A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY

ANTIBACTERIAL AND ANTIBIOFILM EFFECT OF GRAPHENE OXIDE NANOPARTICLES ON GARDNERELLA VAGINALIS ISOLATED FROM IRAQI HOSPITALS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

BY

NOORA SABAH DAWOOD AL-HASHIM

ÇANKIRI

2023

ANTIBACTERIAL AND ANTIBOFILM EFFECT OF GRAPHENE OXIDE NANOPARTICLES ON GARDNERELLA VAGINALIS ISOLATED FROM IRAQI HOSPITALS

By Noora Sabah DAWOOD AL-HASHIM

May 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

Advisor **:** Asst. Prof. Dr. Okan ÜRKER
Co-Advisor **:** Prof. Dr. Suzan Saadi HUSSAIN

Examining Committee Members:

Chairman **:** Asst. Prof. Dr. Okan ÜRKER
Biology
Çankırı Karatekin University

Member **:** Asst. Prof. Dr. Emin BOZKURT
Biology
Çankırı Karatekin University

Member **:** Asst. Prof. Dr. Yasin İLEMİN
Environmental Sciences
Muğla Sıtkı Koçman University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. Hamit ALYAR
Director of Graduate School

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Noora Sabah Dawood AL-HASHIM

ABSTRACT

ANTIBACTERIAL AND ANTIBIOFILM EFFECT OF GRAPHENE OXIDE NANOPARTICLES ON GARDNERELLA VAGINALIS ISOLATED FROM IRAQI HOSPITALS

Noora Sabah DAWOOD AL-HASHIM

Master of Science in Biology

Advisor: Asst. Prof. Dr. Okan ÜRKER

Co-Advisor: Prof. Dr. Suzan Saadi HUSSAIN

May 2023

In this study, data from 250 high vaginal swaps performed on female patients at Al-Karama Hospital and Al-Yarmouk Hospital between July 1, 2022 and October 30, 2022 were gathered and analyzed. the presence of Clue cells in vaginal secretions also serves as persuasive evidence for the presence of G. vaginalis. Out of a total of 200 specimens, 19 (9.5) isolates of G. vaginalis were found with the use of the Vitek 2 Compact. There were only twelve G. vaginalis isolates found out of a total of 100 (50%) specimens that had white secretions and a pH range of 5–6.5. Only 5 (7.4%) of the 68 (34% of the total) specimens had yellow secretions, and G. vaginalis was found in only those with a pH range of 6.0 to 7.0. This indicates that the most common pathogen is G. vaginalis and most of its isolates are biofilm producing (100%), and Pregnant women are most vulnerable to BV than non-pregnant. Meropenem resistance was present in all 19 bacteria isolates (100%). According to the findings, 3/19 (15.8%) were somewhat positive, 13/19 (68.4%) were very positive, and 3/19 (15.8%) were negatively. The results of this study showed that 12/19 (63.2%) isolates of G. vaginalis are highly capable of forming biofilms, 3/19 (15.7%) are somewhat capable of doing so. The results showed that the lowest inhibitory concentration (Sub-MIC) of GO nanomaterials for three of the isolates respectively was 100 g/ml, 125 g/ml, 175 g/ml.

2023, 79 pages

Keywords: *Gardnerella vaginalis***,** Biofilm**,** Graphene oxide nanoparticles

ÖZET

IRAK HASTANELERİNDEN İZOLE EDİLEN GARDNERELLA VAGINALIS ÜZERİNDE GRAFEN OKSİT NANOPARTİKÜLLERİNİN ANTİBAKTERİYEL VE ANTİBİOFİLM ETKİSİ

Noora Sabah Dawood AL-HASHIM
Biyoloji, Yüksek Lisans
Tez Danışmanı: Dr. Öğr. Üyesi Okan ÜRKER
Eş Danışman: Prof. Dr. Suzan Saadi HUSSAIN
Mayıs 2023

Bu çalışmada, 1 Temmuz 2022 ile 30 Ekim 2022 tarihleri arasında Al-Karama Hastanesi ve Al-Yarmouk Hastanesinde kadın hastalar üzerinde gerçekleştirilen 250 yüksek vajinal takastan elde edilen veriler toplanmış ve analiz edilmiştir. Vajinal sekresyonlarda Clue hücrelerinin varlığı da G. vaginalis'in varlığına dair ikna edici bir kanıt görevi görür. Toplam 200 örnekten 19'unda (9,5) Vitek 2 Compact kullanılarak G. vaginalis izolatı bulundu. Beyaz sekresyonlara ve pH aralığı 5-6.5 olan toplam 100 (%50) örnekten yalnızca on iki G. vaginalis izolatı bulundu. 68 örneğin (toplamın %34'ü) yalnızca 5'inde (%7,4) sarı sekresyonlar vardı ve yalnızca pH aralığı 6,0 ila 7,0 olanlarda G. vaginalis bulundu. Bu, en yaygın patojenin G. vajinalis olduğunu ve izolatlarının çoğunun (%100) biyofilm ürettiğini ve hamile kadınların hamile olmayanlara göre BV'ye karşı daha savunmasız olduğunu gösterir. Meropenem direnci 19 bakteri izolatının hepsinde (%100) mevcuttu. Bulgulara göre 3/19 (%15,8) biraz olumlu, 13/19 (%68,4) çok olumlu ve 3/19 (%15,8) olumsuz. Bu çalışmanın sonuçları, G. vaginalis'in 12/19 (%63,2) izolatının yüksek oranda biyofilm oluşturma yeteneğine sahip olduğunu, 3/19 (%15,7) izolatının bunu bir şekilde yapabildiğini gösterdi. Sonuçlar, izolatların üçü için GO nanomateryallerinin en düşük inhibe edici konsantrasyonunun (Sub-MIC) sırasıyla 100g/ml, 125 g/ml, 175 g/ml olduğunu gösterdi.

2023, 79 sayfa

Anahtar Kelimeler: *Gardnerella vaginalis*, Biyofilm, Grafen oksit nanoparçacıkları

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Asst. Prof. Dr. Okan Urker, and Prof. Dr. Suzan Saadi HUSSAIN for their patience, guidance and understanding.

Noora Sabah Dawood AL-HASHIM

Çankırı-2023

CONTENTS

ABSTRACT ..... **i**
ÖZET ..... **ii**
PREFACE AND ACKNOWLEDGEMENTS ..... **iv**
CONTENTS ..... **v**
LIST OF SYMBOLS ..... **viii**
LIST OF ABBREVIATIONS ..... **ix**
LIST OF FIGURES ..... **x**
LIST OF TABLES ..... **xi**
1. INTRODUCTION **1**
1.1 Aim of Study **3**
2. LITERATURE REVIEW **5**
2.1 Bacteriological Characters of Gardnerella Vaginalis **5**
2.2 Etiology **6**
2.3 Epidemiology **9**
2.4 Gardnerella Vaginalis in the Pathogenesis **10**
2.5 Bacterial Vaginosis (BV) **12**
2.6 Virulence Factor of G. vaginalis **14**
2.6.1 Hemolysin production **14**
2.6.2 Sialidase prodaction **15**
2.6.3 Adherence to vaginal epithelial cells **16**
2.6.4 Biofilm production **17**
2.7 Antibiotic Resistance **23**
2.8 Nanomaterials **25**
2.8.1 The organic NPs **26**
2.8.2 The inorganic NPs **26**
2.8.3 Nanomaterials as antimicrobials **27**
2.8.4 Graphene oxide nanomaterials **29**
3. MATERIALS AND METHODS **33**
3.1 Equipments and Apparatus **33**
3.2 Methods **34**

3.2.1 Sterilization methods 34
3.2.2 Preparation of reagents and solutions 35
3.2.3 Solutions 36
3.2.4 Preparation of cultural media 36
3.2.5 Collection of samples 38
3.3 Laboratory Diagnosis 38
3.3.1 Bacterial identification assay 38
3.3.2 Presumptive identification 39
3.3.3 Confirmation identification 40
3.3.4 Identification using vitek 2 system 41
3.4 Maintenance of Bacterial Isolates 42
3.4.1 Short term maintenance 42
3.4.2 Long term maintenance 42
3.4.3 Detection of biofilm production 42
3.4.4 Graphene oxide nanomaterials (GO) 43
4. RESULTS AND DISCUSSION 48
4.1 Selection of Patients 48
4.2 Cultural and Phenotypic Character 50
4.3 Microscopic Findings 51
4.4 Biochemical Test 52
4.5 Susceptibility of *G. aginalis* to Antibiotic by Disk Diffusion Method 52
4.5.1 Kirby bauer test 52
4.6 Phenotyping Etection of Virulence Factor (Biofilm Formation). 53
4.6.1 Congo red agar method (CRA) (Qualitative) 53
4.6.2 Microtiter plate method 54
4.7 Characterization of Graphene Oxide Nanomaterials (GO) 55
4.7.1 Fourier transform-infrared spectroscopy (FT-IR) analysis 55
4.7.2 X-ray Diffraction (XRD) analysis 56
4.7.3 Scanning electron microscopy (SEM) analysis 57
4.7.4 Energy dispersive X-ray spectrometer (EDS) analysis 58
4.8 Determination of Sub-Minimum Inhibitory Concentration (Sub-MIC) of Graphene Oxide Aanomaterials 59

4.8.1 Microdilution method.....59

4.8.2 Well diffusion method 60

4.9 The effect of Sub-Minimum Inhibitory Concentration (Sub-MIC) of Graphene Oxide Nanomaterials (GO) on Biofilm Formations.....61

5 CONCLUSIONS AND RECOMMENDATION.....63

5.5 Conclusion.....63

5.6 Recommendation.....63

REFERENCES.....64

CURRICULUM VITAE.....79

LIST OF SYMBOLS

G	Gram
mg/dL	Milligram / deciliter
mg/mL	Microgram/milliliter
mL	Milliliter
µL	Micro letter

LIST OF ABBREVIATIONS

AFM	Acute Flaccid Myelitis
BV	Bacterial Vaginosis
CRA	Congored Agar
D.W	Distilled water
DNA	Deoxyribonucleic acid
ECM	Extracellular Matrix
EDS	Energy-Dispersive X-ray Spectroscopy
EPS	Extracellular Polymeric Matrix
FT-IR	Fourier Transform-Infrared spectroscopy
GO	Graphene Oxide
HIV	Human Immunodeficiency Virus
MDR	Mutidrug Resistance
MTP	Medical Termination Pregnancy
NPs	Nan Partecls
PTD	Pre-Term Dlivery
SEM	Scanning Electron Microscopy
SWCNTs	Single Long Wrapped Graphene Sheet
XRD	X-ray Diffractometer

LIST OF FIGURES

Figure 2.1 Clinical samples showing evidence of BV-positive and BV-negative vaginal smears (Morrill 2020)..... 13
Figure 2.2 Biofilm life cycle (Karin *et al.* 2022) 19
Figure 2.3 Bactericidal activity of GO against Gardnerella vaginalis (Aunkor *et al.* 2020). 31
Figure 4.1 The distribution of the 200 of the collected specimens according to the color secretions..... 49
Figure 4.2 Gardnerella vaginalis appears Non-hemolytic on blood agar..... 50
Figure 4.3 The gram stain (X1000) of bacterial vaginosis showing clue cell with gram variable *G. Vaginalis* 51
Figure 4.4 Biofilm detection of *G. vaginalis* by Congo red agar method, showing strongly positive..... 54
Figure 4.5 The FT-IR absorption spectrum of GO Nanomaterials 56
Figure 4.6 The X-ray diffraction spectrum of GO Nanomaterials..... 57
Figure 4.7 The SEM image of GO Nanomaterials..... 58
Figure 4.8 EDS pattern of GO Nanomaterials purity..... 59
Figure 4.9 Inhibition Zone for (a) Graphene Oxide Nanomaterials (GO), against G. vaginalis on Mueller-Hinton ag ar plates 61

LIST OF TABLES

Table 3.1 The following equipment's and apparatus 33
Table 3.2 Chemicals 33
Table 3.3 Antimicrobial agents and Antimicrobial agents discs 34
Table 3.4 Cultural media 34
Table 3.5 Kits 34
Table 4.1 The distribution of the 200 of the collected specimens according to the color secretions 49
Table 4.2 The variable susceptibility of *G. vaginalis* isolates towards different locally available antibiotics 53
Table 4.3 Biofilm production of *G. vaginalis* by Congo red method 54
Table 4.4 Biofilm production of *G. vaginalis by* Microtiter plate method 55
Table 4.5 Sub-MIC for GO Nanomaterials 60
Table 4.6 Effect of GO Nanomaterials on biofilm formation 62

1. INTRODUCTION

Gardnerella vaginalis is the sole species of bacteria that belong to the genus Gardnerella. This Gram-variable-staining, non-motile, non-sporing, facultatively anaerobic coccobacilli bacteria is the only member of the genus Gardnerella. (Lyudmila *et al.* 2021) The microorganisms in issue have a diameter that falls somewhere between 1.0 and 1.5 μm, which is a measure of how small they are. They are capable of pleomorphism and take on the morphology of coccobacilli, but they are unable to produce spores and they cannot move. The specimen features a cell wall that is Gram-positive; but, due to the fact that it is so thin, it has the potential to exhibit either a Gram-positive or a Gram-negative morphology when viewed via a microscope. The microscopic correlation between the presence of epithelial cells coated with bacteria, also known as clue cells, has been demonstrated. These cells are usually referred to as "clue cells" (Morrill *et al.* 2020).

Collaborating with trillions of other bacteria to maintain a healthy balance in the vaginal flora and protect against infection. Bacterial vaginosis, also known as bacterial vaginosis (BV), is a common reproductive health issue that can be caused when Gardnerella goes rogue and starts growing at an abnormally high rate (Aved *et al.* 2019). Constitutes a species, along with a large number of other bacteria, the most of which are anaerobic, is implicated in the development of bacterial vaginosis in certain women as a consequence of a disruption in the normal vaginal microbiota (Boyanova *et al.* 2021). G. vaginalis, which was once known as Haemophilus vaginalis and then renamed Corynebacterium vaginalis, can be seen growing as small, circular, convex, gray colonies on chocolate agar. It can also be found growing on human blood bilayer agar media with Tween 80 (commonly known as HBT agar). The use of colistin-oxolinic acid blood agar as a medium makes it possible to selectively culture G. vaginalis (Muktad and Nawfaml 2022).

G. vaginalis. It is also possible to diagnose it based on the vaginal discharge of 40–50% of women who do not have any symptoms. It is the most common type of bacterium

found in women who suffer from vaginosis. Bacterial vaginosis is a disorder that can be avoided by taking appropriate precautions against the overgrowth of potentially harmful bacteria in the vagina (BV) (Gajdács *et al.* 2020). Gardnerella vaginalis is a type of bacteria that can be beneficial to development but is not in and of itself a cause for concern. Gardnerella is already present in your body at low concentrations, but as it multiplies, it wreaks havoc on the microbiota that lives in your vaginal tract. This might even.

Induce symptoms of BV such as discomfort, itching, and unusual discharge from the body. The lactobacillus community in the vaginal environment is facultatively anaerobic, which contributes to the vaginal environment's acidic nature (Pleckaityte *et al.* 2012).

In most cases, the formation of the biofilm is comprised of three stages: the initial adhesion stage, the accumulation stage, and the dispersal stage. The first attachment of BV biofilms to the vaginal epithelium is a necessary step that must be taken before further growth can occur. Despite the fact that considerable quantities of bacteria from a variety of families were found in the biofilm, it was found that G. vaginalis was the most prevalent component of the biofilm. This was identified despite the fact that the biofilm contained significant amounts of bacteria (Lopes *et al.* 2011). As of right now, it is common knowledge that the existence of biofilms in bacterial vaginosis (BV) is highly associated with the presence of Gardnerella vaginalis. This assertion has been supported by a multitude of later research that have provided additional evidence to back up these findings (António and Nuno 2015).

In this day and age, the widespread appearance and transmission of bacteria that are resistant to antibiotics pose a significant threat to public health and call for concerted action on a worldwide scale to combat the potential for further escalation of the problem (Javed *et al.* 2019). The proliferation of G. vaginalis strains that are resistant to antibiotic treatment has made it necessary to search for effective alternatives to antibiotics in order to battle these strains, which constitute a substantial global risk. It is

very necessary to take some kind of action in order to stop the proliferation of these resistant types (Gajdács *et al.* 2019).

In light of this, research into the effects of nanomaterials, such as graphene oxide (GO), was conducted as an alternative to the use of antibiotics in the treatment of bacterial vaginosis (BV), which is caused by germs that are resistant to many drugs (Leos-Alvarado *et al.* 2020). In clinical settings, it is abundantly clear that there is a requirement for the availability of more efficient therapies for bacterial vaginosis (BV). Specifically, treatments that are able to disrupt the biofilms that are integral to the beginning and reappearance of this microbial imbalance are required. These biofilms have been shown to play an important role in both of these events (Abebaw *et al.* 2021).

The research into the etiology of BV and the associated risk factors in Iraq is disorganized, and there is a lack of knowledge on the agent responsible for the disease, its prevalence, the distribution of antibiotic resistance, and related factors. This is as a result of a lack of resources to provide diagnostic materials for aerobic and anaerobic culture and treatment facilities, as well as the fact that researchers have not shown an increased interest in doing research on BV over the course of the past decade (Lyudmila *et al.* 2021). These variables are contributing to a significant increase in the number of maternal deaths, morbidities, and disabilities in nations with minimal resources. As a result, we were motivated to conduct this study on the prevalence of BV among women of reproductive age. The early diagnosis and treatment of BV are the strategies that are used for the prevention and control of the disease (Arroyo-Moreno *et al.* 2022).

1.1 Aim of Study

1. The present study was performed to investigate from
2. The effect of Graphene-oxide-nanoparticles on bacterial growth.
3. The effect of Graphene-oxide-nanoparticles on biofilm formation
4. To achieve those aims, the following steps have been followed:

- Collection of swabs from Bacterial Vaginosis BV and identification of G. vaginalis isolates by biochemical tests and confirmed by using the VITEK 2 compact system.
- Determination of antibacterial susceptibility patterns of G. vaginalis by using Kirby-Bauer (disk).
- Studying the ability of G. vaginalis bacteria to produce biofilms.
- Characterization of the Graphene oxide nanoparticles by FT-IR, XRD, SEM, UV- and EDS analysis.
- Study the effect of graphene-oxide-nanoparticles on isolates. G. vaginalis isolate.
- Studying the effect of Graphene oxide (GO) nanoparticles on biofilm formation.

2. LITERATURE REVIEW

2.1 Bacteriological Characters of Gardnerella Vaginalis

Gardnerella vaginalis is the only member of the Gardnerella genus and belongs to the endogenous vaginal flora found in women of reproductive age. It is a nonsporting, catalase-negative, gram-variable rod that lives in a facultatively anaerobic environment. Smears stained with gram stain show these coccobacilli to be gram-variable rods of a characteristically narrow width. This bacillus has the appearance of granule (Colonna *et al.* 2020).

Although the cell wall of this bacterium (Gardnerella vaginalis) is comparable to that of gram-positive bacteria, the staining reveals that it is a variable or negative gram. In addition to that, it generates an exopolysaccharide as well as a pilli (Randis *et al.* 2013). On chocolate agar, Gardnerella vaginalis forms colonies that are minute, round, convex, and gray in color. Additionally, it can be cultured on human blood bilayer-Tween 80 agar (also known as HBT agar). Because of its capacity for selectivity, the exploitation of colistin-oxolinic acid blood agar has been suggested as a potential method for the isolation of Gardnerella vaginalis. This is because of the selective qualities of the medium. Although the detection of G. vaginalis in a vaginal culture is suggestive of bacterial vaginosis, it is not regarded as the conclusive diagnostic criterion because asymptomatic women can also be carriers of this bacterium (Boyanova *et al.* 2021).

The organism is quite particular about its diet and possesses a sluggish, fermentative metabolism, via which it mostly generates acetate and lactate. However, it is able to ferment glucose, maltose, and sucrose despite the fact that it does not produce catalase or urease (Douglas *et al.* 2022). The organism can grow at temperatures ranging from 25 to 42 degrees Celsius, although it achieves the best growth at temperatures between 35 and 37 degrees Celsius. It can only grow in an environment with a pH between 6-7, and the pH level that is best for its development is anywhere between 6.0 and 6.5 (Wilson 2005).

Gardnerella vaginalis is a stationary organism that may function either aerobically or anaerobically, lacks catalase and oxidase, and has a fermentative metabolic pathway. After subculturing, this bacterium is difficult to cultivate because it grows poorly in liquid medium and is difficult to cultivate overall. In most cases, it becomes visible after 48 hours on Columbia sheep blood agar after being cultured in a humid environment and supplemented with carbon dioxide (Boyanova *et al.* 2021).

Gardnerella agars, which may be produced with either rabbit or human blood, show evidence of beta-type hemolysis when the bacteria are present. The bacteria take the form of very small, spherical, opaque, smooth colonies. MacConkey agar does not support the growth of G. vaginalis. G. vaginalis is an organism that dwells in the vagina of females. Between 20 and 40 % women are considered to be healthy carriers. Although sexual contact is the most common mode of transmission, the infection has on occasion been found in women who have never been sexually active (Morrill *et al.* 2020).

2.2 Etiology

Bacterial vaginosis, which is more commonly abbreviated to BV, is an infection that affects the vaginal tract and is quite prevalent. It is still unclear what exactly causes BV, although the condition is typically associated with a deficiency of lactobacilli in the vaginal canal and the subsequent proliferation of anaerobic polymicrobial organisms as a result of this deficiency. It has been one of the infectious disorders in obstetrics and gynecology that has been the topic of the greatest amount of research as a direct result of the recent significant increase in its prevalence (Kahwati *et al.* 2020). Before delving into the intricacies of the disease's pathophysiology, it is vital to have a good awareness of the typical anatomical or physiological characteristics of the vaginal region. This is because the vaginal region is home to a number of delicate structures. This is due to the fact that the vaginal region houses a number of significant anatomical and physiological aspects (Jane *et al.* 2022).

In 1953, G. vaginalis was first isolated from male patients suffering from inflammation of the urethra (also known as urethritis), inflammation of the prostate (often known as prostatitis), and inflammation of the cervical canal (also known as cervicitis) (Leopold 1953), after then, it was isolated from patients who were diagnosed with non-specific vaginitis, which is an inflammation of the vaginal tract. Researchers at first believed it to be a strain of the Haemophilus bacteria, therefore they gave it the name H. vaginalis. (Norah and Manish 2022).

According to the findings of the study, Gardnerella vaginalis was discovered in only 17.6% of the so-called "healthy" women who did not have a clinical diagnosis of BV (Teixeira *et al.* 2012). However, there was no evidence to suggest which these women fulfilled the Amsel criteria or had an ideal Nugent (Gardnerella vaginalis morphotypes) score of 0 to 4. In other words, the Amsel criteria and the ideal Nugent score are not compatible. Similar to the conclusions drawn from (Burton *et al.* 2003), who discovered that only 19% of women who did not have BV had G. vaginalis in their vaginal microbiomes. The presence of G. vaginalis in these individuals, despite the fact that there was no clinical evidence of BV or even intermediate flora, shows that these individuals most likely have an asymptomatic infection, which is typical of all STDs (Plummer *et al.* 2021). It is not quite obvious which unique host-infection interactions are to blame for restricting the pathogenic potential of the colonizing pathogen. On the other hand, if it were a typical component of the vaginal flora, one might expect to find G. vaginalis in prepubescent girls in addition to women of all ages (Colonna *et al.* 2020).

Despite the fact that Gardnerella is not believed to be contagious to other people, there are still many gaps in our knowledge regarding how the function of transmissibility actually works. It is possible for the transmission of these germs from one person to another during sexual activity to upset the natural balance of bacteria that is normally found in the vagina, and this disruption may even contribute to the formation of BV. This potential for disruption exists because these germs can be passed from one person to another during sexual action. In the majority of cases, BV is caused by an abnormally high number of anaerobic bacteria, such as G. vaginalis, and an abnormally low amount

of the typical Lactobacilli, which are the bacteria that normally produce hydrogen peroxide. BV can also be caused by a combination of these two factors (Jane *et al.* 2022).

Changes in the vaginal environment that occur throughout the menstrual cycle cause major shifts in the ecology of the vaginal microbiota. These shifts can have negative effects on a woman's health (Shipitsyna *et al.* 2013). It is generally agreed upon that lactobacilli species are the primary microorganisms that are responsible for regulating the pH of the vaginal lumen. The DNA of vaginal microflora in women reveals that certain individuals possess bacteria termed Atopobium, Megasphaera, and Leptotrichia that create lactic acid rather than lactobacilli. This is in contrast to the species of lactobacilli that are found in vaginal microflora (Silverberg *et al.* 2021).

The appearance of BV has been connected to a rise in the population of potentially harmful microorganisms and a fall in the number of bacteria that are beneficial to human health. Because there are fewer commensal Lactobacillus spp. Present, the production of lactic acid and hydrogen peroxide, both of which contribute to an environment that is less acidic, is reduced. This, in turn, causes the number of commensal Lactobacillus spp. To decrease. Gardnerella vaginalis and other anaerobic bacteria that have been related to BV are able to enjoy ideal growth rates when the pH level rises. These bacteria have been linked to BV (Srinivasan *et al.* 2012).

Douching, frequent tub baths, having several sex partners, using over-the-counter intravaginal hygiene products, and using a range of intravaginal hygiene products are some of the factors that may contribute to the development of bacterial vaginosis. Other variables that may contribute to the development of bacterial vaginosis include increasing the number of sex acts, frequent tub baths, and having multiple sex partners. (Mijač *et al.* 2006).

2.3 Epidemiology

Gardnerella vaginalis is a type of bacteria that lives in the vagina alongside other bacteria to help maintain the vagina clean and clear of infections. When the Gardnerella bacteria in the vaginal tract reproduce at an abnormally high rate, a disease known as bacterial vaginosis can develop. The most common type of vaginal infection, known as BV, can be swiftly and effectively treated with medication (Muzny *et al.* 2013). Bacterial vaginosis, also known as BV, is the vaginal condition that affects the majority of women between the ages of 15 and 44. In point of fact, it is estimated that one in every three American women will be affected by BV. The incidence rate is highest among women of African descent (Krysanova *et al.* 2021).

In the United States, females aged 14 to 49 who participated in the National Health and Nutrition Examination Survey between the years of 2001 and 2004 had a positive Nugent score for bacterial vaginosis roughly 30 percent of the time, according to the findings of that survey (Pleckaityte *et al.* 2013). There is sufficient epidemiologic evidence to support the theory that G. vaginalis can be sexually transmitted. Nearly eighty percent of the women who had vaginal fluid tested were found to have a positive result for G. vaginalis (Srinivasan *et al.* 2015). Additional frequently discovered BV-associated bacteria include species of Atopobium, Leptotrichia, Megasphaera, Prevotella, and Dialister. These bacteria are recovered from the vaginal microbiome at varying rates. The diversity of severe anaerobes encountered in BV patients is various (Shipitsyna *et al.* 2013).

According to the findings of this research, G. Gardnerella has repeatedly been isolated as a major pathogen in BV, which indicates a high general prevalence of Gardnerella in this group (Castro *et al.* 2018). In addition, bacterial vaginosis, often known as BV, is the most common type of vaginal infection that affects women of reproductive age. Its occurrence rate is reported to be anywhere from 5 to 70 percent. Therefore, it has been hypothesized that Gardnerella could potentially be a component of the normal microbiota that is found in the vaginal area. Diseases like this hardly never strike men.

BV is an illness that can damage a woman's fertility if she is a fertile female (Taylor *et al.* 2013).

It is concerning since BV is linked to preterm delivery of infants with low birth weight, as well as an increase in the spread of the human immunodeficiency virus (HIV), pelvic inflammatory disease, and post-surgical infections. Numerous socioeconomic and racial demographics, the number of sexual partners, the frequency of douching, smoking behaviors, and a high body mass index are all factors that contribute to the risk of BV (Pleckaityte *et al.* 2012).

The vast majority of microorganisms have a certain pH range in which their activity is at their highest. It is possible that the growth of other bacteria will occur from any involvement with the pH of the system (Morrill *et al.* 2020) The similar effect applies to the vaginal system as well, which is densely inhabited with different species of lactobacilli. The vagina of a normal, healthy person has a pH that ranges from 3.5 to 4.5. The lactobacilli are primarily responsible for the generation of lactic acid, which keeps the pH stable (Breshears *et al.* 2015). Any change in pH, whether it be caused by pathophysiological conditions or physical activities like the insertion of contraceptive devices or intercourse, has the potential to result in an increase in the pH within the vaginal lumen. This, in turn, has the potential to result in an increase in the population of lactobacilli and a subsequent increase in the growth of other microbes (Cartwright *et al.* 2018).

2.4 Gardnerella Vaginalis in the Pathogenesis

Since Gardner and Dukes identified Gardnerella vaginalis to be the causative agent of "nonspecific vaginitis," a prevalent illness affecting females that is now known as bacterial vaginosis, there is debate over the clinical significance, Gram stain reaction, and genus affiliation of the organism. This has been the case ever since Gardner and Dukes identified Gardnerella vaginalis as the causative agent of "nonspecific vaginitis." (Cooper 2014). The organism was given the name G. vaginalis when it was determined through taxonomic research that it was genetically distinct from bacteria belonging to

other genera, such as Haemophilus and Corynebacterium. The gram-variable response of the organism has been better understood because to chemistry research and electron imaging (Wilson 2005).

In many cases, it is the sole pathogen responsible for vaginosis, although it can also cause the condition in conjunction with other anaerobic bacteria. A number of genitourinary conditions, including urethritis, cystitis, balanoposthitis, prostatitis, and epididymitis, have been linked to its presence (Castro *et al.* 2018). The incidence of these illnesses is significantly higher in females than to males. It is also possible to spot endometritis and bacteremia in some pregnant women when they are giving birth. There is neonatal involvement in extremely unusual circumstances (Srinivasan *et al.* 2015).

Some women develop bacterial vaginosis as a result of a disruption in the normal vaginal microbiota, which allows G. vaginalis and a wide variety of other bacteria, the vast majority of which are anaerobic, to flourish and contribute to the condition (Janulaitiene *et al.* 2017). The lactobacillus community in the vaginal environment is facultatively anaerobic, which contributes to the vaginal environment's acidic nature (Pessoa *et al.* 2017). Once the anaerobes have taken the place of the normal vaginal bacteria, it is possible that it will be necessary to administer prescription antibiotics that cover anaerobic bacteria in order to restore the ecosystem's equilibrium. It is considered that G. vaginalis is not the cause of bacterial vaginosis, but rather a symptom of the changed microbial ecology that is associated to the overgrowth of several bacterial species (Gaurab 2021).

Studies that used improved media and techniques for the isolation and identification of bacteria in vaginal secretions helped to clarify the disagreement around the genesis of bacterial vaginosis in a large part. These studies were coupled with the standardization of clinical and laboratory diagnosis criteria, which also contributed to the clarification of the matter (Ting *et al.* 2020). In addition to G. vaginalis, other microorganisms, such as Mycoplasma hominis and Mobiluncus spp. As well as a few obligate anaerobes, have been identified as potential causes of bacterial vaginosis. In addition to being passed on through sexual contact, the fact that Mobiluncus species, specifically M. hominis and G.

vaginalis, were found to be dwelling in the rectum raises the possibility of autoinfection (Plummer *et al.* 2021).

Although it cannot be proven, the vast majority of bacterial vaginosis (BV) infections are thought to start with a biofilm produced by G. vaginalis. This biofilm then encourages the growth of other types of opportunistic bacteria. Bacterial vaginosis can be diagnosed based on the presence of clue cells, which are epithelial cells of the cervix that are covered in rod-shaped bacteria (Schellenberg *et al.* 2017). Vaginosis is a pore-forming toxin that is exclusively capable of affecting human cells and is produced by G. vaginalis itself. Vaginolysin is a cholesterol-dependent cytolysin that kicks off complicated signaling cascades that induce target cell lysis and enable Gardnerella's pathogenicity. These cascades are triggered by the presence of vaginolysin (Silverberg *et al.* 2021). Gardnerella has the required virulence characteristics to cling to host epithelia, which enables it to successfully compete with Lactobacilli for dominance in the vaginal environment. Additionally, protease and sialidase enzyme activity frequently accompany this bacterium (Plummer *et al.* 2022).

2.5 Bacterial Vaginosis (BV)

Bacterial vaginosis (BV), formerly known as nonspecific vaginitis, is a disorder that occurs as a consequence of the disruption of the gram-positive Lactobacillus spp.-dominated vaginal microbiota and the subsequent colonization of other bacteria, some of which may be potentially dangerous. Bacterial vaginosis (BV) is a condition that emerges as a result of the disruption of the gram-positive Lactobac (Bebeh *et al.* 2017). Microscopic identification of vaginal clue cells, which are distinguished by the presence of epithelial cells that are surrounding or coated by several gram-positive or gram-negative bacilli or coccobacilli, is made possible by the Amsel criteria and the Nugent scoring systems. Vaginal clue cells are defined by the presence of epithelial cells that are surrounded or coated by many bacilli. The application of these cells in the process of diagnosing BV is depicted in (Figure 2.1). Patients must satisfy three of the following four Amsel criteria in order to be considered for treatment: Signs of aberrant vaginal discharge include a thin, white-yellow, and homogenous discharge; clue cells in the

vaginal discharge investigated microscopically; a vaginal pH greater than 4.5; and the detection of a fishy odor after combining 10% potassium hydroxide with the vaginal discharge (Megan 2018). Gram staining of vaginal exudate is performed with the presence of clue cells serving as the basis for Nugent scoring (Nipunjot *et al.* 2018).

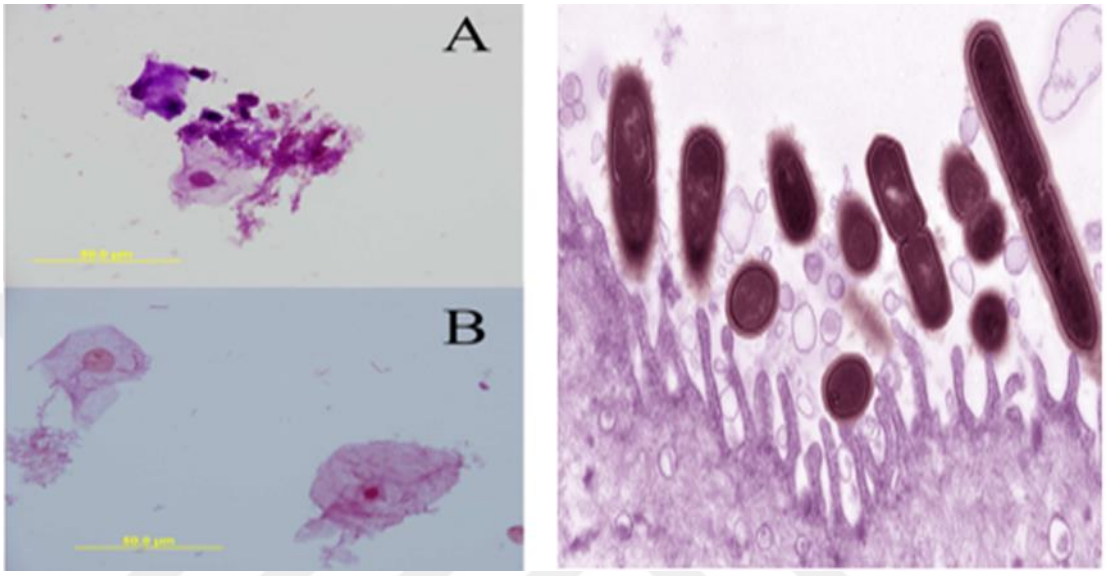


Figure 2.1 Clinical samples showing evidence of BV-positive and BV-negative vaginal smears (Morrill 2020)

Gram staining was performed on the samples, and light microscopy was used to examine the results. (A) Cells taken from a patient who, according to the Amsel criteria for a positive diagnosis of BV, possessed the virüs (Joana *et al.* 2015). Typical examples of clue cell morphology include the excessive coating of vaginal epithelial cells with gram-variable bacteria. (B) Cells taken from a patient who did not fulfill the requirements of the Amsel criteria for a positive diagnosis of BV. Epithelial cells that have gram-positive bacilli distributed throughout them can be seen (Frincy *et al.* 2014).

An imbalance in the microbiological environment of the lower genital tract is the root cause of the common ailment known as bacterial vaginosis. The reported frequency of bacterial vaginosis among pregnant women in the United States ranges from 5.8% to 19.3%, with some races and ethnic groups having greater rates than others (Kahwati *et al.* 2020). Poor obstetric outcomes have been associated to a number of different

conditions, including preterm birth, early miscarriage, postpartum endometritis, low birth weight, and bacterial vaginosis. The majority of people who have bacterial vaginosis do not experience any symptoms, the condition may clear up on its own, and it regularly returns even after therapy. The majority of medical professionals address bacterial vaginosis in pregnancy as a symptom in their practice. The screening for asymptomatic bacterial vaginosis during pregnancy is given primary importance in the current recommendations (Douglas *et al.* 2020).

2.6 Virulence Factor of G. vaginalis

2.6.1 Hemolysin production

In a test tube, exotoxins that are produced by bacteria and are called hemolysins lyse red blood cells (Teixeira *et al.* 2012). Hemolysins are a type of bacterial protein that allow bacteria to acquire nutrients from their host cells. Iron, for instance, has the potential to act as a growth inhibitor for a wide variety of bacteria that are potentially harmful. When red blood cell hemolysis is apparent in agar plates, it is simpler to classify certain dangerous bacteria, such as Gardnerella vaginalis, which can cause infection (Srinivasan *et al.* 2015).

It has been determined that the hemolytic activity of G. vaginalis is caused by a single protein that is released by G. vaginalis cells during the exponential growth phase of the bacteria. Only human erythrocytes, neutrophils, and endothelial cells are susceptible to the lytic activity of the toxin (Rivero-Segura *et al.* 2022). G. vaginalis has the potential to form a decomposition zone surrounding colonies that grow on Human Blood Agar as it works on the analysis of red blood cells and cells with nuclei (Nucleated Cells), such as the cells that line the uterus. This occurs because G. vaginalis releases protein into the medium and works on the analysis of Nucleated Cells. Cytolysin is a protein with a molecular weight of between 61 and 63 Dalton, which can be found in endothelial cells and neutrophils (Vick 2014).

By including 0.1% starch and 0.3% Tween 80 into the culture medium, one can achieve the optimum conditions necessary for the production of hemolysin by these bacteria. When the pH is at 7.4, this factor operates at its highest level of efficiency. Hemolysin or cytolysin, which are both products of G. vaginalis, are represented by the symbol "Ctox." Anamphiphilic protein was halted in its tracks by protein-digesting enzymes such trypsin, -chymotrypsin (Schellenberg *et al.* 2017).

This protein is particularly skilled at working with red blood cells and performs analysis on human red blood cells, neutrophil cells, and nuclear cells found in the uterine lining (Norah Kairys 2022). Since the membrane of human red blood cells contains glycoprotein with a molecular weight of 57 kDa or has a specialized site for Ctox, G. vaginalis cytolysines differ from cytolysin produced by another bacteria found in vaginal mucosa, like Streptococcus group B, and as a result, in the majority of cases, it does not affect other types of blood cells but rather only affects human blood cells. This is because (Morrill *et al.* 2020).

According to Onderdonk *et al.* (2016), The process of cytolysine degradation is unrelated to the oldest type of cytolysin-like toxins connected with cholesterol produced by Gram positive bacteria, cell deterioration due to differentiating osmosis, and the effectiveness of phospholipase. According to (Rivero-Segura *et al.* 2022). This toxin's analytical mechanism involves the creation of a channel between the cells, the incorporation of Ctox into the membrane lipids, and the fact that two or more Ctox-monomers contribute to the complete breakdown when pressure forms a hole in the membranes of the target cells (Zvirbliene *et al.* 2010).

2.6.2 Sialidase prodaction

Gardnerella vaginalis, a potentially pathogenic inhabitant of the female genital canal, was shown to contain and produce a sialidase, which was subsequently isolated. The optimal pH for the enzyme was measured to be 5.5, and its molecular weight was in the range of 75,000 (Haddad *et al.* 2018). Sialidases could be called enzymatic agents because they not only degrade mucins, which increases bacterial adherence, but they

also cleave sialic acid, which reduces the effectiveness of mucosal immune defenses (Hu *et al.* 2017).

The enzyme sialidase and the presence of Gardnerella vaginalis have both been proposed as potential diagnostic markers for bacterial vaginosis. Sialidase production and negative pregnancy outcomes have been connected, and there may be a connection between the synthesis of this enzyme and (symptomatic) bacterial vaginosis and the development of biofilms. Sialidase production has also been associated to unfavorable pregnancy outcomes (Kırmusaoğlu 2019).

2.6.3 Adherence to vaginal epithelial cells

Because it enables a potential pathogen to evade elimination by host defense mechanisms such as the mucociliary escalator, urine flow, or vaginal secretion flow, the act of adherence plays a pivotal role in the initial stages of pathogenesis. This is because adherence allows a potential pathogen to adhere to a surface. In addition, the capacity of bacteria to produce a biofilm on polystyrene is not important to the process of pathogenesis unless the bacteria are first capable of adhering to the vaginal epithelium. This is because adherence is the first step in the process of producing a biofilm, and pathogenesis requires adhesion (Joo *et al.* 2022).

Pili are typically involved in the process of mediating epithelial adhesion. It is possible that G. vaginalis is an early colonizer that sets circumstances for the establishment of other species with low inherent pathogenic potential given that it can connect to and develop a biofilm on vaginal epithelia. This is a possibility given that G. vaginalis can create a biofilm on vaginal epithelia (Jang *et al.* 2017).

As is the case with many infections, the adherence of G. vaginalis to epithelial cells may be a critical stage in the pathogenesis of bacterial vaginosis. Factors influencing the adhesion of G. vaginalis to exfoliated vaginal epithelial cells invitro were studied. Additionally, the effect of G. vaginalis being exposed to subinhibitory amounts of

ampicillin and metronidazole on its ability to adhere in vitro was investigated (Adela *et al.* 2022).

2.6.4 Biofilm production

It is common knowledge that G. vaginalis creates a biofilm that is exceptionally long-lasting (Jane *et al.* 2022). Biofilm formation is an essential component of virulence because of its improved resistance to antibiotics and its ability to withstand the immunological defenses of the host. Because the immune system is unable to completely eliminate biofilm bacteria and the bacteria cannot be completely eradicated by drugs, biofilm-related illnesses frequently have a nature that is either chronic or recurrent (Morrill *et al.* 2020). According to Patterson *et al.* (2010), The majority of people who have BV have a chronic infection that has a high risk of either relapsing or coming back. Even in circumstances in which lactobacilli are present, the establishment of a biofilm in the vagina has the potential to result in colonization. This is because biofilms increase bacterial resistance to lactic acid and hydrogen peroxide, which in turn raises the likelihood of colonization (Kim *et al.* 2021)

A biofilm is an organized assemblage of microorganisms (multicellular aggregates of sessile cells) that are permanently attached to a living or non-living surface, on solid or liquid surfaces, or on soft tissue in living animals. Biofilms can be found on solid surfaces, in liquid surfaces, or on soft tissue in living organisms. They inhabit an extracellular polymeric matrix (EPS) that they themselves build and use as their home. (Gaurab *et al.* 2021). In point of fact, it is widely believed that biofilms are the fundamental reason for the bulk of microbial diseases that affect humans, as well as a significant factor in the persistence of bacterial infections (Flemming *et al.* 2016).

The advantageous adaptation known as biofilm makes it possible for bacteria to live in harsh environments. Additionally, the removal of individual cells is a simpler process than the elimination of germs from a biofilm (Hu *et al.* 2017). Bacterial biofilm is a structured microbial community made up of multilayered cell clusters embedded in an extracellular polysaccharide matrix that promotes microorganism adhesion or displays a

different phenotype from planktonic bacteria in terms of growth rate and gene expression. The term "bacterial biofilm" was first coined in 1978 by Costerton and colleagues. They described it as a structured microbial community made up of multilayered cell clusters embedded in an extracellular polys (Moon *et al.* 2022).

Gardnerella vaginalis is the most common source of infections that are linked with biofilms on indwelling medical devices. It is able to switch between existing as single free-floating cells and multicellular biofilms, and it has a number of different processes and virulence factors that allow it to avoid being destroyed by the immune system. There are many essential microbial virulence factors, and biofilm is one of them (Lyudmila *et al.* 2021).

On the epithelial lining of the vagina, biofilms are frequently observed to develop, and G. vaginalis is the species that predominates in the majority of these instances. In the pathophysiology of bacterial vaginosis, the conventional wisdom holds that the development of a biofilm is one of the most important contributors to the condition (Castro *et al.* 2017). This study provides an overview of the significance of the development of biofilm by G. vaginalis and other anaerobic microbes in the polymicrobial etiology of BV, beginning with the point at which these microorganisms initially adhere to a surface and continuing on to the creation of biofilm. In addition to this, it investigates the commensal and synergistic interactions that take place between these microorganisms in order to shed light on the phenotypic shift that occurs during the formation of the G. vaginalis biofilm that ultimately results in the establishment of BV (Joo *et al.* 2022).

- Composition stage of biofilm formation: A matrix of self-produced hydrated extracellular polymeric substances (EPS) provides support for the local habitat. This matrix serves as a home for the bacteria that are found in biofilms, which are also referred to as slime (William *et al.* 2022). Polysaccharides, proteins, nucleic acids Extracellular DNA, also known as eDNA, as well as lipids and other types of biopolymers, such as humic substances, make up the majority of EPS. In addition to providing mechanical support, EPS also forms a cohesive matrix and sequesters

nutrients. In most cases, bacteria account for less than 10% of the dry mass of biofilms, but the matrix may constitute more than 90% of the total (Qian *et al.* 2021).

In addition to this, EPS is responsible for the promotion of horizontal gene transfer, the maintenance of the population of biofilm cells in close proximity to one another (quorum sensing), the facilitation of cell-to-cell communication, the protection of biofilm cells from host immune responses, and the resistance of biofilm cells to antimicrobial treatments (Arroyo-Moreno *et al.* 2022). (eDNA) is a potential target for bacterial diseases despite the fact that it stabilizes bacterial biofilms and protects them from the effects of both physical and chemical stress (Boyanova *et al.* 2021).

➢ Stage of biofilm formation: Bacteria attaching themselves to the surface, the production of microcolonies, the maturation of the biofilm, and finally separation (also known as dispersal), which allows bacteria to leave the biofilm and colonize new areas, are the four primary stages of biofilm formation (Figure 2.2) (Karin *et al.* 2022).

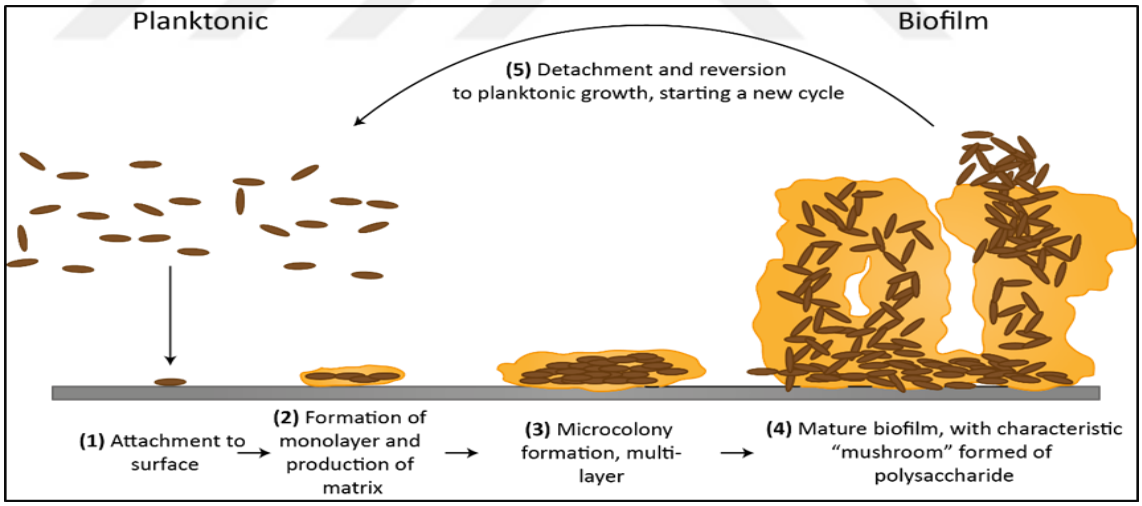


Figure 2.2 Biofilm life cycle (Karin *et al.* 2022).

Attachment /colonization of bacteria to the surface: The initial stage in the development of a biofilm is the attachment of microorganisms to a surface. When the surrounding environment is moist, it is possible for planktonic bacteria to attach themselves to a wide variety of surfaces, such as metals, plastics, soil particles, medical implant

materials, and the flesh of humans and animals. When the surrounding environment is damp, these bacteria will also begin to secrete a material that is similar to glue (Babics *et al.* 2015).

Fimbriae, pili, and flagella were present in the earliest bacterial colonies that attached themselves to a surface. These appendages strengthen the interface between the bacteria and the surface by producing van der Waals forces, which are weak connections that can be broken and made again (Santiago *et al.* 2011). Cell adhesion molecules are proteins that are found on the surfaces of cells and are responsible for the process known as cell attachment. If the colonists are not rapidly removed off the surface, they will be able to more securely anchor themselves by utilising cell adhesion molecules. The ability of germs to adhere to surfaces can be affected by environmental conditions such as pH and temperature. Hydrophobic surfaces are more prone to attract bacteria, whereas hydrophilic surfaces are less likely to do so (Abebaw *et al.* 2021).

Microcolony formation (Growth and development): After the formation of the microcolonies, the bacteria that are contained within the biofilm will begin to communicate with one another. This connection kicks off reactions that impact gene expression and activate components that promote sassily, such as those involved in the creation of extracellular matrix (ECM). These responses are triggered as a result of this interaction (Kostakioti *et al.* 2013). During this phase, there is an increase in the production of extracellular matrix (ECM), the development of three-dimensional structures, and the emergence of intricate diffusion channels. These channels are used to move waste materials and dead cells in addition to nutrients, oxygen, and other growth-critical elements for bacteria (Choudhary *et al.* 2020).

- Biofilm detachment and dispersal: Detachment is the final step in the process of biofilm formation. When biofilms reach their full maturity, they become denser and create an anaerobic environment on the inside, while the layers on the outside may begin to split apart. Detachment and dispersion are both potential outcomes of a nutrient deficiency in a population (Ivanova *et al.* 2021). After the maturation process is complete, the biofilm will become thicker so that it can colonize new surfaces and

inhibit Gardnerella vaginalis. There is a possibility that the mature biofilm will suffer from density-mediated hunger, which may result in an anaerobic environment from the inside and lead to the decomposition of the biofilm. Some examples of mechanisms that cause biofilm to detach from their host are seed distribution, erosion, and sloughing. These processes of detachment are required in order to form new biofilms in new niches (Patterson *et al.* 2010).

Shear tension acts as a mediator between the sloughing and erosion modes of biofilm detachment, which are collectively referred to as passive detachments. Sloughing is the process of separating a major percentage of a biofilm from the initial bulk of the biofilm, whereas erosion is the process of removing a very little amount of biomass or bacteria off the surface of an area. Sloughing is often referred to as "sloughing off." (Udayalaxmi *et al.* 2011). P. aeruginosa undergoes an active detachment process during the process of seed dissemination. A variety of substances, including metal chelators, anthranilate, and other surfactants, can assist in the dispersal of biofilms by causing the release of individual planktonic cells or microcolonies from the center of the biofilm and producing an empty space in their stead (Lee and Yoon 2017).

Treatment of BV: Because BV is caused by a wide variety of microorganisms, treating it can be difficult. According to (Curran and Rivlin 2012), BV often occurs when hormonal swings produce a drop in the vaginal pH, hence altering the vaginal flora. In addition, BV can go away on its own, which is a phenomena that occurs in one-third of women who are not pregnant and in one-half of women who are pregnant (Boyanova *et al.* 2021).

Since the cause of the condition has not been identified, effective treatment is difficult to come by, which contributes to a high rate of relapse (Cartwright *et al.* 2018). As the goal of BV treatment is to alleviate symptoms and ensure that the follow-up test is negative, it would not be beneficial to treat patients who were asymptomatic because this would defeat the purpose of the treatment. Furthermore, treating asymptomatic women can cause their vaginal yeast infection to become noticeable to the patient. However, in the case of asymptomatic patients who are scheduled to undergo

gynecologic procedures, it is recommended that they receive preventative treatment (Castro *et al.* 2018).

It is common practice to treat BV with either metronidazole or clindamycin, administered either systemically or topically (Hu *et al.* 2017). Clindamycin is a bacteriostatic antibiotic that targets aerobes in addition to Gram-positive and Gram-negative anaerobes. Whereas metronidazole is bactericidal and often efficient in killing Gram-negative anaerobes, clindamycin is a bacteriostatic antibiotic that targets Gram-positive and Gram-negative anaerobes (Gaurab 2021).

In spite of the success of treatment, 25–30% of women will experience recurrence within one to three months, and 50% will do so within six to twelve months. There are two different possible causes of recurrence: re-infection with a biotype that is distinct from the biotype that caused the initial infection; poor eradication of the organisms responsible for the infection; and thus, the inability of normally protective lactobacilli to re-establish themselves (Arroyo-Moreno *et al.* 2022).

Due to metronidazole has no effect on Lactobacillus species, one of the other options is to use bacteriotherapy, which involves replacing harmful microorganisms with non-harmful ones in order to treat the infection. The use of this antibiotic does not dramatically disrupt the natural ecology of the vaginal tract. Researchers from a variety of institutions have investigated the potential benefits of lactobacillary probiotics for the treatment of BV (Vitali *et al.* 2012, Schellenberg *et al.* 2017).

Probiotics are live strains of microorganisms that, when supplied in sufficient quantities, have the ability to improve a patient's health in a positive way. Not only can probiotics be effective in the treatment of BV because they can replace low lactobacilli concentrations, but they can also be useful in the therapy of BV because they can stimulate an immunomodulation process. When probiotics are used to treat BV, there is a forty percent chance that the patient will be cured (Teixeira *et al.* 2012).

2.7 Antibiotic Resistance

In light of the fact that BV is associated with an increased risk of preterm delivery, it is imperative that effective treatment strategies be developed.PTD. Through the use of antibiotic treatment, pregnancy-related BV has been completely eliminated (MacDorman 2011). According to the Centers for Disease Control and Prevention (CDC), the antibiotics metronidazole or clindamycin should be taken either intravenously or orally. The implementation of these regiments has been quite successful, despite the fact that it is only a temporary solution. (Nagaraja, , 2008) After receiving therapy with either antibiotic, approximately 30–70% of BV cases will display recurrence within three months, and approximately 50–80% of cases will do so within one year. A has been fingered as the cause of repeated infections. Vaginae exhibiting irregular patterns of metronidazole susceptibility, A. vulvare and G. vaginalis are resistant to antimicrobial therapy as well as re-infection by a different biotype. Vaginae displaying irregular patterns of metronidazole susceptibility (Ting *et al.* 2020).

The primary concern is the increased potential for the development of antibiotic resistance following subsequent administration of the same drug. Because it is the only medication that is effective against anaerobic bacteria while also protecting the natural vaginal ecology, metronidazole has become the treatment of choice for BV (Breshears *et al.* 2015). Since the early 1980s, metronidazole has been successfully utilized as a treatment for BV. Nonetheless, over the course of the past ten years, metronidazole resistance has grown increasingly prevalent, including against G. vaginalis (Mittal *et al.* 2012). Another pattern that emerged from this analysis was that 99% of the G. vaginalis isolates were resistant to metronidazole. According to (Norah Kairys and Manish 2022). In spite of the fact that metronidazole has proven to be an effective treatment for BV, in the past 10 years, strains of G. vaginalis that are immune to the effects of the medication have evolved that were not previously known to exist.

Women who had encountered PTD in the past were more likely to have biotypes 2, 5, and 7 as resistant to metronidazole, while women who had experienced FTD in the past were more likely to have biotypes 3, 4, and 8. (Vitali *et al.* 2012). The fact that G.

vaginalis is resistant to metronidazole in this study clearly suggests that resistant strains of G. vaginalis, and not just A. vaginae as was previously described, may be responsible for the resistance to metronidazole in the treatment of BV. A. vaginae is not the only organism that has been shown to be resistant to metronidazole in the treatment of BV (Plummer *et al.* 2021).

As resistance is becoming more common, researchers have been looking into alternative therapy approaches. Nifuratel recently demonstrated its efficacy in vitro by demonstrating activity against a group of bacteria that is known to contribute to the development of BV. This activity was observed without causing any harm to the normal lactobacilli flora (Togni *et al.* 2011). Two clinical trials are currently being carried out with the purpose of comparing it to more traditional BV treatments. Because taking probiotics during pregnancy does not pose any danger to either the mother or the fetus, it is possible that in the future, preventative treatments will be administered differently as a result of their use (Togni *et al.* 2011).

In spite of the widespread efficacy of medicines, there are still a great deal of challenges associated with the treatment of bacterial infections, the most significant of which is the evolution of antibiotic resistance. Many different types of inorganic nanoparticles, polymeric nanoparticles, dendrimers, and liposomes have all been used as drug delivery platforms for nanoparticles in recent years. This has been done in an effort to improve the therapeutic efficacy of existing antibiotics (Tomusiak *et al.* 2011). In this particular review, we will be concentrating on those aspects of the treatment of bacterial illnesses that stand to benefit the most from the application of nanoparticle-based approaches. Some of these subjects include the delivery of antibiotics in a targeted manner, the distribution of antibiotics in a way that adapts to the environment, the delivery of antibiotics in a combinatorial manner, antibacterial vaccinations that are made possible by nanoparticles, and nanoparticle-based bacterial detection. In each of these areas, we focus on the most cutting-edge antimicrobial nanoparticle systems and investigate the level of effectiveness those systems have had in warding off bacterial diseases. (Udayalaxmi *et al.* 2011)

2.8 Nanomaterials

The word "nano" comes from the Greek word "Nanos," which literally translates to "dwarf." In the context of the scientific community, this refers to items that are approximately 10 to 9 nanometers in size, which is one billionth the normal size. In other words, the diameter of a tennis ball and the diameter of the Earth have the same relationship to a nanometer as a meter has to a meter. A nanometer is equal to one billionth of a meter (Zhou *et al.* 2022).

Due to the one-of-a-kind qualities that nanostructures possess across a variety of business sectors and possible application areas, such as the field of nanomedicine, they have received a lot of attention recently. The aforementioned phenomenon has led in the introduction of straightforward procedures for the generation and aggregation of metal particles at the nanoscale. These methodologies have been made possible as a direct outcome of the phenomenon. Nanomaterials and nanoparticles are characterized by their overall dimensions, shape, size distribution, chemical content, and degree of dispersion. These factors have a substantial impact on the physicochemical properties of the nanomaterials and nanoparticles. Nanomaterials and nanoparticles present a range of potential answers to a variety of technological and environmental difficulties that are present in a variety of sectors (Behzad *et al.* 2021).

The combination of nanotechnology with biotechnology has given rise to the field of nano-biotechnology, which aims to foster the development of biosynthetic and environmentally friendly technologies for the synthesis and manufacture of nanomaterials and nanoparticles (NPs) (Shahriari *et al.* 2021). In comparison to physical and chemical methods, the current method offers an alternative that is significantly more cost-effective for the production of nanomaterials and nanoparticles. Professor Norio Taniguchi of Tokyo Science University coined the phrase "nanotechnology" in 1974. He was one of the first people to use the term (Srivastava and Bhargava 2022). NPs can be broadly grouped into two, namely:

2.8.1 The organic NPs

In this category are newly discovered allotropic carbon types that have found applications in a wide variety of scientific sectors over the past 20 years (Ma *et al.* 2021). Nanoparticles made of graphene oxide (GO) and nanotubes made of single-walled carbon are both instances of organic nanoparticles (SWCTs). As well as carbon nanoparticles (fullness) that showed strong microcidal capabilities (Vadlapudi and Kaladhar 2014).

The size and amount of surface area of carbon Nanomaterials are among the most important factors that determine the antibacterial activity of a substance; hence, increasing the surface area of the nanomaterials or NPs by reducing their size results in increased activity for interacting with bacteria (Kırmusaoğlu 2019).

2.8.2 The inorganic NPs

Magnetic NPs, NPs made of noble metals (such as gold and silver), and semi-conductor NPs (such as zinc oxide and titanium oxide) are all included in this category. Inorganic nanoparticles are rapidly gaining popularity as a result of the remarkable material characteristics and diverse functional applications they offer (Ibisanmi 2022).

Metallic nanoparticles, which are formed mostly from fullerenes, copper, zinc, gold, carbon, silver, titanium, iron, and palladium, are the most important biological agents. The ever-increasing demand for ecologically responsible business practices is being met through the application of biological processes and other forms of green synthesis (Shahriari *et al.* 2021). For their versatility as biological marker probes, several NPs are highly sought after:

The modest size is in the range of one to one hundred nanometers. The significant ratio of volume to surface area. The biological and chemical features, in particular those pertaining to size and structure, have a strong affinity for the target, in particular those

aspects of the target that are protein-based. Despite its atomic fineness, the structural endurance of the material. Enhanced photoemission as well as the nature of surface modification are the two factors that determine how quickly or slowly particles agglomerate. The increased catalytic activity on the surface, along with the high heat and electrical conductivity (Sahayaraj and Rajesh 2011).

2.8.3 Nanomaterials as antimicrobials

The comeback of infectious diseases is a serious potential challenge that society may have to face in the future, as does the developing resistance of bacteria to traditional antibiotics. Both of these challenges will require humanity to find solutions. The development of bacteria and the rise of so-called "superbugs" are contributing to an increase in both the prevalence of diseases and the severity of their symptoms. Stays in the hospital that are either frequent or protracted are contributing to rising medical expenditures, which is problematic for the industry as a whole (Joshi *et al.* 2019).

Even the most potent medicines are no longer successful in combating superbugs. It is absolutely necessary to develop strategies that are powerful, efficient, and successful in order to put a stop to the proliferation of antibiotic resistance (Ghosal *et al.* 2018). Antibiotic resistance is caused by a number of variables, one of which is human activity at various societal levels, which impacts people all over the world (Nakamura *et al.* 2017).

Antibiotic resistance has been the subject of a significant number of research, which have shed light on the problem and suggested potential solutions (World Health Organization 2014). The development of bacterial and fungal resistance to conventional antibiotics in immunocompromised individuals, as well as those with AIDS and cancer, has raised significant clinical concerns. In addition, problems associated with antibiotic-resistant bacterial infections greatly increase morbidity and mortality rates (cassini *et al.* 2019).

On the other hand, developing antibiotics that are more effective is a task that is both time-consuming and difficult. In order to combat diseases that have developed resistance to several drugs, new innovative techniques and novel biomaterials are currently being developed as part of an initiative known as multidrug resistance (MDR). The utilization of nanoparticles is a cutting-edge method that is becoming increasingly common in biomedical research. Research is now being done to investigate the antibacterial capabilities of graphene and other carbon nanostructures (Jindal and Goswami 2020).

The use of nanotechnology in the development of novel, non-traditional antimicrobial agents known as Nano-antibiotics has many advantages over the use of traditional antimicrobial agents. These advantages include the absence of side effects, increased effectiveness against species that have evolved to become drug-resistant, and the ability to prevent the emergence of resistance by interfering with multiple biological pathways. Traditional antimicrobial agents have many of these same advantages (De La Fuente 2014).

These nanomaterials, by producing high local concentrations of antibiotics, either exhibit antibacterial action on their own or improve the efficacy and safety of the administration of traditional antibiotics (Megan 2018). Traditional antibiotics are obviously outclassed by antimicrobial nanoparticles, which surpass them in a variety of areas, including reduced toxicity, the capacity to overcome resistance, and lower cost. (Nipunjot *et al.* 2018). Antimicrobial nanoparticles (NPs) have the potential to mechanically disrupt bacterial membranes and potentially infiltrate cells "unnoticed" by the bacterial defense system. This is because NPs are so small (Muktad *et al.* 2022).

These NPs destroy bacteria by the following mechanisms:

- The production of reactive oxygen species (ROS) such as H2O2, $O2^{-}$, and OH^{-}.
- The disorder of bacterial cell wall membrane.
- The inhibition of DNA synthesis and intracellular enzymes activity.

- The interruption of energy transduction (Xie *et al.* 2011).

As antibiotic treatment is often ineffective, numerous nanomaterials and NPs that possess antibacterial properties have been developed as a direct result of the development of nanotechnology in order to fill the void that this failure leaves (Beyth *et al.* 2015).

2.8.4 Graphene oxide nanomaterials

Given its capacity to form covalent bonds and lengthy chains of interconnected carbon atoms, which exhibit extraordinary stability and strength, carbon is a chemical element that serves as the basis for a wide variety of compounds. Carbon's versatility makes it an ideal candidate for this role. Diamond and graphite were the two forms of pure carbon that were thought to be the only ones that could survive for a significant amount of time, despite contrary evidence. The scope of carbon chemistry has recently been enlarged greatly thanks to recent findings. Examples of different types of carbon nanoforms include graphene, carbon nanotubes, and fullerenes (Joshi *et al.* 2019).

The family of graphene is a noteworthy example of the class of carbon nanomaterials. In 2004, micromechanical exfoliation of graphite (Gt) was used for the first time to successfully separate graphene. In recognition of their seminal contributions to the field of graphene research, Andres Geim and Konstantin Novoselov were awarded the Nobel Prize in 2010 (James 2022). Since that time, graphene has been the subject of extensive research, and the findings of that research have been published in a variety of forms. Graphene is defined as a single layer of carbon atoms that are conjugated and sp2-hybridized and are organized in hexagonal rings. The merging of many single layers results in the formation of a two-dimensional (2D), honeycomb-shaped sheet structure that has an extremely large surface area (2630 m2/g). It excels in the electrical, optical, thermal, and mechanical qualities that are measured (Ghosal and Sarkar 2018).

Given its new properties, graphene is an ideal candidate for use in a wide variety of sectors, including biomedicine and the delivery of medications (Nakamura *et al.* 2017). In terms of its chemical and physical properties, graphene differs from other carbon allotropes such as graphite and fullerenes. Recently, graphene and graphene oxide (GO) have come to light as promising nanoplatforms with enormous potential for biomedical applications and translational research. This is because graphene differs from other carbon allotropes such as carbon nanotubes, graphite, and fullerenes (Zhen *et al.* 2019). As a result of its design and production, it has a high Young's modulus, a large specific surface area, excellent biocompatibility, a high diffraction strength, quick charge carrier mobility, and outstanding thermal and electrical conductivity. In addition to these characteristics, it has rapid charge carrier mobility (Ogunshe an Falode 2021).

Antibacterial drugs have a variety of applications, including sensing, biological imaging, and molecular imaging (Zhou *et al.* 2022). Graphene and graphene oxide (GO) have an extremely high specific surface area, which makes them useful for medication and gene delivery, as well as cancer therapy and tissue scaffolds (Shadjou and Hasanzadeh 2016). The direct contact mechanism, oxidative stress, and the wrapping/trapping of bacteria in aggregated nanoparticles resulting to cell suffocation and so restricting their proliferation are some of the proposed explanations for the inhibitory action of GO suspended NPs. Another one of the offered explanations is that GO suspended NPs inhibit the multiplication of bacteria (Veisi *et al.* 2015).

According to the findings of the experiments, the synthetic GO possessed sharp edges that had the potential to function as instruments (Nano knives). When this comes into touch with the bacterium, it has the potential to cause damage to the cell wall as well as the membrane that surrounds the cell (Chen *et al.* 2014, Pham *et al.* 2015). It is possible for lipid injuries to occur as a result of the (Figure 2.3), in which considerable quantities of phospholipids are removed from the bacterium as a result of increased van der Waals contacts and the formation of hydrophobic bonds with the charged groups of GO (Tu *et al.* 2013). As a consequence of this, the capacity of the pathogens to carry out essential functions like as respiration, the movement of material, the maintenance of osmotic

equilibrium, and the transmission of energy may be compromised (Akhavan and Ghaderi 2012, Pham *et al.* 2015, Chong *et al.* 2017).

If the surface of the bacterial cell is suitably ringed by GO sheets, the bacterial cell will become physiologically inactive and will finally die. This will result in the formation of a distinct zone of inhibition in the culture plate (Figure 2.3). Cell entrapment is a viable alternative explanation for the antibacterial action of GO, which brings us to our second point. When bacteria come into touch with GO sheets, there is a possibility that the cells will become obstructed (Breshears *et al.* 2015). It is possible that the bacteria that have been confined will lose contact with their surroundings, and as a result, their growth will be stunted. Additionally, they may have limited access to nutrients. According to the findings of this study, the size of the GO may have a substantial impact on the total number of cells that are entrapped, with larger lateral GO sheets demonstrating a greater degree of inhibition (Veisi *et al.* 2015).

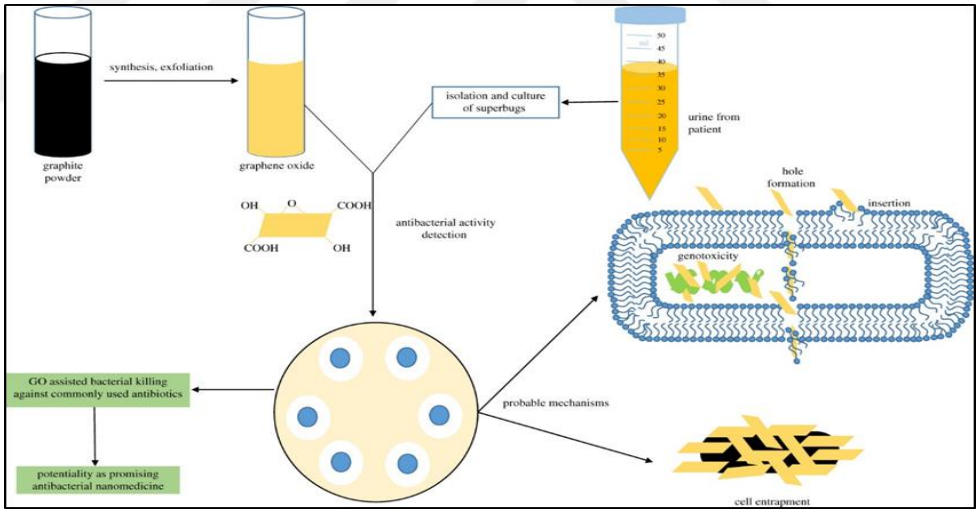


Figure 2.3 Bactericidal activity of GO against Gardnerella vaginalis (Aunkor *et al.* 2020)

According to UV-vis and AFM studies, the lateral size of the GO is comparable to or slightly larger than that of the round-shaped bacteria, S. aureus, which may measure up to one meter in length. This indicates that entrapment may be possible. As a result, it is not out of the question that the GO nanosheets can eliminate germs by both entrapping

them and slicing them to pieces with their edges. The advantage that GO possesses over other antibacterial materials in terms of its ability to kill or suppress bacteria may be broken down into three distinct properties: The antibacterial activity of GO is affected by both chemical oxidation and physical degradation, both of which reduce bacterial resistance. Unlike other carbon nanomaterials, their low production costs, simplicity of processing, and mass production ensured their efficacy as an antibacterial agent. Low doses of the GO have some cytotoxic effects on mammalian cells (Boyanova *et al.* 2018).

3. MATERIALS AND METHODS

3.1 Equipments and Apparatus

The following equipment's, Antimicrobial agents and apparatus were used (Table 3.1, Table 3.2 and Table 3.3) during the present study:

Table 3.1 The following equipment's and apparatus

No.	Apparatus	Company
1.	Autoclave	Hirayama
2.	Vortex	Labcoo
3.	Light microscope	Lomo (micmeds 2)
4.	Water bath	Memmert
5.	Incubator	
6.	Centrifuge	Gallenkamp
7.	Electric oven	
8.	Hot plate with magnetic stirrer	
9.	Micropipettes	Hirschmann
10.	Balance	Sartorious
11.	Millipore filter-unit	Difco
12.	Melting point apparatus	Gallen kamp MFB-600

Table 3.2 Chemicals

No.	Chemicals	Company
1	Beef extract	Oxoid
2	Peptone	Oxoid
3	Urea agar supplement	Oxoid
4	Sodium Hippurate	BDH
5	N,N,N,N. (Tetramethyl-p-phenylenediamine dihydrochloride)	
6	Methyl red	
7	Alpha-naphthol	
8	Barium chloride	
9	KOH	
10	Glycerol	
11	NaCl	
12	Phenol red	
13	Potassium hydroxide	Fluka
15	H_2O_2	Fluka
16	Potassium nitrate	Sigma
17	Absolute ethanol	
18	Ethyl alcohol 95%	
19	Hydrochloric acid	
20	Ether	

Table 3.3 Antimicrobial agents and Antimicrobial agents discs

No.	Antimicrobial agents discs	code	Concentration on disk	Company and origin
1.	Nitrofurantoin	F	100	**Bioanalyse / Turkey**
2.	Erythromycin	E	15	**Bioanalyse / Turkey**
3.	Gentamycin	GN	10	**Bioanalyse / Turkey**
4.	Meropenem	MEM	10	**Bioanalyse / Turkey**
5.	Doxycycline	DO	10	**Bioanalyse / Turkey**
6.	Amikacin	AK	10	**Bioanalyse / Turkey**
7.	Amoxicillin/clavulanic	AMC	20/10	**Bioanalyse / Turkey**
8.	Trimethoprim /sulphamethoxid	STX	75	**Bioanalyse / Turkey**
9.	Ciprofloxacin	CIP	10	**Bioanalyse / Turkey**
10.	Cefixime	CFM	5	**Bioanalyse / Turkey**

Table 3.4 Cultural media

No.	Media	Company
1.	Blood agar	Himedia
2.	BHI broth	Oxoid
3.	BHI agar	
4.	MR-VP broth	
5.	Tryptophan broth	
6.	Urea agar base	
8.	Columbia blood agar	

Table 3.5 Kits

No.	Kit	Company
1.	Kovacs reagent	Hi-media
2.	Gram stain,s (crystal violet, iodine, acetone and safranin)	Syrbio

3.2 Methods

3.2.1 Sterilization methods

In order to sterilize media and some solutions that are resistant to the effects of heat, a method of sterilization called moist heat was utilized. This goal was reached by

subjecting the material to pressures of 15 bar/in2 and temperatures of 121°C inside of an autoclave for a period of fifteen minutes. In contrast, the sterilisation of glassware was accomplished by subjecting it to dry heat for a period of 3 to 2 hours at temperatures ranging from 160 to 180° C. Filtration with millipore filters having a diameter of 0.22 µm was the procedure that was utilized while dealing with solutions that are prone to becoming denatured when subjected to heat (William and Davi 2008).

3.2.2 Preparation of reagents and solutions

Methyl red reagent :In order to prepare methyl red reagent, 0.1 gm of methyl red needed to be dissolved in 300 ml of ethanol that had a concentration of 95%. Through the utilization of distilled water, the volume was brought up to a total of 500 ml. In order to determine whether or not glucose had been completely hydrolyzed, the method in question was put to use (Brown 2007).

Vogas-proskauer reagent (Barrett,s reagent) :It was composed of two solutions as follows:

I. Five grams of alpha nephthol were dissolved in ninety-five milliliters of ethanol that was 99.99 percent, and the mixture was then placed in a container that was shielded from light.

II. A solution was made by dissolving forty grams of potassium hydroxide (KOH) in sixty milliliters of distilled water. This produced the solution. After that, additional distilled water was added to the solution until it reached the desired volume of 100 milliliters (McFaddin 2000).

Oxidase test reagent :The mix 1.0 gm of N,N,N,N. (Tetramethyl-p-phenylenediamine dihydrochloride) in 100 ml of distilled water (Brown 2007).

Catalase test reagen: Prepare by 3% H2O2 (Brown 2007).

Sodium hippurate reagent: Manufactured by dissolving 12 gm of FeCl3.6H2O in 2.5 ml of hydrochloric acid. The volume was then brought up to its final, predetermined level of 100 ml by the addition of distilled water. In order to detect sodium hippurate hydrolysis, it was utilized. (Brown 2007).

3.2.3 Solutions

Normal saline solution: It was made by dissolving 9 gm of sodium chloride in a tiny amount of distilled water, then bringing the total volume up to 1000 ml, adjusting the pH to 7.2, sterilizing it in an autoclave at 121°C for 15 min, and storing it at 4°C (McFaddin 2000).

- MacFrland standard solution:

1. Solution (A): 1% aqueous barium chloride (1gm barium chloride 100 ml distilled water).
2. Solution (B): 1% aqueous solution of sulfuric acid, add 0.1 ml of component (A) to 9.9 ml of component (B), and then store the resulting solution in ampoules that are clean and dry. The diameter of the ampoules ought to be the same as that of the test tube that will afterwards be employed in the determination of the density (Brown 2007).

3.2.4 Preparation of cultural media

Ready-made media: Ready-made media, such as Brain heart infusion agar and Brain heart infusion broth, Columbia blood agar (selective media), Blood agar, MR-VP broth, Tryptophan broth, and Kliglar iron agar, were prepared in accordance with the instructions provided by the manufacturing companies. These instructions are typically affixed to the container containing the ready-made media. After being sterilized in an autoclave at 121°C for 15 min at a pressure of 15 bars/in2, they were incubated at 37°C for 24 hours to ensure their sterility, after which they were stored at 4°C until they were utilized (Silva 2013).

- Laboratory prepared media

Blood agar: The preparation of blood agar medium involved the dissolution of 40gm of blood agar base powder in 1000ml of D.W. The pH of the solution was adjusted to 7 and the medium was sterilized through autoclaving at 121 ^{0}C and 15 pound / inch^{2} for a duration of 15 min. According to the manufacturer's instructions. After cooling to 50-55°C, fresh human blood (5%) was added to the media, carefully mixed and poured into sterilized Petri dishes which were then stored at 4°C until used (Silva 2013).

Brain Heart Infusion broth with sucrose 2%: For the purpose of biofilm detection, this medium was created by first dissolving 2 g of sucrose in 100 ml of B.H.I. broth, and then being sterilized in an autoclave (Babapour *et al.* 2016).

Congo red (CR) agar: The Congo red medium was made by dissolving 0.8 g of Congo red stain and 37 g of brain heart agar in 900 ml of deionized water, bringing the mixture to a boil in order to dissolve the medium thoroughly, and then sterilizing it in an autoclave at 121° C, 15 pounds per square inch for 15 minutes. after it had cooled to between 50 and 55 degrees Celsius. After the process of sterilization by filtration (2.2.2.1.5), add the sucrose solution, thoroughly mix, and then pour into the sterilized Petri dishes (Kırmusaoğlu 2019).

Urea agar: This medium was made by dissolving 2.4 g of urea agar base in 95 ml of distilled water and then heating the mixture until all of the constituents were completely dissolved. After the medium had been autoclaved to ensure its cleanliness, it was cooled to 50°C and given a urea supplement before being dispensed into sterilized test tubes of a volume of 5 ml and allowed to harden into a slant form (Michael and Burton 2011).

Sodium hippurate medium: After dissolving 25 g of brain heart infusion broth and 10 grams of sodium hippurate in 1000 milliliters of distilled water, this medium was then distributed in tubes at a volume of 10 ml per tube and sterilized in an autoclave for 15 min at 121°C and 15 bars (Woo *et al.* 2015).

3.2.5 Collection of samples

The information necessary for each patient was documented, and data from 250 high vaginal swaps performed on female patients at Al-Karama Hospital and Al-Yarmouk Hospital between July 1, 2022 and October 30, 2022 were gathered and analyzed. These patients have experienced itching as well as a change in the color of their vaginal discharge. Their ages range from 15 to 55, and their specimens were collected using a speculum and sterile cotton swabs. We took two swabs and placed the first one in transport media for culture, while the second one was placed in normal saline for immediate microscopic wet mount examination, Gram stain, and KOH testing.

The samples were gathered in accordance with the procedure recommended by Macfaddin (2000). In the case of BV, the sample swabs were obtained from the vaginal discharge with a moistened little amount of brain heart infusion or saline. This was done in order to collect the necessary information (McFadden 2000).

Without a moment's pause, each sample was swiftly sent to the laboratory. After inoculating the sample in nutrient agar, blood agar, and MacConkey agar for one full day at 37°C in an aerobic environment, chosen colonies were separated from the blood agar medium and subcultured on mannitol salt agar (McFadden 2000).

3.3 Laboratory Diagnosis

3.3.1 Bacterial identification assay

Swab was cultured in selection media plate and incubated in candle jar contains candle to provide 5-10% CO2, 37°C, 48 hours, prepare wet quantity and Gram stain, Whiff test and pH test from swap without transport media. In the first phase, swab was cultured in transport media and incubated in candle jar contains candle to provide 5-10% CO2. (Woo *et al.* 2015).

According to the diagnostic procedures recommended by (Woo *et al.* 2015). The isolation and identification of *G. vaginalis* divided to:

3.3.2 Presumptive identification

The development of colonies on Columbia blood agar that are surrounded by zones of transparent hemolysisn and have diffuse margins, the typical appearance of Gram-stained cells, and a negative catalase and oxidase reaction are the hallmarks of this organism. (Michael and Burton 2011), moist surface and overall look *G. vaginalis* cell is a clue cell, and the vaginal secretion has a strongly acidic pH (Saunders *et al.* 2007) as well as the Whiff test. These tests provide a presumptive identification of *G. vaginalis*, and its minimal criteria may be sufficient for determining whether or not strains isolated from vaginal fluid are *G. vaginalis* (Woo *et al.* 2015).

- **Oxidase test:** After touching a sterile swab to the growth and delivering several drops of oxidase reagent to the cells on the swab, wait 10 to 30 seconds and observe the color of the reagent; a change that occurs after 30 seconds is regarded to be a negative reaction. (Michael and Burton 2011).

- Catalase test: In order to determine whether or not catalase is formed, a small quantity of growth is moved from a plate to a clean microscope slide using a wooden stick. Once the growth is on the slide, a few of drops of hydrogen peroxide with a concentration of 3% are given to the cells. If catalase is formed, there will be intense bubbling owing to the breakdown of hydrogen peroxide and the formation of oxygen gas. This will occur whether or not catalase is created (Woo *et al.* 2015).

- Whiff test: Mix vaginal secretion with KOH 10% on microscopic slide. If the test is +ve, the smell will be fishy (Michael and Burton 2011).

- pH test of secretion: By immersion pH paper in vaginal secretions by using medical forceps (Catlin 1992).

3.3.3 Confirmation identification

Methyl red test, Voges-proskaur test, urea test (Holt *et al.* 1994), indol test hydrolysis of hippurate and Starch.

- Methy Red Test: When 3-4 drops of methyl red are added to a culture that has been cultivated in MR-VP medium, the positive result tube should turn red almost immediately. If the test comes back positive, it shows that the organism has undergone mixed-acid fermentation. (Michael and Burton 2011).

- Voges-proskaur Test: After adding three to four drops of Barritt's reagent to a culture that has been established in MR-VP medium, the test tube is let to stand at room temperature for thirty minutes. If acetonin is present, the test tube will change color from pink to red during this period (Woo *et al.* 2015).

- Urea hydrolysis: Examine the urea slant that has been infected with the organism; a positive test will cause the tube to turn pink, whilst a negative test will cause the tube to remain yellow. (Woo *et al.* 2015).

- Tryptophan degradation: When 10 to 12 drops of Kovac's reagent are added to the culture of organisms in tryptone broth, a coating of a red organic substance should appear on top of the culture. in this instance, it refers to the presence of indole (Michael and Burton 2011).

- Hippurate hydrolysis: The tubes were made with 10 ml of hippurate sodium and ten hours of incubation at 37° C. After centrifugation at 6,000 revolutions per minute, 0.8 milliliters of the filtrate were taken, and 0.2 ml of a reagent was added. The mixture was well combined, and the presence of turbidity for more than 10 min indicated a favorable result (Michael and Burton 2011).

3.3.4 Identification using vitek 2 system

Vitek 2 compact systems were able to identify every bacterial isolate that was a part of this study. The VITEK 2 compact system was created for the detection and measured susceptibility testing of bacteria that have been clinically isolated. The Compact apparatus, a computer, and a printer are all components of the VITEK 2 system. A number of tools for data analysis and management are included in the software that comes packaged with the VITEK 2 Compact system (Hernández-Durán *et al.* 2017).

Detection of antibiotic susceptibility test by discs diffusion method: The Kirby-Bauer method was used to determine the antibiotic resistance of 19 different isolates of *G. vaginalis* to a total of 10 different antibiotics (nitrofurantoin, erythromycin, gentamicin, metropenem, doxycycline, amikacin, amoxicillin/clavulanic, trimethoprim sulphamrthoxid, ciprofloxacin, and ceflixime). The colonies were removed from the nutrient agar plates after an overnight culture and then inserted in a tube with three milliliters of normal saline solution. The turbidity was increased to 0.5 57 McFarland tubes, which is equal to 1.5x018 CFU per milliliter of solution. After letting a cotton swab that had been cleaned and sterilized soak in bacterial suspension, the swab was pressed against the interior of the test tube to remove any excess fluid that had accumulated. The bacterial suspension was then dispersed across the surface of the Mueller-Hinton agar plates after they had been allowed to dry for ten minutes. The plates were then incubated at 37 degrees Celsius. The technique began with the sterile placing of antibiotic discs onto the surface of the medium using forceps that had been through the sterilization process. This was followed by the incubation of the plates at 37°C for a period of 24 hours. The guidelines published by the Clinical Laboratory Standards Institute (CLSI *et al.* 2020) were used to compare the results of the measurements that were obtained with a metric ruler. After that, the results were recorded as susceptible (S), resistant (R), or intermediate (I).

3.4 Maintenance of Bacterial Isolates

3.4.1 Short term maintenance

The bacterial isolates were grown on brain heart agar slant and incubated at 37°C for 24 hours. After that, they were maintained on slant agar using screw caped test tubes for a period of time that ranged from one to three months, and they were stored at 4°C. (Veroniek *et al.* 2008)

3.4.2 Long term maintenance

Freezing the bacterial isolates in a medium that contained 15% glycerol allowed them to be kept for an extended amount of time. To produce the medium, glycerol was added to the Brain heart infusion broth until the final amount was 10 ml. The medium was then poured into a tiny bottle with a screw-capped and autoclaved to kill any bacteria. After being cooled, the medium was injected with a single colony of each isolate, then it was incubated at 37°C for twenty-four hours, and the tubes were kept in a deep freezer at a temperature of -20°C (Veroniek *et al.* 2008).

3.4.3 Detection of biofilm production

Congo red test: In order to determine whether or not pathogenic bacteria are capable of producing biofilms, bacterial isolates were streaked over Congo red agar (2.2.3.2.3) and then placed in an incubator at 37°C for one night. A good result can be identified by the presence of a black colony that has a dry and crystalline quality. The presence of pink colonies after testing indicates a negative outcome. (Sevan and Fattma 2016).

Microtiter plate method: Through the use of the microtiter plate method, the current investigation sought to determine whether or not bacteria have a tendency to form biofilms. To be more specific, a 96-well flat bottom Microtiter plate was used, and into each well was put 180 µl of brain heart infusion broth that had been sublimated with 2%

sucrose. After that, 20 ml of the bacterial culture that had been cultured for twenty-four hours was injected into the wells. The colony-forming units (CFU) of bacteria in this sample were equivalent to 0.5 McFarland standard, as determined by the Dens check. The control wells each received an additional 200 µl of the brain heart infusion broth. In the course of the overnight incubation at 37°C, the Microtiter plate was covered (this procedure was carried out three times for each sample). After washing the wells with phosphate buffer saline with a pH of 7.2 to remove any cells that were not adhered to the well, the cells that were attached to the well were allowed to dry at room temperature. The wells each received around 200 ml (0.1%) of crystal violets, and each received 20 mints. After removing the crystal violet and washing the wells three times with PBS (pH 7.2) in order to get rid of any unbound dye, the wells were allowed to dry at room temperature before being read by ELISA. After the wells were filled with ethanol at a concentration of 95%, the reader measured the absorbance of each well at 630 nm (Joana *et al.* 2015 Hu *et al.* 2017). O.D values were classified into the following to determine the biofilm formation.

$OD \leq ODC$ (NONE)

$OD_C < OD \leq 2OD_C$ (Weak)

$2OD_C < OD \leq 4OD_C$ (Moderate)

$4ODC < OD$ (High) according to (Babapour *et al.* 2016).

3.4.4 Graphene oxide nanomaterials (GO)

Characterization of graphene oxide nanomaterials (GO): Through the utilization of X-ray diffractometer (XRD) pattern analysis, Nano Research Lab, Alkhora Company for General Trading was able to successfully complete the characterization of GO Nanomaterials. The samples' molecular compositions were analyzed by means of Fourier Transform Infrared Spectroscopy (FT-IR) using a Bruker - Tensor 27 equipped with an ATR unit. Emission Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDS) were utilized by the Nano Research Lab Alkhora

Company for General Trading in order to conduct an analysis of the morphology and particle size of the sample.

Graphene oxide Nanomaterials: Determination of the Sub-Minimum Inhibitory Concentration (Sub-MIC): The determination of sub-MIC done by the different methods include (CLSI 2020):

- Microdilution method

The broth micro-dilution method recommended by the Clinical and Laboratory Standards Institute was used to determine the minimum inhibitory concentration of the formulated GONPs against clinically isolated *G. vaginalis*. This was done in accordance with the criteria provided by the Clinical and Laboratory Standards Institute. In a nutshell, the GONPs formulation was subjected to a process of serial dilution in nutritional broth within the wells of a 96-well plate. At first, a total of twelve different bacterial suspensions were added, and then the plates were put into an incubator at a temperature of 37°C for a period of twenty-four hours. The Minimum Inhibitory Concentration (MIC) was determined by visually recognizing the wells that did not show any apparent bacterial growth in order to find the wells that met the criteria. - For this purpose, GO Nanomaterials were weighed as 10 mg, and then they were dissolved in 10 ml of dimethyl sulfoxide (DMSO) to create stock solutions of a concentration of 1 mg/ml. The required concentration was then calculated from this solution, and it included the following: (25-50-75-100-125-150-175-200-225-250-275-300-325-350-375-400) µg/ml had been prepared for the determination of Sub MIC.

- In each well, there is nutritional broth, and varying concentrations of GO Nanomaterials (ranging from 25 to 400 µg/ml) are prepared from stock suspension in order to achieve a final volume of 200 µl in each well. This is done in accordance with the protocol (Babapour *et al.* 2016).
- A well of MTP without GO Nanomaterials (as positive control).
- Finally, a bacterial suspension with turbidity equivalent to 0.5 Mc-Farland was added to the wells that contained various concentrations of GO Nanomaterials,

ranging from 25 to 400 g/ml. The volume of the bacterial suspension was 2 µl. Following a 24-hour incubation period at 37°C, 0.1µl of each well's contents were removed, cultured on nutrient agar, and then re-incubated at 37°C for another 24-hour period in order to determine the Sub-MIC concentration.

- ❖ To determine the sub mic concentration, add 5 µl of the Tetrazolium solution to each well. This concentration is determined based on the turbidity and pink color that occur after one hour of incubation at 37 ° C.
- ❖ In order to confirm that the DMSO solvent did not inhibit the development of the bacteria, a control experiment was carried out in which infected broth containing just DMSO solvent was subjected to the same dilutions.

➢ well diffusion method

Following the completion of the MIC determination using the microtiter plate and tube method, the same concentration was selected alongside several other concentrations that were in close proximity. employing a mechanism called well diffusion, as will be shown in the following:

- A bacterial suspension was prepared by transferring a small number of bacterial colonies (2-4) from an overnight culture into 2 milliliters of normal saline. The turbidity of the suspension was adjusted to 0.5 McFarland, which corresponds to 1.5 108 CFU per milliliter.
- A sterile cotton swab was utilized in the process of inoculating a bacterial suspension into Muller-Hinton agar plates .
- The medium's surface was streaked three times using a swab, and after each application, the plate was rotated at an angle of sixty degrees to remove any residual staining. After that, the swab was moved around the edge of the agar surface in a circular motion.
- The plates were covered and allowed to dry at room temperature for a few minutes before the lid was put back on the container.

- A number of distinct holes, up to a maximum of six in number, were drilled into the surface of the medium, and each one was filled with a certain concentration.
- The plates were kept in an incubator for 24 hours at 37° C .
- The effect of the nanomaterial made of (GO Nanomaterials) is measured based on the development of the clear zone, also known as the inhibitory zone, around the holes.

➢ The effect of Sub-Minimum Inhibitory Concentration (Sub-MIC) of Graphene oxide nanomaterials (GO) on biofilm formation.

Co-incubation studies were used to quantify the effect of GO nanomaterials on *G. vaginalis* isolates to determine their sub-minimal inhibitory concentrations (Sub-MIC).

- To 96-well flat-bottomed plastic tissue culture plates, 100 µl of each *G. vaginalis* bacterial suspension in brain heart infusion broth with 2% sucrose were added, along with 100 µl of a Sub-MIC concentration of GO Nanomaterials at 125 g/ml.
- In the control wells, there was 180 µl of brain heart infusion broth containing 2% sucrose and 20 µl of bacterial suspensions that did not include GO. The covered microtiter plate was sealed with parafilm for the 24-hour incubation period at 37 degrees Celsius **.**
- The wells were washed three times with phosphate buffer stain (pH:7.2), which was used to eliminate any bacterial cells that were not adherent **.**
- Following drying at room temperature, 200 l of crystal violet solution containing 0.1 % was added to each well for a period of twenty minutes **.**
- After allowing the stained adherent bacterial cells to dry at room temperature, they were washed three times with phosphate buffer slain (pH:7.2), then extracted twice with 200 µl of 95% ethanol, and the absorbance of each well was measured at 630 nm using an ELISA reader**.**

- Before and after treatment with various concentrations of GO Nanomaterials (100 and 125 g/ml), the ability of biofilm formation by the majority of the bacteria that were recovered (*G. vaginalis*) was investigated (Qian *et al.* 2021).

4. RESULTS AND DISCUSSION

4.1 Selection of Patients

BV is one of the vaginal conditions that affects women of childbearing age more frequently than any other in the world. More than 200 unique bacterial species are required for a healthy microbiome in the vaginal area (Walker *et al.* 2016). Changes in the vaginal microbiota are invariably followed with a reduction in the number of Lactobacillus sp. and an increase in the number of anaerobic bacteria when seen under certain conditions. *G. vaginalis* is the most common agent responsible for BV, and it possesses virulence factors such as vaginolysin and sialidase (Lopes *et al.* 2011). Rebuilding the vaginal flora using Lactobacillus species has been shown to be an effective treatment for BV as well as a means of reducing the likelihood that the condition would reoccur (Cartwright *et al.* 2018, Castro *et al.* 2018).

At Yarmouk Hospital and AL Karama Hospital, a total of 200 samples, including those from pregnant and non-pregnant women, were collected for the purpose of this study between July 1, 2022 and October 30, 2022. They underwent examination and classification based on the number of Clue cells present in the vaginal secretions, as well as the color, odor, and pH of those secretions. Secretions from the vaginal canal might be white, yellow, or a greenish-yellow color. The pH of the vaginal secretions ranged from 3.8 to 4.2, and every single specimen was able to pass both the Whiff and Clue cell tests with flying colors. On each and every specimen, the results of the wet mount test and the whiff test came back positive. Because Clue cells were seen in the vaginal secretion Gram stain and a significant quantity of coccobacilli was found in the vaginal secretion, there is a lot of evidence to suggest that *G. vaginalis* is present.

There were 19 (9.5) isolates of *G. vaginalis* identified out of a total of 200 specimens. There were only twelve *G. vaginalis* isolates found out of a total of one hundred (50%) specimens that had white secretions and a pH range of 5–6.5. Only 5 (7.4%) of the 68 (34% of the total) specimens with yellow secretions were found to be *G. vaginalis*, and their pH ranged from 6.0 to 7.0. Only two (6.3%) of the 32 (16%) specimens of

secretions tested had *G. vaginalis* as the causative agent, despite all of the specimens having a greenish yellow hue and a pH of 6.5. The distribution of the 200 specimens that were collected in accordance with the color discharges is presented in (Table 4.1).

Table 4.1 The distribution of the 200 of the collected specimens according to the color secretions

Color of secretion	Collected specimens		Isolates of *G. vaginalis*	
	Percentage	Number	Percentage	Number
White	50 %	100	12.0 %	12
Yellow	34 %	68	7.4 %	5
Greenish- yellow	16 %	32	6.3 %	2

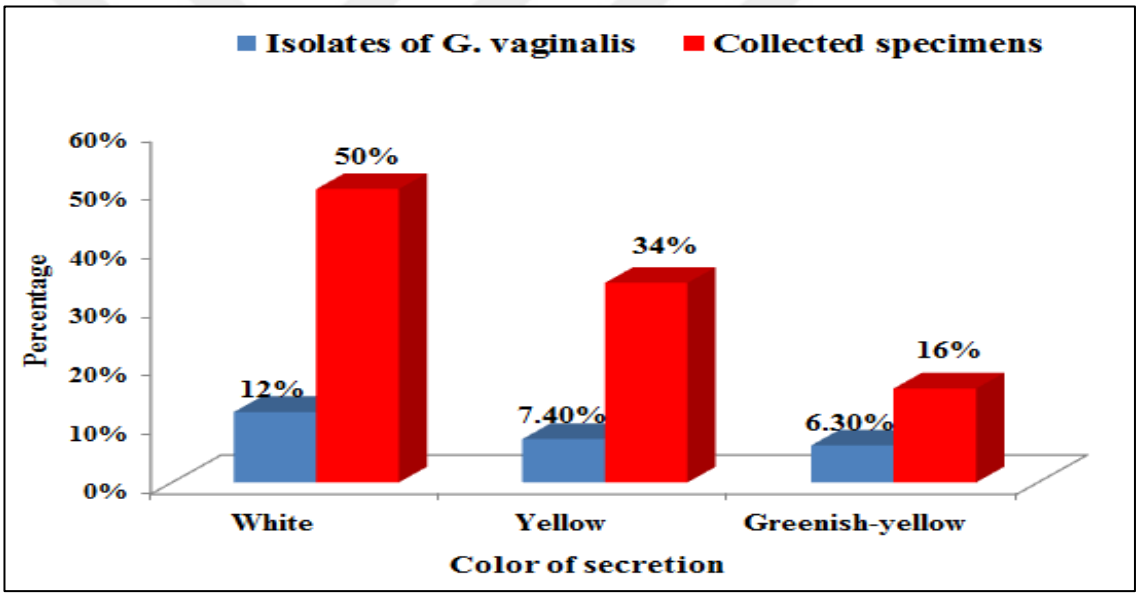


Figure 4.1 The distribution of the 200 of the collected specimens according to the color secretions

Epithelial cells and a sizeable number of gram-positive lactobacilli, often known as normal flora, are seen, on the other hand, in vaginal discharge that is considered to be normal. The gram staining of typical vaginal discharge reveals epithelial cells in addition to a considerable number of gram-positive lactobacilli, which are considered to be normal flor (Dowd and West 1987), the prevalence percentage is mentioned as being 49%. Gram staining of vaginal discharge and the detection of clue cells in moist amounts are two diagnostic procedures that can be used to establish that *G. vaginalis* is

present. Only 1% of cases were found to have secretions that were a greenish-yellow color, while 30 % of all cases were reported to have yellow secretions (Pessoa *et al.* 2015).

4.2 Cultural and Phenotypic Character

G. vaginalis colonies develop at 37°C for 24 hours on Columbia blood agar that contains 15 g/ml of nalidixic acid. These colonies are pinpoint-sized, spherical, gray to white in color, and are surrounded by hemolysis (MacFaddin 2000). These colonies are grown on chocolate agar and blood agar under the same conditions to reveal the same colonies. However, these colonies do not have a green zone, which is what differentiates them from the colonies of *Haemophilus spp*. An examination with a microscope reveals that *G. vaginalis* is a polymorphic cell that can include a single Gram-negative, variable, or double bacillus. This information was obtained from the analysis of a sample (Holt *et al.*1994, Arroyo-Moreno *et al.* 2022).

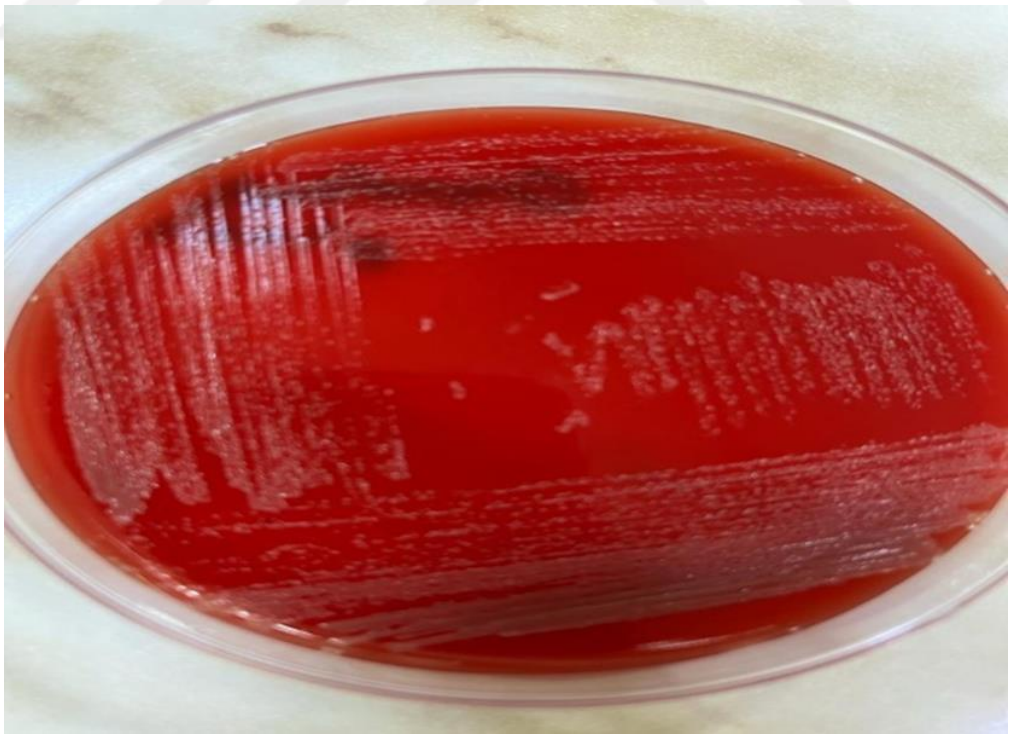


Figure 4.2 Gardnerella vaginalis appears Non-hemolytic on blood agar

4.3 Microscopic Findings

It was highlighted how crucial it is to do microscopic investigations of vaginal secretions (Arroyo-Moreno *et al.* 2022) ,who considered the presence of exfoliated squamous epithelial cells in a wet mount to be a valuable clue that *G. vaginalis* was present. The adhesion of several, microscopic bacteria results in typical clue cells having indistinct borders and a granular appearance. This is because of the presence of multiple bacteria. In the vaginal discharges of most or all patients diagnosed with bacterial vaginosis, numerous researchers found cells that could be considered clues. Consequently, we arrived at the realization that clue cells are the most accurate predictor of bacterial vaginosis as a result of this. The findings of other investigations, on the other hand, did not support this hypothesis (Spiegel 1991).

The diagnosis of bacterial vaginosis in the laboratory is based on an analysis of morphotypes found in Gram-stained smears of vaginal secretions. It was suggested that the Kopeloff modification of the Gram stain, as well as the utilization of basic fuchsine as the counterstain, be utilized as a method for avoiding excessive decolorization (Rivero-Segura *et al.* 2022). According to (Holt *et al.* 1994) There is a high level of confidence that *G. vaginalis* is present when there are both Clue cells and a considerable number of coccobacilli seen in vaginal discharge (Figure 4.2).

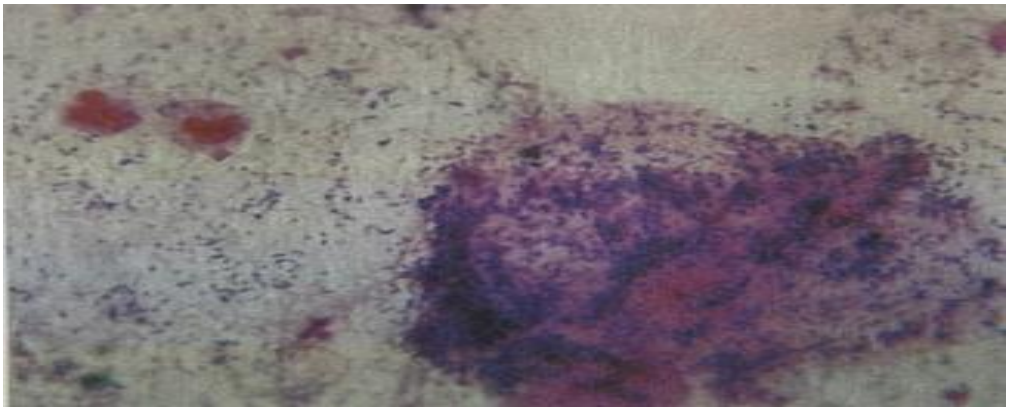


Figure 4.3 The gram stain (X1000) of bacterial vaginosis showing clue cell with gram variable *G. Vaginalis*

4.4 Biochemical Test

In order to classify the clinical isolates of *G. vaginalis*, standard biochemical testing was performed by hand in addition to being performed using a Vitek compact device. The methyl red test, the lactose fermentation test, the maltose fermentation test, the hemolysin production test, and the methyl red (MR) test were all carried out, and the results of all of them came back positive (Onderdonk *et al.* 2016). On the other hand, the tests for catalase, oxidase, indole, urease production, nitrate reduction, rafinose fermentation, and mannitol fermentation yielded negative results (Michael 2012). *G. vaginalis* did not show any symptoms of growth when cultured on Kligler agar. These findings were in line with the information presented in Bergey's Manual Determinative Bacteriology, an essential resource for making the diagnosis (William *et al.* 2012).

4.5 Susceptibility of *G. aginalis* to Antibiotic by Disk Diffusion Method

4.5.1 Kirby bauer test

It was determined whether 19 *G. vaginalis* isolates were susceptible to any of 10 different antibiotics. According to the research, all of the bacterial strains that were identified shown a one hundred percent (100%) sensitivity to the antibiotics erythromycin, gentamycin, ciprofloxacin, and cefixime. Due to the lack of these bacteria, which is responsible for the transfer of antibiotic resistance genes between bacterial cells (Catlin *et al.* 1992), this result was in agreement with the report of (Al-saady *et al.* 2001). Despite the fact that every single bacterial strain tested was completely resistant (100%) to meropenem.

According to the data presented in (Table 4.2), it was discovered that each of the isolates exhibited a unique response when exposed to amikacin, doxycycline, trimethoprim/sulfamethoxid, amoxicillin/clavulanic, and nitrofurantoin.

Table 4.2 The variable susceptibility of *G. vaginalis* isolates towards different locally available antibiotics

Antibiotics	Resistance %	Sensitivity %
Amikacin	44.2	55.8
Doxycycline	35.2	46.8
Trimethoprim /sulphamethoxid	9.6	90.4
Amoxicillin/clavulanic	51.6	48.4
Nitrofurantoin	35.7	64.3

4.6 Phenotyping Etection of Virulence Factor (Biofilm Formation).

This assay, which involves both qualitative and quantitative investigation of the biofilm biomass produced by *G. vaginalis*, makes use of two tests, which are described in more detail below:

4.6.1 Congo red agar method (CRA) (Qualitative)

We tested 19 different vaginal discharge isolates of *G. vaginalis* for their ability to produce biofilms. The investigation was evaluated using a CRA culture that was conducted for 24 hours at 37°C. According to the synthesis of the black pigment, the findings were divided into three reference strains: those that produced positive biofilm, those that produced negative biofilm, and those that produced moderate biofilm. This is illustrated in (Figures 4.4).



Figure 4.4 Biofilm detection of *G. vaginalis* by Congo red agar method, showing strongly positive

The results showed that 13 of 19 samples (68.4%) were extremely positive, 3 of 19 (15.7%) were moderately positive, and 3 of 19 (15.7%) were negative. The results were close comparable with what (Lisalotte 2017), had discovered, where 90 of 120 (75%) of *G. vaginalis* clinical strains developed black colonies. This method was utilized to give quantitative results about the potential of these isolates to create biofilm, as can be seen in (Table 4.3), which shows the outcomes of this method.

Table 4.3 Biofilm production of *G. vaginalis* by Congo red method

Biofilm formation	No. of isolate	%
Strong biofilm formation	13	68.4%
Moderate biofilm formation	3	15.8%
Weak biofilm formation	3	15.8%
Total	19	100

4.6.2 Microtiter plate method

Quantification of the biomass of the biofilm that has attached itself to the walls of the microtiter plate is performed here. Quantification of the amount of biofilm that had developed in each well was performed after the removal of planktonic cells, the application of the appropriate stain to the surface-attached bacterial cells, and the

utilization of LSD for statistical analysis (Mirsada *et al.* 2018). The findings of this study indicated that there was a difference that could be considered statistically significant between all 19 of the isolates. The results of the current investigation showed that 12/19 (63.2%) isolates of *G. vaginalis* had a strong ability to biofilm formation, while 3/19 (15.7%) has the moderate ability for biofilm formation, and 4 / 19 (21.1%) has the weak ability for biofilm production. These findings are presented in (Table 4.4):

Table 4.4 Biofilm production of *G. vaginalis by* Microtiter plate method

Biofilm formation	No. of isolate	Percentage%
strongly positive (black pigment)	12	(63.2 %)
moderately positive (mild blackening)	3	(15.7 %)
Weak (no pigment)	4	(21.1%)
Total	19	100%

4.7 Characterization of Graphene Oxide Nanomaterials (GO)

Several different methods, including Fourier Transform-Infrared spectroscopy (FT-IR), X-ray Diffractometer (XRD), Scanning Electron Microscopy (SEM), and Energy-Dispersive X-ray Spectroscopy (EDS), were utilized in order to investigate the morphological properties of the sample.

4.7.1 Fourier transform-infrared spectroscopy (FT-IR) analysis

A pectra analysis that was based on Fourier transform infrared spectroscopy was carried out in order to determine the functional groups that are associated with GO NMs. It can be seen in figure (3-8) that the carbide oxide has a decompression (Stretch) at 3550-3700 cm-1 (O-H), and it also belongs to the groups (C-OH). This information was collected from the FT-IR spectra of GO NMs, which were obtained in the region of 400 to 4000 cm-1. At 1700 cm-1 (C = O), it is a representation of (carbonyl / carboxy), at 1625 cm-1 (C = C), it is an aromatic compound (Atari), and at 1350 cm-1 (C-O), it is a representation of (carboxy). Because of the presence of moisture in the air, carboxylic acid has the possibility of containing some amount of water. (AL-Saadi and Jihad 2016)

in Iraq came to similar conclusions, and their findings are compatible with those findings.

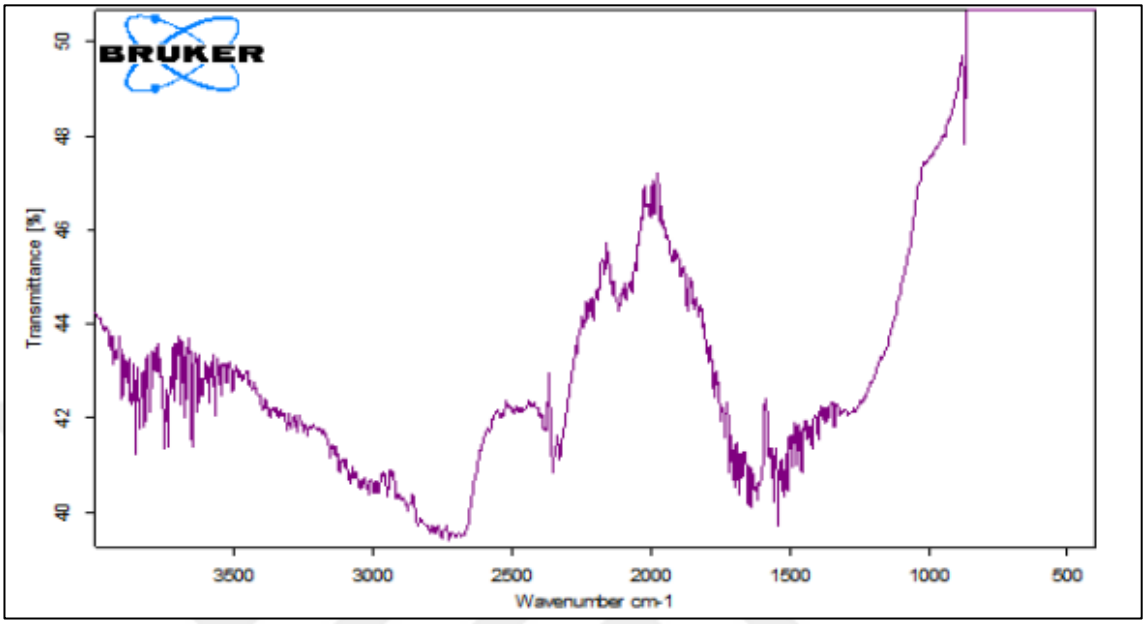


Figure 4.5 The FT-IR absorption spectrum of GO Nanomaterials

4.7.2 X-ray Diffraction (XRD) analysis

The measurement pattern of X-ray diffraction is utilized in order to investigate the intersystem behavior of crystalline behavior in films subjected to particular conditions. In order to scan the incident and reflected angles, these parameters are used: the target is CuK radiation, which has a wavelength of (1.5406 A), a current of (30 mA), and a voltage of (40 KV). In order to scan both the incident and reflected angles, the 2 technique is utilized. The XRD spectrum of GO Nanomaterials is depicted in Figure (3-6). This spectrum featured strong peaks at 26.58o, 44.02o, 54.66o, 64.35o, and 77.46o, all of which were characteristic signals for this molecule. These findings are largely consistent with those of the study by (Osama *et al.* 2021), when graphite oxide was subjected to X-ray diffraction analysis using Cu Ka1 radiation (= 1.54056), a well-defined, sharp peak for graphite that has a 3.36 d-spacing (interlayer spacing) appeared at 26o (Myat *et al.* 2018a).

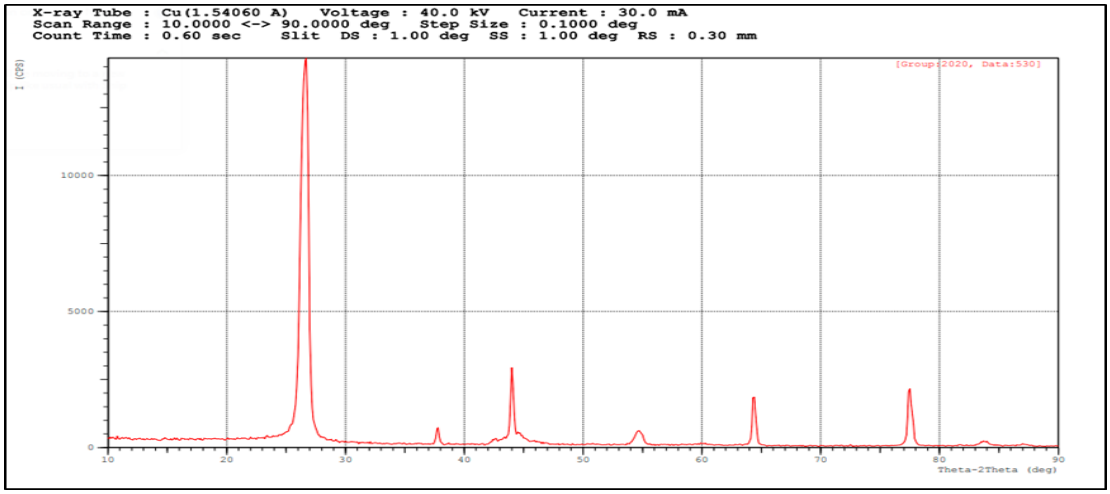


Figure 4.6 The X-ray diffraction spectrum of GO Nanomaterials

4.7.3 Scanning electron microscopy (SEM) analysis

A scanning electron microscope, often known as a SEM, is a specific kind of electron magnifying lens that generates images of a substance by subjecting it to an active electron beam for examination (Myat *et al.* 2018).The interaction between the particles in the specimen and the electrons in the environment produces a number of unique indicators that offer information about the structure of the sample and the surface geology. The following picture was captured with a scanning electron microscope (SEM) and shows GO Nanomaterials. The size of GO Nanomaterials was determined to be between 23.49 to 49.45 nm, in accordance with the findings (Figure 4.7).

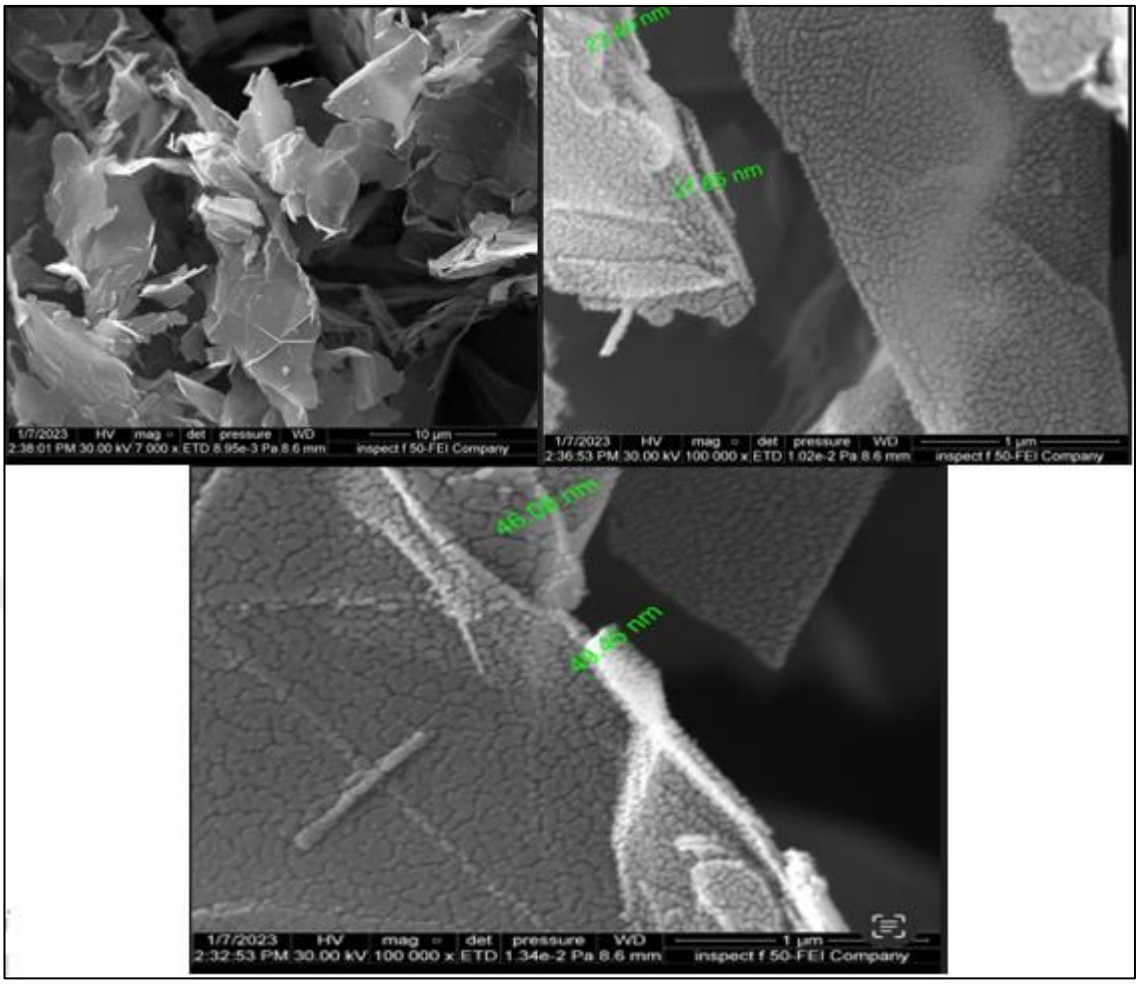


Figure 4.7 The SEM image of GO Nanomaterials

4.7.4 Energy dispersive X-ray spectrometer (EDS) analysis

The energy-dispersive X-ray spectrometer (EDS) study that was attached to the scanning electron microscope (SEM) instrument for the validation of elemental GO Nanomaterials showed that component of GO Nanomaterials as follows: Where it appears on a high degree of purity, C=81.0 and O=19.0, as shown in (Figure 4.8). The findings of (AL-Saadi and Jihad 2016), in which his results likewise contained carbon by 90.71 and oxygen by 8.66, are in line with these findings (Manoratne *et al.* 2017).

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	85.0	0.5	81.0	0.5
O	15.0	0.6	19.0	0.8

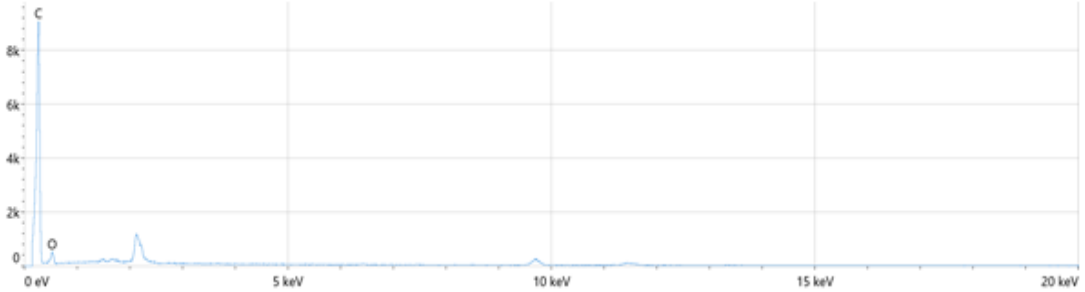


Figure 4.8 EDS pattern of GO Nanomaterials purity

4.8 Determination of Sub-Minimum Inhibitory Concentration (Sub-MIC) of Graphene Oxide Aanomaterials

The purpose of this study was to estimate the Sub-Minimum Inhibitory Concentration (Sub-MIC) of Graphene Oxide Nanomaterials by using a number of methodologies, including biofilm production, and six *G. vaginalis* bacteria isolates that had the highest levels of antibiotic resistance and strong biofilm-producing skills:

4.8.1 Microdilution method

Different concentrations ranging from 25 to 400 g/ml were used in this experiment. According to the results of the study, the sub-minimal inhibitory concentration (Sub-MIC) for GO Nanomaterials ranged from g/ml for three isolates to 125 g/ml for two isolates, 150 g/ml for one isolate, and 175 g/ml for three isolates (Table 4.5). Utilize Triphenyl Tetrazolium Chloride, also known as TTC, so that the results of your experiment can be more clearly understood. In addition, a control test was performed by utilizing inoculation broth containing just DMSO solvent at the same dilutions that the result showed had no influence in the culture medium. This test was carried out to ensure that the DMSO solvent did not affect the growth of the bacteria. In order to verify the MIC and sub-MIC values, add 10 µl of bacterial suspension to nutrient agar and record the results in (Tables 4.5).

Table 4.5 Sub-MIC for GO Nanomaterials

No.	No. of isolates	Sub-MIC of GO (µg/ml)
2.	2	125
3.	1	150
4.	3	175
Total	6	

How GO Nanomaterials interact with bacterial cells and how those interactions alter the cells' structure, viability, and metabolism are all affected by the length of time the cells are exposed to the materials, the concentration of the materials, the chemical and physical properties of the materials, and the characteristics of the microorganisms. (Faria *et al.* 2014). According to the findings of several investigations, the bursting of membranes and cell walls is caused by either the production of reactive oxygen species (ROS) or the sharp edges of the GO (Aunkor *et al.* 2020).

4.8.2 Well diffusion method

A 175 µg/ml of (GO and DMSO) was the most common concentration used to show the clear zone around *G. vaginalis* bacterial colonies as shown in the (Figure 4.9).

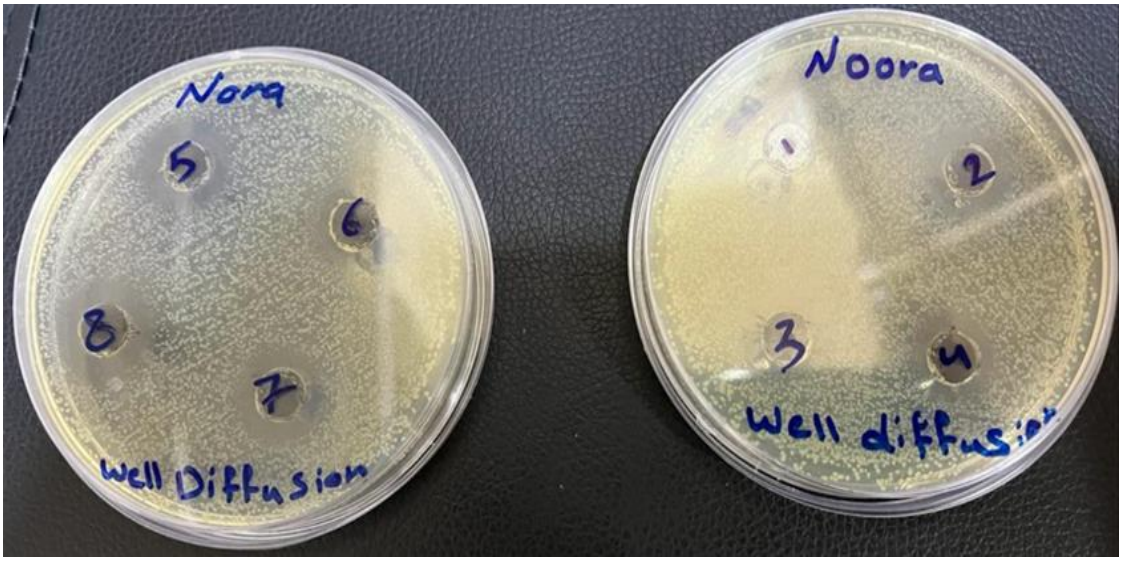


Figure 4.9 Inhibition Zone for (a) Graphene Oxide Nanomaterials (GO), against G. vaginalis on Mueller-Hinton agar plates

4.9 The effect of Sub-Minimum Inhibitory Concentration (Sub-MIC) of Graphene Oxide Nanomaterials (GO) on Biofilm Formations

In order to investigate the effects of nanomaterial on the formation of biofilm by bacteria, six different bacterial isolates of *G. vaginalis* were selected, each of which had a high level of antibiotic resistance as well as strong biofilm formation properties. Therefore, following treatment with GO MNs, the samples were analyzed to determine whether or not biofilms had formed. Following treatment with GO Nanomaterials, the ability of bacterial isolates to produce biofilm was found to be impacted, as determined by the findings of a study that investigated the effect of GO Nanomaterials on the formation of biofilm in pathogenic bacteria at various concentrations below the minimal inhibitory concentration (Sub-MIC). Because of this, five of the bacterial isolates that had the highest levels of biofilm production and antibiotic resistance were downgraded to the weak category, while just one of them was downgraded to the moderate category (Tables 4.6).

Table 4.6 Effect of GO Nanomaterials on biofilm formation

No. of isolate	Before treatment (biofilm)	After treatment (biofilm)
1	Strong	Weak
2	Strong	Weak
3	Strong	Moderate
4	Strong	Weak
5	Strong	Weak
6	Strong	Weak

5 CONCLUSIONS AND RECOMMENDATION

5.5 Conclusion

1. Pregnant women are most vulnerable to BV than non-pregnant.
2. The most frequent etiological agent of BV was *G. vaginalis*.
3. All bacteria isolates were 100 % resistance to: meropenem while Erythromycin, Gentamycin, Ciprofloxacin, cefixime are the antibiotics that showed high antibacterial activity against all *G. vaginalis* isolates, compared with varying activities of other antibiotics against *G. vaginalis*.
4. Most *G. vaginalis* isolates are biofilm producers (100%) ranging from strong to moderate and weak producers.
5. The Graphene oxide nanoparticles could potentially be an antibacterial reagent against *G. vaginalis*.
6. The current study indicates that Graphene oxide nanoparticles have strong anti-*G. vaginalis* biofilms activity.

5.6 Recommendation

1- Studying another nanoparticles in pharmaceutical preparation as antivaginosis agent.

2- Studying Synergistic effect of nanoparticles with antibiotic as a new mixed against resistance isolate of *Gardnerella vaginosis.*

3- Studying the usage of graphene oxide NPs as an ointment or vaginal wash.

REFERENCES

Abebaw B., Mengist, A., Belew, H., Aschale, Y. and Reta, A. 2021. The prevalence, antibiotic resistance pattern, and associated factors of bacterial vaginosis among women of the reproductive age group from felege hiwot referral hospital, Ethiopia. Infection and Drug Resistance, 5(8): 2685-2696.

Azad, K. A. and Rasul, M. 2019. Advanced biofuels: Applications, technologies and environmental sustainability. Woodhead Publishing.

Adela M., Mohamed A. A., Mohamed A.E. and Ashraf A.M. 2022. Removal of heavy metals and dyes from wastewater using graphene oxide-based nanomaterials: A critical review. Environmental Nanotechnology, Monitoring and Management, 18: 1007019.

Akhavan, O. and Ghaderi, E. 2012. Escherichia coli bacteria reduce graphene oxide to bactericidal graphene in a self-limiting manner. Carbon, 50(5): 1853-1860.

Al-Saadi, T. M. and Jihad, M. A. 2016. Preparation of graphene flakes and studying its structural properties. Iraqi Journal of Science, 57(1): 145-153

Alves, P., Castro, J., Sousa, C., Cereija, T. B. and Cerca, N. 2014. Gardnerella vaginalis outcompetes 29 other bacterial species isolated from patients with bacterial vaginosis, using in an in vitro biofilm formation model. The Journal of Infectious Diseases, 210(4): 593-596.

António Machado, A. and Nuno, C. 2015. Influence of biofilm formation by Gardnerella vaginalis and other anaerobes on bacterial vaginosis. The Journal of infectious diseases, 212(12): 1856-1861.

Arroyo-Moreno, S., Cummings, M., Corcoran, D.B., A. Coffey, R.R. and McCarthy, Y. 2022. Identification and characterization of novel endolysins targeting Gardnerella vaginalis biofilms to treat bacterial vaginosis. NPJ Biofilms Microbiomes, 8 (1): s41522-022.

Aunkor, M. T. H., Raihan, T., Prodhan, S. H., Metselaar, H. S. C., Malik, S. U. F. and Azad, A. K. 2020. Antibacterial activity of graphene oxide nanosheet against multidrug resistant superbugs isolated from infected patients. Royal Society Open Science, 7(7): 200640.

Aved, A., Parvaiz, F. and Manzoor, S. 2019. Bacterial vaginosis: an insight into the prevalence, alternative treatments regimen and it's associated resistance patterns. Microb Pathog, 1(27): 21–30.

Babapour, E., Haddadi, A., Mirnejad, R., Angaji, S. A. and Amirmozafari, N. 2016. Biofilm formation in clinical isolates of nosocomial Acinetobacter baumannii and its relationship with multidrug resistance. Asian Pacific Journal of Tropical Biomedicine, 6(6): 528- 533.

Babics, A., Roussellier, P. 2015. Gardnerella vaginalis: An overlooked pathogen in male patients? Med Mal Infect, 45(10): 423-424.

Baljinder K., Balgir, P., Mittu, B., Chuahan, A., Kumar, B. and Garg, N. 2013. Antimicrobial spectrum of Anti-Gardnerella vaginalis bacteriocin producing Lactobacillus fermentum HV6b against bacterial vaginosis associated organisms. American Journal of Drug Discovery and Development, 3(1): 1-12.

Bebeh N. W., Nurjanah, R., Mahen, E. C. S. and Nuryantini, A. Y. 2017. Synthesis and characterization of carbon nanoparticle/PVA/chitosan for security ink applications. Materials Research Express, 4(3): 034003.

Behzad, F., Naghib, S. M., Tabatabaei, S. N., Zare, Y. and Rhee, K. Y. 2021. An overview of the plant-mediated green synthesis of noble metal nanoparticles for antibacterial applications. Journal of Industrial and Engineering Chemistry, (94): 92-104.

Beyth, N., Houri-Haddad, Y., Domb, A., Khan, W. and Hazan, R. 2015. Alternative antimicrobial approach: nano-antimicrobial

Boyanova, L., Marteva-Proevska, Y., Gergova, R. M., Ghanim, R., Mohammad, M. R. and Abdul Hussien, A. M. 2018. Antibacterial Activity and Morphological Characterization of Synthesis Graphene Oxide Nanosheets by Simplified Hummer's Method. Biosciences, Biotechnology Research Asia, 15(3): 627-33.

Boyanova, L., Marteva-Proevska, Y., Gergova, R. and Markovska, R. 2021. Gardnerella vaginalis in urinary tract infections, are men spared? Anaerobe, (72): 102438

Breshears, L.M., Edwards, V.L., Ravel, J. and Peterson, M.L. 2015. Lactobacillus crispatus inhibits growth of Gardnerella vaginalis and Neisseria gonorrhoeae on a porcine vaginal mucosa model. BMC Microbiol, (15): 276–287.

Bretelle, F. 2015. High Atopobium vaginae and Gardnerella vaginalis Vaginal Loads Are Associated with Preterm Birth. Clin. Infect. Dis., (60): 860–867.

Brown, A. E. 2007. Benson's microbiological application Laboratory Manual in General Microbiology. (10th ed). McGraw-Hill Companies. New York.

Burton, J. P., Cadieux, P. A. and Reid, G. 2003. Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. Appl Environ Microbiol, (69): 97–101.

Cartwright, C. P., Pherson, A. J., Harris, A. B., Clancey, M. S. and Nye, M. B. 2018. Multicenter study establishing the clinical validity of a nucleic-acid amplification-based assay for the diagnosis of bacterial vaginosis. Diagn Microbiol Infect Dis., 92(3): 173-178.

Cassini, A., Högberg, L. D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G. S. and Hopkins, S. 2019. Attributable deaths and disability-adjusted life-years caused by infections with antibioticresistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. The Lancet Infectious Diseases, 19(1): 56-66.

Castro, J., França, A., Bradwell, K.R., Serrano, M.G., Jefferson, K.K., and Cerca, N. 2017. Comparative transcriptomic analysis of Gardnerella vaginalis biofilms vs. planktonic cultures using RNA-seq. Npj Biofilms Microbiomes, (3): 3.

Castro, J., Paula, A., Elisa, M. and Cerca, N. 2018. Anaerobe Lactobacillus crispatus represses vaginolysin expression by BV associated Gardnerella vaginalis and reduces cell cytotoxicity. Anaerobe, (50): 60–63.

Catlin, B. W. 1992. Gardnerella vaginalis characteristics, clinical consid- erations, and controversies. Clin Microbiol Rev, 5(3): 213.

Chen, J., Peng, H., Wang, X., Shao, F., Yuan, Z. and Han, H. 2014. Graphene oxide exhibits broad-spectrum antimicrobial activity against bacterial phytopathogens and fungal conidia by intertwining and membrane perturbation. Nanoscale, 6(3): 1879-1889.

Chong, Y., Ge, C., Fang, G., Wu, R., Zhang, H., Chai, Z. and Yin, J. J. 2017. Light-enhanced antibacterial activity of graphene oxide, mainly via accelerated electron transfer. Environmental Science and Technology, 51(17): 10154-10161.

Choudhary, S., Malik, Y. S. and Tomar, S. 2020. Identification of SARS-CoV-2 cell entry inhibitors by drug repurposing using in silico structure-based virtual screening approach. Frontiers in immunology, 11: 1664.

CLSL, CLSL Supplement M100s. and Wane, PA. 2020. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing. 30thed.

Colonna, S., Caitlin, H., Steelman, G. and Michael, T. 2020. Amsel Criteria. Pubmed. PMID 31194459.

Connie, M. R., Lehman, D. C. and Manuselis, G. 2007. Staphylococci. Text book of Diagnostic Microbiology, 3: 367.

Cooper D. R. 2014. Experimental Review of Graphene. Hindawi Publishing Corporation, 3 Nov. 2011. Web. 16 Apr. 2014. http://www.hindawi.com/journals/isrn/2012/ 501686/.

Curran, D. and Rivlin, M. E. 2012. Bacterial vaginosis, E-medicine (Online) Accessed: 13 November 2012.

De La Fuente, J. 2014. "Graphene." Graphenea. Web. 26 Apr. 2014. http://www.graphenea.com/pages/graphene#. U1xxufldWSo.

Di Rosa, Q., Cheng, L. and Zhang, H. 2016. Identification of vaginal bacteria diversity and its association with clinically diagnosed bacterial vaginosis by denaturing gradient gel electrophoresis and correspondence analysis. Infection, Genetics and Evolution, (44): 479–486.

Douglas, K., Owens, C. W. and Encina, C. 2022. Screening for Bacterial Vaginosis in Pregnant Persons to Prevent Preterm Delivery. JAMA., 323(13): 1286-1292.

Faria, A. F. D., Moraes, A. C. M. D. and Alves, O. L. 2014. Toxicity of nanomaterials to microorganisms: mechanisms, methods, and new perspectives. In Nanotoxicology, 363-405.

Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., and Kjelleberg, S. 2016. Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology, 14(9): 563-575.

Frincy, Kh. B., Ajanta, S., Chanakya, D., Naba, K. H, and Jasmin, H. H. 2014. Role of Gardnerella vaginalis as an etiological agent of bacterial vaginosis. Iran J Microbiol, 6(6): 409–414.

Gajdács, M. 2019. The concept of an ideal antibiotic: implications for drug design. Molecules, 24(5): 892.

Gaurab, K. 2021. Gardnerella vaginalis associated Bacterial Vaginosis (BV): clinical, cultural and Biochemical diagnosis. December 22: 2022

Ghosal, K. and Sarkar, K. 2018. Biomedical applications of graphene nanomaterials and beyond. ACS Biomaterials Science and Engineering, 4(8): 2653-2703.

Gottschick, C., Szafranski, S. P. and Kunze, B. 2016. Screening of compounds against Gardnerella vaginalis biofilms. PLoS One. 11(4): 0154086 - e0154086.

Hernández-Durán, M., López-Jácome, L. E., Colín-Castro, C. A., Cerón-González, G., Ortega-Peña, S., Vanegas-Rodríguez, E. S., Mondragón-Eguiluz, J. and Franco-Cendejas, A. 2017. Comparison of the MicroScan WalkAway and VITEK 2 Compact systems for the identification and susceptibility of clinical Gram-positive and Gram-negative bacteria. IX International Congress on Rehabilitation Research. Presentation, 8: 57.

Holt, J. G. 1994. Bergey's manual of determinative bacteriology. 9th Edition, Lippincott Williams and Wilkins, Baltimore.

Hu, Y., Zhu, Y. and Lu, N. H. 2017. Primary Antibiotic Resistance of Helicobacter pylori in China. Dig. Dis. Sci, (62): 1146–1154.

Hukić, M., Seljmo, D., Ramovic, A., Ibrišimović, M. A., Dogan, S., Hukic, J. and Bojic, E. F. 2018. The effect of lysozyme on reducing biofilms by Staphylococcus aureus, Pseudomonas aeruginosa, and Gardnerella vaginalis: an in vitro examination. Microbial Drug Resistance, 24(4): 353-358.

Ibisanmi, T. A. 2022. Nanotechnology Strategy as Antibacterial: a Primer for the Notice

Ivanova, A., Ivanova, K. and Tzanov, T. 2021. Simultaneous Ultrasound-Assisted Hybrid Polyzwitterion/Antimicrobial Peptide Nanoparticles Synthesis and Deposition on Silicone Urinary Catheters for Prevention of Biofilm-Associated Infections. Nanomaterials, 11(11): 3143.

James, B. 2022. Graphene: The New Kid on the Block. In Graphene; Jenny Stanford Publishing, 1: 25-31.

Jane, R. Schwebke, C., Muzny, A. and William, E. J. 2022. Role of Gardnerella vaginalis in the Pathogenesis of Bacterial Vaginosis: A Conceptual Model. J Infect Dis., 210(3): 338-43.

Jang, S. E., Jeong, J. J., Choi, S. Y., Kim, H., Han, M. J. and Kim, D. H. 2017. Lactobacillus rhamnosus HN001 and Lactobacillus acidophilus La-14 Attenuate Gardnerella vaginalis-infected bacterial vaginosis in mice. Nutrients, 9: 17.

Jang, S. E., Jeong, J. J., Choi, S. Y., Kim, H., Han, M. and Kim, D. H. 2017. Lactobacillus rhamnosus HN001 and Lactobacillus acidophilus La-14 Attenuate Gardnerella vaginalisInfected Bacterial Vaginosis in Mice. Nutrients, (9): 531.

Janulaitiene, M., Paliulyte, V., Grinceviciene, S., Zakareviciene, J., Vladisauskiene, A., Marcinkute, A. and Pleckaityte, M. 2017. Prevalence and distribution of Gardnerella vaginalis subgroups in women with and without bacterial vaginosis. BMC Infect Dis, 17(1): 394.

Javed, A., Parvaiz, F. and Manzoor, S. 2019. Bacterial vaginosis: an insight into the prevalence, alternative treatments regimen, and its associated resistance patterns. Microbial Pathogenesis, (127): 21–30.

Jindal, K. and Goswami, M. 2020. Sensitivity testing of antibiotics: A brief review. Annals of Tropical Medicine and Public Health, (23): 231-536.

Joana, C., Patrícia, A., Cármen, S., Tatiana, C., Ângela, F., Kimberly, K. J. and Nuno, C. 2015. Using an in-vitro biofilm model to assess the virulence potential of bacterial vaginosis or non-bacterial vaginosis Gardnerella vaginalis isolates, (5): 11640.

Joo K. Y., Moon, E. C., Kim, J. Y., Kim, H. J., Heo, K., Shim, J. J. and Lee, J. L. 2023. Lactobacillus helveticus HY7801 ameliorates bacterial vaginosis by inhibiting biofilm formation and epithelial cell adhesion of Gardnerella vaginalis. Food Science and Biotechnology, 32(4): 507-515.

Joshi, K., Mazumder, B., Chattopadhyay, P., Bora, N. S., Goyary, D. and Karmakar, S. 2019. Graphene family of nanomaterials: Reviewing advanced applications in drug delivery and medicine. Current Drug Delivery, 16(3): 195-214.

Kahwati, L. C., Clark, R. and Berkman, N. 2020. Screening for bacterial vaginosis in pregnant adolescents and women to prevent preterm delivery: updated evidence report and systematic review for the US Preventive Services Task Force. JAMA., (10): 1001-0233.

Karin S., Stoodley, P., Goeres, D. M., Hall-Stoodley, L., Burmølle, M., Stewart, P. S. and Bjarnsholt, T. 2022. The biofilm life cycle: Expanding the conceptual model of biofilm formation. Nature Reviews Microbiology, 20(10): 608-620.

Khanna, A., Khanna, M. and Aggarwal, A. 2013. Serratia Marcescens- A Rare Opportunistic Nosocomial Pathogen and Measures to Limit it's Spread in Hospitalized Patients. Journal of Clinical and Diagnostic Research, 7(2): 243-246.

Kharsany, A. B., Hoosen, A. A. and Van den Ende, J. 1993. Antimicrobial susceptibilities of Gardnerella vaginalis, Antimicrobal Agents Chemother, 37(12): 2733–2735.

Kim, H., Kim, Y. and Kang, C. H. 2021. In vivo confirmation of the antimicrobial effect of probiotic candidates against Gardnerella vaginalis. Microorganisms, 9(8): 1690.

Kırmusaoğlu, S. 2019. The methods for detection of biofilm and screening antibiofilm activity of agents. Antimicrobials, antibiotic resistance, antibiofilm strategies and activity methods, 1-17.

Kostakioti, M., Hadjifrangiskou, M. and Hultgren, S. J. 2013. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harbor perspectives in medicine, 3(4): a010306.

Krysanova, A. A., Gushchin, A. E., Savicheva, A. M. 2021. Significance of Gardnerella vaginalis genotyping in diagnosis of recurrent bacterial vaginosis. Medical alphabet. 1(30): 48-52.

Lagacé-Wiens, P.R.S., Ng, B., Reimer, A., Burdz, T., Wiebe, D. and Bernard, K. 2008. Gardnerella vaginalis bacteremia in a previously healthy man: A case report and characterisation of isolate. Journal of Clinical Microbiology, 46(2): 804-806

Lee, K. and Yoon, S. S. 2017. Pseudomonas aeruginosa biofilm, a programmed bacterial life for fitness.

Leopold, L.B. and Maddock, T. 1953. The Hydraulic Geometry of Stream Channels and Some Physiographic Implications. USGS Professional Paper No, (252): 1-57.

Leos-Alvarado, C., Llaca-Díaz, J., Flores-Aréchiga, A., Pérez-Chávez, F. and Casillas-Vega, N. 2020. Male urethritis. A review of the ideal diagnostic method. Actas Urol Esp (Engl Ed), 44(8):523-8.

Livengood, C. H. 2009. Bacterial Vaginosis: An Overview for 2009. Reviews in Obstetrics and Gynaecology, 2(1): 28-36

Lopes, S. S. G., Deschaght, P., Aila, N. E., Kiama, T. N. Verstraelen, H. Jefferson, K. K., Temmerman, M. and Vaneechoutte, M. 2011. Gardnerella vaginalis comprises three distinct genotypes of which only two produce sialidase. Am. J. Obstet. Gynecol, 204: 450.

Lyudmila B., Marteva-Proevska, Y., Gergova, R. and Markovska, R. 2021. Gardnerella vaginalis in urinary tract infections, are men spared?. Anaerobe, 72: 102438.

Ma, J., Li, Y., Grundish, N. S., Goodenough, J. B., Chen, Y., Guo, L. and Wan, L. J. 2021. The 2021 battery technology roadmap. Journal of Physics D: Applied Physics, 54(18): 183001.

Ma, W., Li, J., Li, X., Bai, Y. and Liu, H. 2021. Nanostructured Substrates as Matrices for Surface Assisted Laser Desorption/Ionization Mass Spectrometry: A Progress Report from Material Research to Biomedical Applications. Small Methods, 5(10): 2100762.

MacDorman, M. F. 2011. Race and ethnic disparities in fetal mortality, preterm birth, and infant mortality in the United States: an overview. Seminars in Perinatology, 35(4): 200-208

MacFaddin, J. F. 2000. Biochemical tests for identification of medical bacteria, (3rded). Lippincott Williams and Wilkins. Philadelphia, PA.

Manoratne, C., Rosa, S. and Kottegoda, I. 2017. Xrd-Hta, Uv Visible, Ftir And Sem Interpretation Of Reduced Graphene Oxide Synthesized From High Purity Vein Graphite. Material Science Research India, (14): 19-30.

Megan, R. 2018. Bacteria against bacteria: Isolation, identification and characterization of bacteria with antimicrobial activities against Gardnerella vaginalis. A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science (MSc) in Biology.

Menard, J. P. 2011. Antibacterial treatment of bacterial vaginosis: current and emerging therapies. International Journal of Women's Health, (3): 295–305

Michael, G. 2012. Bergey's Manual of Systematic Bacteriology. editors, volume 5; William B. Whitman, director of the editorial office; Aidan C. Parte, managing editor. Volume 5, The Actinobacteria. New York: Springer

Michael, J. and Burton, E. 2011. A Photographic Atlas for the Microbiology Laboratory. 4th EDITION. Publisher: Douglas N. Morton. Copyright © 2011 by Morton Publishing Company. ISBN: 978-089582-872-9

Mirsada H., Seljmo, D., Ramovic, A., Ibrišimović, M. A., Dogan, S., Hukic, J. and Bojic, E. F. 2018. The effect of lysozyme on reducing biofilms by Staphylococcus aureus, Pseudomonas aeruginosa, and Gardnerella vaginalis: an in vitro examination. Microbial Drug Resistance, 24(4): 353-358.

Mittal, V., Jain, A. and Pradeep, Y. 2012. Development of modified diagnostic criteria for bacterial vaginosis at peripheral health centres in developing countries. Journal of Infections in Developing Counties, 6(5): 373-377

Moon, E. C., Park, M. S., Lim, T., Kim, R. H., Ji, G. E., Kim, S. Y. and Hwang, K. T. 2022. Antibacterial effect of cell-free supernatant fraction from Lactobacillus paracasei CH88 against Gardnerella vaginalis. Scientific Reports, (12): 4763

Morrill, S., Gilbert, N. M. and Lewis, A. L. 2020 Gardnerella vaginalis as a cause of bacterial vaginosis: appraisal of the evidence from in vivo models. Front Cell Infect Microbiol, (10): 168.

Muktad, F. A. and Nawfal, H. A. 2022. Antibacterial and antibiofilm activity of bacterially reduced Graphene oxide against some MDR bacterial pathogens isolated from urinary tract infections. AIP Conference Proceedings, 2386(1): 2386 -1063.

Muzny, C. A. and Schwebke, J. R. 2013. Gardnerella vaginalis: still a prime suspect in the pathogenesis of bacterial vaginosis. Curr Infect Dis Reports, (15): 130–135.

Myat, S., Wah, W. Htwe, D. and Pho, K. 2018. Synthesis of Graphene Oxide using Modified Hummer's Method and its Characterizations. See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/326449999

Nagaraja, P. 2008. Antibiotic resistance of Gardnerella vaginalis in recurrent bacterial vaginosis. Indian Journal of Medical Microbiology, 26(2): 155-157.

Nakamura, M., Tahara, Y., Fukata, S., Zhang, M., Yang, M., Iijima, S. and Yudasaka, M. 2017. Significance of optimization of phospholipid poly (ethylene glycol) quantity for coating carbon nanohorns to achieve low cytotoxicity. Bulletin of the Chemical Society of Japan, 90(6): 662-666.

Nikhil, K., Beauty, B., Sai, S. S., Kunal, P., Sirsendu, S. R. and Saroj, R. 2011. Bacterial vaginosis: Etiology and modalities of treatment—A brief note. J Pharm Bioallied Sci, 3(4): 496–503.

Nipunjot, G., Mahajan, A. and Bajaj, J. K. 2018. The effect of supplementation of standard antibiotic therapy with oral probiotics for bacterial vaginosis.

Norah, K. and Manish, G. 2022. Gardnerella. Treasure Island (FL): StatPearls Publishing; 2022 Jan. Norah Kairys; Manish Garg.

Dowd, T. C. and West, R. R. 1987. Clinical prediction of Gardnerella vaginalis in general practice. J. Royl Coll. Gen. Pract, 37(295): 59–61

Ogunshe, A. A. O. and Falode, O. A. 2021. The Intriguing Extrapolations of Haemolysis Assay as Screening Criterion for Selecting Biosurfactant-Producing Microorganisms in Petroleum Industries Process-Conditions. J Pet Environ Biotechnol, (8): 431.

Onderdonk, A. B., Delaney, M. L. and Fichorova, R. N. 2016. The Human Microbiome during Bacterial Vaginosis. Clin. Microbiol. Rev. 29: 223–238.

Osama, H. R., Mohamed, A. A. and Ashraf, A. M. 2021. An eggshell hydroxyapatıte-graphene oxıde nanocomposıte for the removal of heavy metals from waste water. Journal of Environmental Science, 50(2): 1-33.

Othman, M. 2012. ProB Trial: Probiotics and the prevention of preterm labour: A randomised controlled trial protocol. WebmedCentral Obstetrics and Gynaecology, 3(6): WMC003535.

Page, A. J., Cummins, C. A., Hunt, M., Wong, V.K., Reuter, S. and Holden, M.T.G. 2015. Roary: rapid large-scale prokaryote pans genome analysis. Bioinforma. Oxf. Engl, (31): 3691–3693.

Patterson, J. L., Stull-Lane A., Girerd, P. H. and Jefferson K. K. 2010. Analysis of adherence, biofilm formation and cytotoxicity suggests a greater virulence potential of Gardnerella vaginalis relative to other bacterial-vaginosis-associated anaerobes. Microbiology, 156(2): 392-399.

Pessoa, W.F.B., Melgaço, A.C.C., de Almeida, M.E., Ramos, L.P., Rezende, R.P. and Romano, C.C. 2017. In Vitro Activity of Lactobacilli with Probiotic Potential Isolated from Cocoa Fermentation against Gardnerella vaginalis. BioMed Res. Int, (20): 1–10.

Pham, V. T., Truong, V. K., Quinn, M. D., Notley, S. M., Guo, Y., Baulin, V. A. and Ivanova, E. P. 2015. Graphene induces formation of pores that kill spherical and rod-shaped bacteria. ACS Nano, 9(8): 8458-8467.

Pleckaityte, M., Janulaitiene, M., Lasickiene, R. and Zvirbliene, A. 2012. Genetic and biochemical diversity of Gardnerella vaginalis strains isolated from women with bacterial vaginosis. FEMS Immunol Med Microbiol, 65(1): 69-77.

Pleckaityte, M., Zilnyte, M. and Zvirbliene, A. 2012. Insights into the CRISPR/Cas system of Gardnerella vaginalis. BMC microbiology, 12: 1-11.

Plummer, E. L., Vodstrcil, L. A., Doyle, M., Danielewski, J. A., Murray, G. L. and Fehler, G. A. 2021. Prospective, open-label pilot study of con- current male partner treatment for bacterial vaginosis. mBio, 12(5): e0232321.

Qian, Z., Zhu, H., Zhao, D., Yang, P., Gao, F., Lu, C. and Chen, D. 2021. Probiotic Lactobacillus sp. strains inhibit growth, adhesion, biofilm formation, and gene expression of bacterial vaginosis-inducing Gardnerella vaginalis. Microorganisms, 9(4): 728.

Randis, T. M., Zaklama, J., LaRocca, T. J., Los, F. C. and Lewis, E, L. 2013. Vaginolysin drives epithelial ultrastructural responses to Gardnerella vaginalis. Infect Immun.Dec, 81(12): 4544-50.

Rivero-Segura, N. A., Morales-Rosales, S. L. and Rincón-Heredia, R. 2022. Microscopy Principles in the Diagnosis of Epidemic Diseases. In Principles of Genetics and Molecular Epidemiology, 6: 87-105.

Sahayaraj, K. and Rajesh, S. 2011. Bionanoparticles: synthesis and antimicrobial applications. Science against microbial pathogens: communicating current research and technological advances, 23: 228-244.

Santiago, G. L., Deschaght, P., El Aila, N., Kiama, T. N., Verstraelen, H., Jefferson, K. K., Temmerman, M. and Vaneechoutte, M. 2011. Gardnerella vaginalis Comprises three distinct genotypes of which only two produce sialidase. Am J Obstet Gynecol, 204(5): 450.

Saunders, S., Bocking, A., Challis, J. and Reid, G. 2007. Effect of Lactobacillus challenge on Gardnerella vaginalis biofilms. Colloids Surf B Biointerfaces, 55(2): 138-142.

Schellenberg, J. J., Patterson, M. H. and Hill, J. E. 2017. Gardnerella vaginalis diversity and ecology in relation to vaginal symptoms. Res Microbiol, 168(9-10):837-844.

Sevan, H. B. and Fattma, A. A. 2016. Comparison of Different Methods for Detection of Biofilm Production in Multi-Drug Resistance Bacteria Causing Pharyngotonsillitis. International Journal of Research in Pharmacy and Biosciences, 3(2): 2394-5893

Shadjou, N. and Hasanzadeh, M. 2016. Graphene and its nanostructure derivatives for use in bone tissue engineering: Recent advances. J. Biomed. Mater. Res. A, 104(5): 1250–1275.

Shahriari, S., Sastry, M., Panjikar, S. and Raman, R. S. 2021. Graphene and Graphene Oxide as a Support for Biomolecules in the Development of Biosensors. Nanotechnology, Science and Applications, (14): 197.

Shipitsyna, E., Roos, A. and Datcu, R. 2013. Composition of the vaginal micro- biota in women of reproductive age sensitive and specific molecular diagnosis of bacterial vaginosis is possible? PloS One, (8): e60670.

Shopova, E., Nikolov, A. and Dimitrov, A. 2011. Susceptibility to antibiotics of microorganisms related with recurrent bacterial vaginosis. Akush Ginekol (Sofiia), 50(7): 20-21

Silva, D., Taniwaki, N. and Gomes, R. 2013. Preparation of media and reagents, in: Microbiological Examination Methods of Food and Water. CRC Press, 6: 347–436.

Silverberg, B., Moyers, A., Hinkle, T., Kessler, R. and Russell, N. G. 2021. CDC Update: treatment and complications of sexually trans- mitted infections (STIs). Venereology, 1(1): 23-46.

Sobel, J.D, Barberi, R.L. and Barss, V.A. 2012. Bacterial vaginosis (Online) www.uptodate.com/contents/bacterial-vaginosis Accessed: 8 November 2012

Spiegel, C. A. 1991. Bacterial vaginosis. Clinical microbiology reviews, 4(4): 485-502.

Srinivasan, S., Hoffman, N. G. and Morgan, M. T. 2012. Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. PloS One, (7): e37818.

Srinivasan, S., Morgan, M. T., Fiedler, T. L., Djukovic, D. and Hoffman, N. G. 2015. Metabolic signatures of bacterial vaginosis. MBio, (14): 16-20.

Srivastava, S. and Bhargava, A. 2022. Green nanoparticles: the future of nanobiotechnology, pp. 1-352, Berlin/Heidelberg, Germany: Springer.

Taylor, B. D., Darville, T. and Haggerty, C. L. 2013. Does bacterial vaginosis cause pelvic inflammatory disease. Sex Transm Dis, 40(2): 117-122.

Teixeira, G. S., Carvalho, F. P. and Arantes, R. M. 2012. Characteristics of Lactobacillus and Gardnerella vaginalis from women with or without bacterial vaginosis and their relationships in gnotobiotic mice. J Med Microbiol, (61): 1074-1081.

Ting, L., Zhang, Z., Wang, F., He, Y., Zong, X., Bai, H. and Liu, Z. 2020. Antimicrobial susceptibility testing of metronidazole and clindamycin against Gardnerella vaginalis in planktonic and biofilm formation. Canadian Journal of Infectious Diseases and Medical Microbiology, 2: 20.

Togni, G., Battini, V., Bulgheroni, A., Mailland, F., Caserini, M. and Mendling, W. 2011. In vitro activity of nifuratel on vaginal bacteria: could it be a good candidate for the treatment of bacterial vaginosis? Antimicrobial Agents and Chemotherapy, 55(5): 2490-2492

Tomusiak, A., Strus, M. and Heczko, P. B. 2011. Antibiotic resistance of Gardnerella vaginalis isolated from cases of bacterial vaginosis. Ginekologia Polish, 82(12): 900-904

Tu, Y., Lv, M., Xiu, P., Huynh, T., Zhang, M., Castelli, M. and Zhou, R. 2013. Destructive extraction of phospholipids from Escherichia coli membranes by graphene nanosheets. Nature Nanotechnology, 8(8): 594-601.

Udayalaxmi, J., Bhat, G. K. and Kotigadde, S. 2011. Biotypes and virulence factors of Gardnerella vaginalis isolated from cases of bacterial vaginosis. Indian Journal of Medical Microbiology, 29(2): 165-168

Vadlapudi, V. and Kaladhar, D. S. V. G. K. 2014. Green synthesis of silver and gold nanoparticles. Middle East J Sci Res, 19(6): 834-842.

Veisi, K., Farajnia, S., Zarghami, N., Khorshid, H. R. K., Samadi, N., Khosroshahi, S. A. and Jaliani, H. Z. 2015. Chaperone-assisted soluble expression of a humanized anti-EGFR ScFv antibody in E. Coli. Advanced Pharmaceutical Bulletin, 5(1): 621.

Veroniek, S. M., Saegeman, N. L., Ectors, D., Lismont, B. and Verduyckt, V. 2008. Short- and long-term bacterial inhibiting effect of high concentrations of glycerol used in the preservation of skin allografts. Burns, 34(2): 205-211.

Vick, E. J. 2014. Characterization of the human inflammatory response to Gardnerella vaginalis. Doctoral dissertation, Middle Tennessee State University, Murfreesboro, TN.

Vitali, B, Cruciani, F, Baldassarre, M.E, Capursi, T, Spisni, E, Valerii, M.C, Candela, M, Turroni, S and Brigidi, P. 2012. Dietary supplementation with probiotics during late pregnancy: outcome on vaginal microbiota and cytokine secretion. BMC Microbiology, (12): 236.

Walker, A.W. 2016. Microbiota of the Human Body. Implic. Health., (902): 5–32.

William, A., Rutala, David J. and Weber, H. 2008. Guideline for Disinfection and Sterilization in Healthcare Facilities. Healthcare Infection Control Practices Advisory Committee (HICPAC).

William, B., Whitman, J. and Sons, W. 2012. Bergey's Manual of Systematic of Archaea and Bacteria, First Edition.

William, J. A., Wareab, W., Frederique, K., Christopher, D., Jason, L., Brownbc, S., Hagend, D., Corcorane, M., Cummingse, G. and Ramagebc, R. K. 2022. In vitro bacterial vaginosis biofilm community manipulation using endolysin therapy. Biofilm, (5): 100101

Wilson, M. 2005. Microbial Inhabitants of Humans: Their Ecology and Role in Health and Disease, (1ed) Press Syndicate of the University of Cambridge, Cambridge_Unitedkingdome.

Woo, K., Sik, H. and Young, K. 2015. In Guk Hwang, Sang Hoon Lee. and Heon Sang Jeong. 2015. Characteristics of the Thermal Degradation of Glucose and Maltose Solutions. Prev Nutr Food Sci, 20(2): 102-9.

Xie, Y., He, Y., Irwin, P. L., Jin, T. and Shi, X. 2011. Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni. Applied and Environmental Microbiology, 77(7): 2325-2331.

Zhen, X., Lundborg, C. S., Sun, X., Hu, X. and Dong, H. 2019. Economic burden of antibiotic resistance in ESKAPE organisms: a systematic review. Antimicrobial Resistance and Infection Control, 8(1): 1-23.

Zhou, H., Zou, F., Koh, K. and Lee, J. 2022. Antibacterial Activity of Graphene-Based Nanomaterials. In Multifaceted Biomedical Applications of Graphene, 5: 233-250.

Zvirbliene, A., Pleckaityte, M., Lasickiene, R., Kucinskaite-Kodze, I. and Zvirblis, G. 2010. Production and characterization of monoclonal antibodies against vaginolysin: mapping of a region critical for its cytotoxic activity. Toxicon, (56): 19–28.

CURRICULUM VITAE

Personal Information

Name and Surname : Noora Sabah DAWOOD AL-HASHIM

Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Biology	2019-2023
Undergraduate	Baghdad University Faculty of Science Department of Biology	1996 -2000