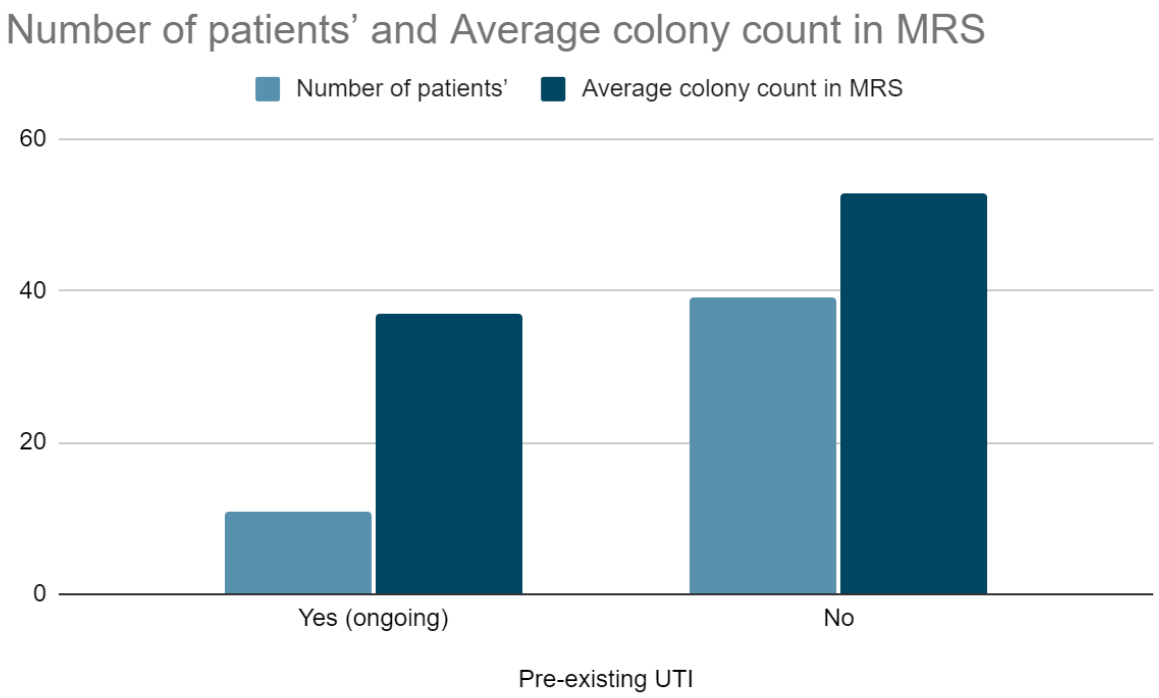


Fig 3.18 pre-existing UTI patients & colony count of *Lactobacillus species*

3.19 Co-relationship between pre-existing Vaginal discharge patients & colony count of *Gardnerella vaginalis*

According to co-relationship between patients who had pre-existing vaginal discharge & colony count of *Gardnerella vaginalis*, it can be said that average colony count in HBT is highest in case of patients who had pre-existing vaginal discharge.

Table 3.19 pre-existing Vaginal discharge patients & colony count of Gardnerella vaginalis

Vaginal discharge	Number of patients	Average colony count in HBT
Yes	42	116.19
No	8	96.37

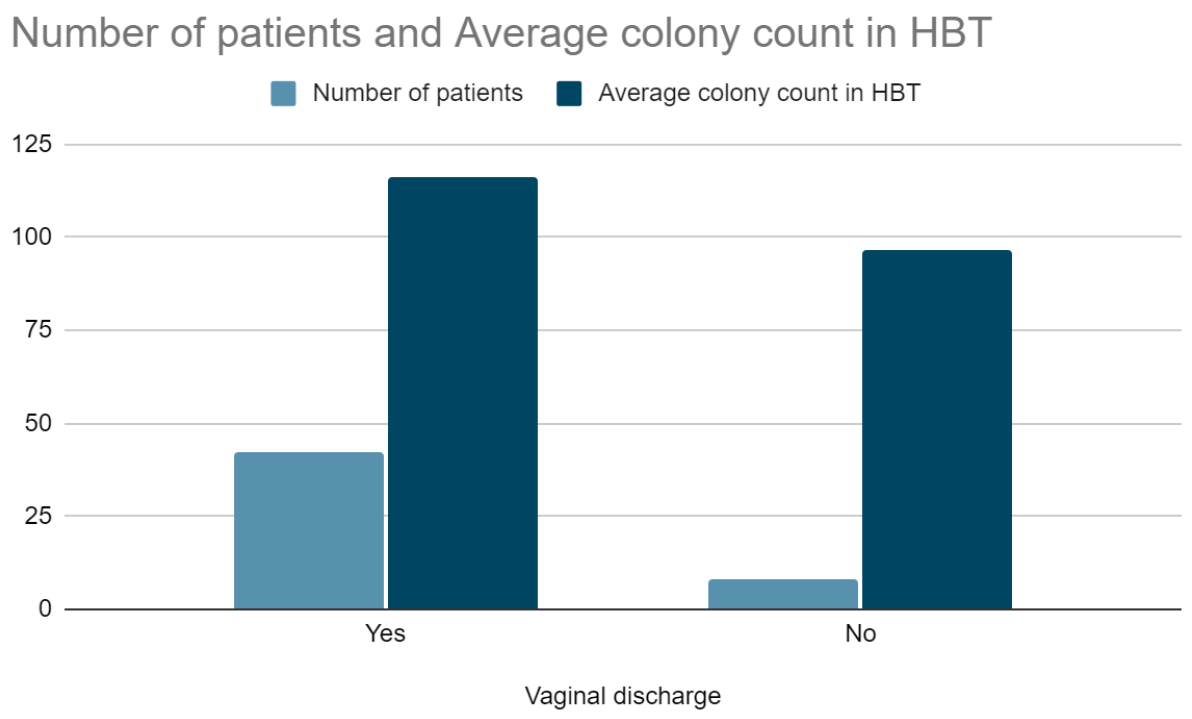


Fig: 3.19 co-relationship between pre-existing Vaginal discharge patients & colony count of *Gardnerella vaginalis*

3.20 Co-relationship between pre-existing Vaginal discharge patients & colony count of *Atopobium vaginae*

According to co-relationship between patients who had pre-existing vaginal discharge & colony count of *Atopobium vaginae*, it can be said that average colony count in BA is highest in case of patients who had pre-existing vaginal discharge.

Table 3.20 pre-existing Vaginal discharge patients & colony count of *Atopobium vaginae*

Vaginal discharge	Number of patients	Average colony count in BA
Yes	42	70.07
No	8	66.25

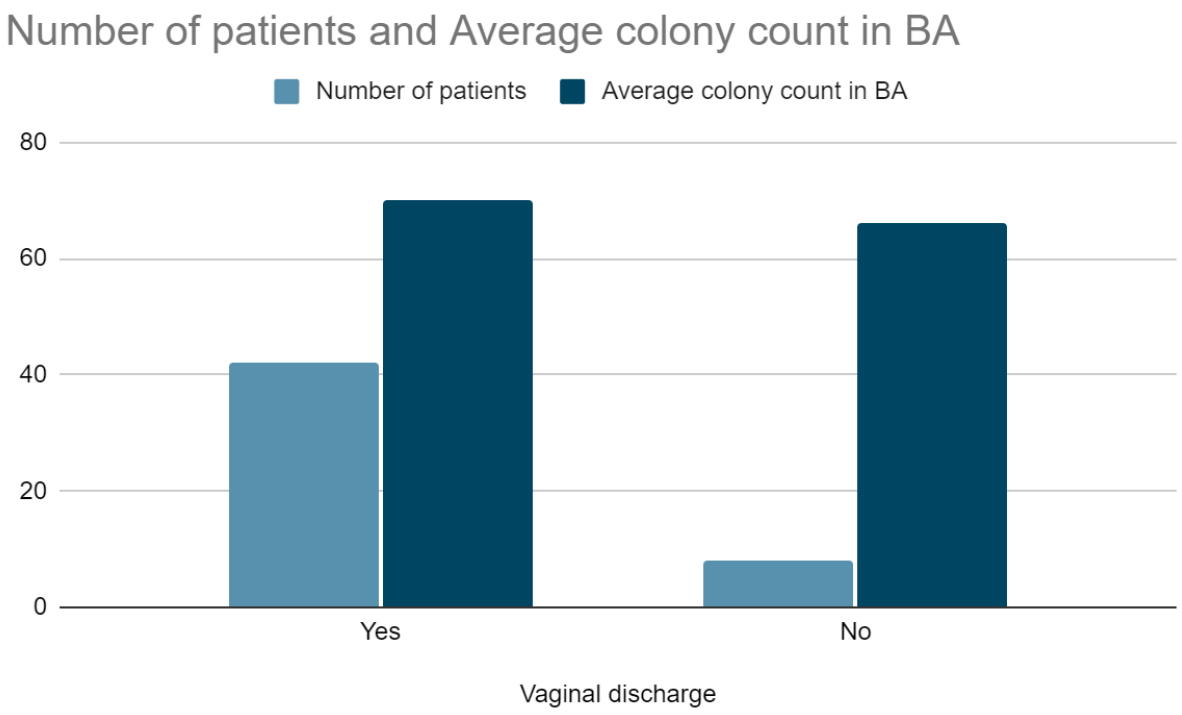


Fig: 3.20 pre-existing Vaginal discharge patients & colony count of *Atopobium vaginae*

3.21 Co-relationship between pre-existing Vaginal discharge patients & colony count of *Lactobacillus species*

According to the co-relationship data of patients who had pre-existing vaginal discharge & colony count of *Lactobacillus species*, it can be said that average colony count in MRS is highest in case of patients who did not have any pre-existing vaginal discharge. The chart & data is given below:

Table 3.21 pre-existing Vaginal discharge patients & colony count of *Lactobacillus species*

Vaginal discharge	Number of patients	Average colony count in MRS
Yes	42	33.61
No	8	65.37

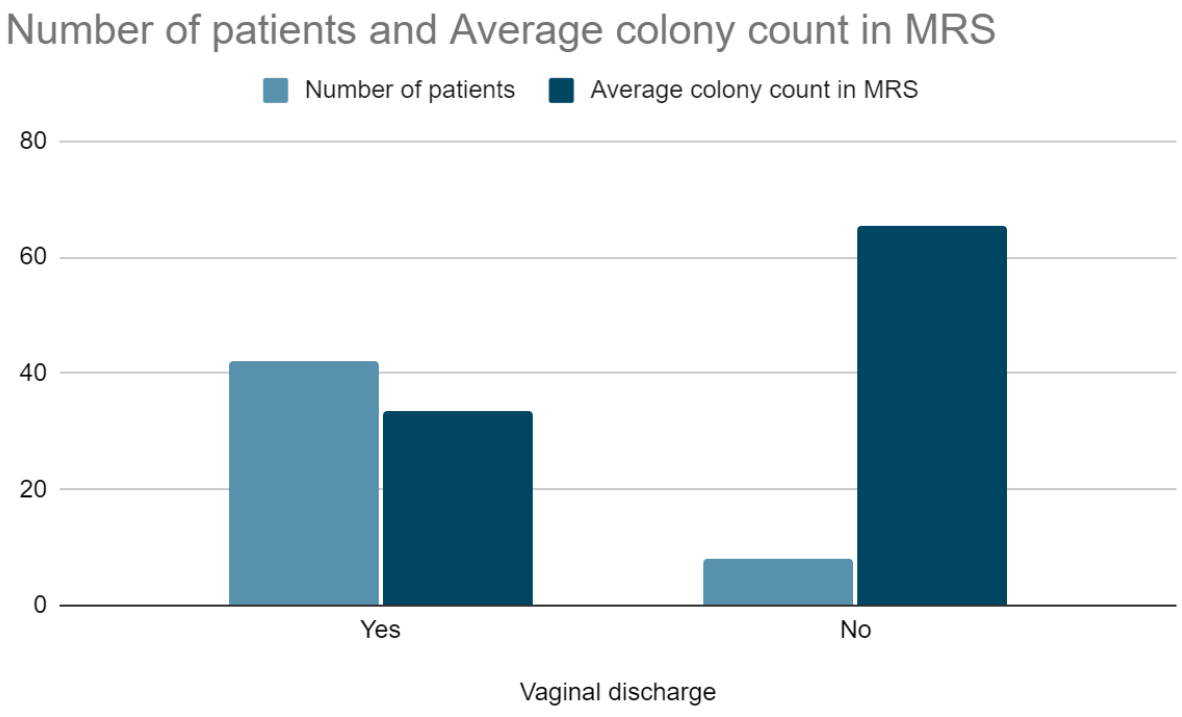


Fig: 3.21 Co-relationship between pre-existing Vaginal discharge patients & colony count of *Lactobacillus species*

-Discussion-

4.0 Discussion: The initial purpose of this study was to define the vaginal flora associated with bacterial vaginosis using clinical indicators as well as laboratory methods.

In multivariable analyses, three microorganisms consistently appeared more frequently among women with bacterial vaginosis: *Garderenella vaginalis, Atopobium vaginae* and *prevotella bivia*. Because these organisms are present in the vagina of pregnant women as a normal microbiota, their presence does not indicate that the patient is suffering from bacterial vaginosis. However, if the bacterial load of *Gardeneralla vaginalis, Atopobium vaginae,* and *Prevotella bivia* is high while the load of lactobacillus species is low, we can conclude that the patient has Bacterial vaginosis. Sociodemographic variables, previous health history, previous UTI and BV record, vaginal discharge, and weight have all been linked to variations in bacterial vaginosis prevalence rates. The majority of the patients in the study (n=50) were young adults between the ages of 25 and 30, who had the highest average colony count in both HBT and Blood agar, respectively 152.94 and 65.29, but also the lowest average colony count of 25.29, indicating that the age group between 25 and 30 has the highest risk of bacterial vaginosis, as reported in a study. (Ibrahim et al. 2014)However, this study also demonstrated that, as in earlier investigations, the highest prevalence of bacterial vaginosis was found in women when they are pregnant. The bulk of the patients in the study group (n=50) had a pregnancy duration of 35-40 weeks (78 percent) had the highest prevalence record of bacterial vaginosis and this finding is similar with a study by Goldenberg RL, Klebanoff MA, Nugent R, et al. In our study, 82% of patients are from urban areas, whereas only 18% of women are from rural areas and this finding supports the fact that patients who are from rural areas have a greater chance of suffering from bacterial vaginosis. Furthermore, according to our findings, 11 out of 50 patients have a prior UTI, and these patients had a higher average colony count than those who did not have a prior UTI of 135.54 and 28.63 for HBT and Blood agar, respectively, but had a very low average colony count of 37 for Lactobacillus species, which is lower than 52.70 for patients without prior UTI, indicating that patients with a prior UTI have a higher risk of bacterial vaginosis. However, this finding is exactly matching with other study of *Hillebrand L, Harmanli OH, Whiteman V, Khandelwal M* where they are claiming that women suffering from bacterial vaginosis (BV) are at a greater risk of urinary tract infections than others. Furthermore, according to our findings, 44 out of 50 patients had no previous BV history, with only 6 women having a history of BV which indicates that previous record of bacterial vaginosis did not affect that much in causing bacterial vaginosis during

pregnancy. Most of the patients in the study cohort (n=50) weigh between 55 and 65 kg, accounting for 56 percent which claim that women with medium weight have a higher chance of developing bacterial vaginosis.

According to our findings, 84 percent of patients have a primarily white vaginal discharge, which is a clinical hallmark of BV, while 16 percent have no vaginal discharge. Furthermore, patients with vaginal discharge had a higher average colony of 116.19 and 70.67 in HBT and Blood agar, respectively, than patients without vaginal discharge, who had average colony count of 96.37 and 66.25 in HBT and Blood agar, respectively. Furthermore, individuals with vaginal discharge have a lower average Lactobacillus species colony count of 33.61 compared to patients without vaginal discharge who had a colony count of 65.37 on MRS agar, indicating that having vaginal discharge is a presumptive sign of bacterial vaginosis is similar to the study of Ibrahim SM, Bukar M, Galadima GB, Audu BM, Ibrahim HA et al findings. After culturing the vaginal swab in HBT, Blood agar and MRS nutrition plate for detection of *Garderella vaginalis, Atopobium vaginae* and *lactobacillus spp* growth was observed in most of the culturing plate. However, the CFU number varied from patient to patient for Gardnerella vaginalis. Most (64.4%) of the patients have CFU between 0-100 and heavy growth of the bacteria was seen in 17.8% patients. This result is quite similar for *Atopobium vaginae* where most (71.1%) of the patients have CFU between 0-100 and heavy growth of the bacteria was seen in 8.9% patients. Facultative lactobacilli were isolated less frequently from women with bacterial vaginosis, regardless of the method used to diagnose it. In our study, Growth was seen in the majority of plates after culturing on MRS for the detection of Lactobacillus species. The amount of CFUs, on the other hand, differed from patient to patient. The majority of the patients (86.7%) had CFU between 0 and 100, while 6.7% have significant bacterial growth. Furthermore, patients with higher cultural growth for Gardnerella vaginalis and *Atopobium vaginae* have lower growth for lactobacillus spp, implying that the majority of the patients have bacterial vaginosis. This finding of excessive presence of *Gardnerella vaginalis* and *Atopobium vaginae* lower the bacterial load of *Lactobacillus* species is an indicator of bacterial vaginosis is consistent with a study by. Martius, J., M. The study had a few limitations. Firstly, the sample size was too small to come down to a specific conclusion. Second, after cultivating *Atopobium vaginae* in Blood agar, an API biochemical test is required for precise confirmation.

However, bacterial vaginosis identification by screening though culture-based method is least sensitive compared to other method such as Nugent's scoring method which is considered as gold standard method for screening of bacterial vaginosis (Bhat et al.2011), we have used culture-based method because our ultimate target is to screen those suspected bacteria through molecular level by performing PCR which has the highest accuracy rate. Furthermore, Nugent scoring can be quite difficult method for beginners those have no previous experience in this field and can contribute to False positive results.

-References-

References:

1.Backer, Ellen De, Rita Verhelst, Hans Verstraelen, Mohammed A Alqumber, Jeremy P Burton, John R Tagg, Marleen Temmerman, and Mario Vaneechoutte. 2007. "Quantitative Determination by Real-Time PCR of Four Vaginal Lactobacillus Species, Gardnerella Vaginalis and Atopobium Vaginae Indicates an Inverse Relationship between L. Gasseri and L. Iners." *BMC Microbiology* 7 (1): 1–13.

2.Barry, A L, G D Fay, and R L Sauer. 1972. "Efficiency of a Transport Medium for the Recovery of Aerobic and Anaerobic Bacteria from Applicator Swabs." *Applied Microbiology* 24 (1): 31–33.

3.Begum, Afroza, Sofia Nilufar, Khaleda Akther, Abdur Rahman, Fatema Khatoon, Motiur Rahman, and Fatema Khatun. 2003. "Prevalence of Selected Reproductive Tract Infections among Pregnant Women Attending an Urban Maternal and Childcare Unit in Dhaka, Bangladesh." *Journal of Health, Population and Nutrition*, 112–16.

4.Bhat, Gopalkrishna, Subbannayya Kotigadde, and Shalini Shenoy. "Comparison of the methods of diagnosis of bacterial vaginosis." *Journal of Clinical and Diagnostic Research* 5, no. 3 (2011): 498-501.

5.Boustouller, Yvonne L, A P Johnson, and D Taylor-Robinson. 1987. "Pili on Gardnerella Vaginalis Studied by Electronmicroscopy." *Journal of Medical Microbiology* 23 (4): 327–29.

6.Brook, Itzhak. 1998. "Microbiology of Common Infections in the Upper Respiratory Tract." *Primary Care* 25 (3): 633–48.

7.Casari, Erminia, Antonella Ferrario, Emanuela Morenghi, and Alessandro Montanelli. 2010. "Gardnerella, Trichomonas Vaginalis, Candida, Chlamydia Trachomatis, Mycoplasma Hominis and Ureaplasma Urealyticum in the Genital Discharge of Symptomatic Fertile and Asymptomatic Infertile Women." *The New Microbiologica* 33 (1): 69.

8.Donders, G G, Kristel Van Calsteren, G Bellen, R Reybrouck, T Van den Bosch, I Riphagen, and S Van Lierde. 2009. "Predictive Value for Preterm Birth of Abnormal Vaginal Flora, Bacterial Vaginosis and Aerobic Vaginitis during the First Trimester of Pregnancy." *BJOG:*

An International Journal of Obstetrics & Gynaecology 116 (10): 1315–24.

9.ESBROECK, MARJAN VAN, Peter Vandamme, Enevold Falsen, Marc Vancanneyt, Edward Moore, Bruno Pot, Françoise Gavini, Karel Kersters, and Herman Goossens. 1996. "Polyphasic Approach to the Classification and Identification of Gardnerella Vaginalis and Unidentified Gardnerella Vaginalis-like Coryneforms Present in Bacterial Vaginosis." *International Journal of Systematic and Evolutionary Microbiology* 46 (3): 675–82.

10.Eschenbach, David A, Pamela R Davick, Betsy L Williams, Seymour J Klebanoff, Karen Young-Smith, Cathy M Critchlow, and King K Holmes. 1989. "Prevalence of Hydrogen Peroxide-Producing Lactobacillus Species in Normal Women and Women with Bacterial Vaginosis." *Journal of Clinical Microbiology* 27 (2): 251–56.

11.Eschenbach, David A, Sharon Hillier, Cathy Critchlow, Claire Stevens, Timothy DeRouen, and King K Holmes. 1988. "Diagnosis and Clinical Manifestations of Bacterial Vaginosis." *American Journal of Obstetrics and Gynecology* 158 (4): 819–28.

12.Fredricks, David N, Tina L Fiedler, and Jeanne M Marrazzo. 2005. "Molecular Identification of Bacteria Associated with Bacterial Vaginosis." *New England Journal of Medicine* 353 (18): 1899–1911.

13.Garrett, Wendy S, and Andrew B Onderdonk. 2015. "Bacteroides, Prevotella, Porphyromonas, and Fusobacterium Species (and Other Medically Important Anaerobic Gram-Negative Bacilli)." In *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 2773–80. Elsevier.

14.Goldenberg, Robert L, Jay D Iams, Brian M Mercer, Paul J Meis, Atef H Moawad, R L Copper, Anita Das, Elizabeth Thom, Francee Johnson, and Donald McNellis. 1998. "The Preterm Prediction Study: The Value of New vs Standard Risk Factors in Predicting Early and All Spontaneous Preterm Births. NICHD MFMU Network." *American Journal of Public Health* 88 (2): 233–38.

15.Guerra, Brunella, Tullio Ghi, Simona Quarta, Antonio Maria Morselli-Labate, Tiziana Lazzarotto, Gianluigi Pilu, and Nicola Rizzo. 2006. "Pregnancy Outcome after Early Detection of Bacterial Vaginosis." *European Journal of Obstetrics & Gynecology and Reproductive Biology* 128 (1–2): 40–45.

16.Hay, Phillip E, Ronald F Lamont, David Taylor-Robinson, D John Morgan, Catherine Ison, and Josephine Pearson. 1994. “Abnormal Bacterial Colonisation of the Genital Tract and Subsequent Preterm Delivery and Late Miscarriage.” *Bmj* 308 (6924): 295–98.

17.Holmes, King K, Kirk C S Chen, Carolyn M Lipinski, and David A Eschenbach. 1985. “Vaginal Redox Potential in Bacterial Vaginosis (Nonspecific Vaginitis).” *Journal of Infectious Diseases* 152 (2): 379–82.

18.Ibrahim, S M, M Bukar, G B Galadima, B M Audu, and H A Ibrahim. 2014. “Prevalence of Bacterial Vaginosis in Pregnant Women in Maiduguri, North-Eastern Nigeria.” *Nigerian Journal of Clinical Practice* 17 (2): 154–58.

19.Kurki, Tapio, Aulikki Sivonen, OLLI-VEIKKO Renkonen, EEva Savia, and Olavi Ylikorkala. 1992. “Bacterial Vaginosis in Early Pregnancy and Pregnancy Outcome.” *Obstetrics and Gynecology* 80 (2): 173–77.

20.Lamont, Ronald F, Jack D Sobel, Robert A Akins, Sonia S Hassan, Tinnakorn Chaiworapongsa, Juan Pedro Kusanovic, and Roberto Romero. 2011. “The Vaginal Microbiome: New Information about Genital Tract Flora Using Molecular Based Techniques.” *BJOG: An International Journal of Obstetrics & Gynaecology* 118 (5): 533–49.

21.Łaniewski, Paweł, and Melissa M Herbst-Kralovetz. 2021. “Bacterial Vaginosis and Health-Associated Bacteria Modulate the Immunometabolic Landscape in 3D Model of Human Cervix.” *NPJ Biofilms and Microbiomes* 7 (1): 1–17.

22.Larsen, Jeppe Madura. 2017. “The Immune Response to Prevotella Bacteria in Chronic Inflammatory Disease.” *Immunology* 151 (4): 363–74.

23.Leitich, Harald, Barbara Bodner-Adler, Mathias Brunbauer, Alexandra Kaider, Christian Egarter, and Peter Husslein. 2003. “Bacterial Vaginosis as a Risk Factor for Preterm Delivery: A Meta-Analysis.” *American Journal of Obstetrics and Gynecology* 189 (1): 139–47.

24.Machado, António, and Nuno Cerca. 2015. “Influence of Biofilm Formation by Gardnerella Vaginalis and Other Anaerobes on Bacterial Vaginosis.” *The Journal of Infectious Diseases* 212 (12): 1856–61.

25.Martius, J. O. A. C. H. I. M., MARIJANE A. Krohn, SHARON L. Hillier, WALTER E. Stamm, KING K. Holmes, and DAVID A. Eschenbach. "Relationships of vaginal Lactobacillus

species, cervical Chlamydia trachomatis, and bacterial vaginosis to preterm birth." *Obstetrics and gynecology* 71, no. 1 (1988): 89-95.

26.McGregor, James A. 2000. “Bacterial Vaginosis in Pregnancy.” *Obstetrical & Gynecological Survey* 55 (5): 1–19.

27.Reid, Gregor, and Alan Bocking. 2003. “The Potential for Probiotics to Prevent Bacterial Vaginosis and Preterm Labor.” *American Journal of Obstetrics and Gynecology* 189 (4): 1202–8.

28.Spiegel, Carol A, Richard Amsel, David Eschenbach, Fritz Schoenknecht, and King K Holmes. 1980. “Anaerobic Bacteria in Nonspecific Vaginitis.” *New England Journal of Medicine* 303 (11): 601–7.

29.Srinivasan, Sujatha, Noah G Hoffman, Martin T Morgan, Frederick A Matsen, Tina L Fiedler, Robert W Hall, Frederick J Ross, Connor O McCoy, Roger Bumgarner, and Jeanne M Marrazzo. 2012. “Bacterial Communities in Women with Bacterial Vaginosis: High Resolution Phylogenetic Analyses Reveal Relationships of Microbiota to Clinical Criteria.” *PloS One* 7 (6): e37818.

30.Stewart-Tull, D E S. 1964. “Evidence That Vaginal Lactobacilli Do Not Ferment Glycogen.” *American Journal of Obstetrics and Gynecology* 88 (5): 676–79.

31.Thorsen, Poul, Inge Panum Jensen, Bernard Jeune, Niels Ebbesen, Magnus Arpi, Annie Bremmelgaard, and Birger R Møller. 1998. “Few Microorganisms Associated with Bacterial Vaginosis May Constitute the Pathologic Core: A Population-Based Microbiologic Study among 3596 Pregnant Women.” *American Journal of Obstetrics and Gynecology* 178 (3): 580–87.

32.Totten, PATRICIA A, R Amsel, J Hale, P Piot, and K K Holmes. 1982. “Selective Differential Human Blood Bilayer Media for Isolation of Gardnerella (Haemophilus) Vaginalis.” *Journal of Clinical Microbiology* 15 (1): 141–47.

33.Verstraelen, Hans, and Alexander Swidsinski. 2013. “The Biofilm in Bacterial Vaginosis: Implications for Epidemiology, Diagnosis and Treatment.” *Current Opinion in Infectious Diseases* 26 (1): 86–89.

34.Verstraelen, Hans, Rita Verhelst, Geert Claeys, Marleen Temmerman, and Mario

Vaneechoutte. 2004. “Culture-Independent Analysis of Vaginal Microflora: The Unrecognized Association of Atopobium Vaginae with Bacterial Vaginosis.” *American Journal of Obstetrics and Gynecology* 191 (4): 1130–32.