SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

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Detection of Aerobic Bacterial Pathogens Causing Bacterial Vaginitis among Pregnant Women in The Third Trimester, in Khartoum state, Sudan

الكشف عن البكتريا الهوائية الممرضة المسببة لالتهاب المهبل بين النساء الحوامل في الثلث الثالث من الحمل في ولاية الخرطوم

A Thesis Submitted in Fulfilment of the Requirements

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بسم الله الرحمن الرحيم

الاية

((يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِير))

صدق الله العظيم

سورة المجادلة، آية"11"

Dedication

Every challenging work needs self efforts as well as guidance of Elders especially those who were very close to our heart.

My humble effort I dedicate to My sweet and loving

Supporting Father & caring Mother

Whose affection, love, encouragement and prays of day and night make me able to get such success and honor,

Along with all hard working and respected

My supervisor

Acknowledgment

All thanks to Allah the worthy of all praises for all that we are sincore thanks and gratitude.

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ABSTRACT

Bacterial vaginosis, candidiasis and trichomoniasis, and bacterial vaginitis along with sexually transmitted infection are the common vaginal infection in women of reproductive age group in developing countries.Vaginal colonization with some species of bacteria during the last term of pregnancy can affect the health of fetuses and newborns resulting in high morbidity and mortality among newborns. Aerobic vaginitis microbiological common pathogens are Group B *Streptococcus, Enterococcus faecalis, Escherichia coli and Staphylococcus aureus.* Aerobic vaginitis is an endogenous opportunistic infection brought about by the disruption of the normal vaginal microbiota. The early diagnosis and treatment during pregnancy may reduce the risk of negative pregnancy out come.

The Present study was carried out to determine the prevalence of vaginal infection and isolation of pathogens involved in vaginitis.

A cross sectional study was conducted at Khartoum state, Sudan during the period of March-September 2022. Consecutive patients were approached to participate in the study, Socio demographic and clinical data were obtained from each participant a total of 100 third trimester pregnant women using pre tested questionnaires. High Vaginal Swab were collected and cultured for detection of aerobic bacterial pathogens.

A total of one hundred high vaginal swabs were collected from pregnant women which 49% showed positive growth constituted of various type of pathogens while 51% showed no growth. The percentages of positive high vaginal swab culture were include different type of pathogens 16(16%) was *E. Coli* highest growth,15 (15%) was *S aureus* second highest growth, 7 (7%) was *E.faecalis*, 3(3%) was *Klebsiella* Spp ,6 (6%) was *Candida* Spp and 2 (2%) was *S.agalactiae* .

The result of the research showed that there was a significant association between growth of bacteria and following symptoms (fatigue, vaginal discharge, vaginal irritation). The most frequent symptoms among pregnant women were vaginal discharge 61 (61%) the growth observed on 48 (78%) from pregnant women with vaginal discharge and 13(22%) had no growth. Also vaginal irritation showed significant association with bacterial growth 44(83%) and 9(17%) showed no growth and 46 (46%) had fatigue showed positive growth percentages 28 (60%).

There was association between vaginal discharge and bacterial growth, *E.coli* and *Staphylococcus* were the predominant bacteria found in present study.

ملخص الدراسة

التهاب المهبل البكتيري وداء المبيضات وداء التريكوموناس والتهاب المهبل الجرثومي بالإضافة إلى العدوى المنقولة جنسياً هي العدوى المهبلية الشائعة لدى النساء في سن الإنجاب في البلدان النامية ، ويمكن أن يؤثر الاستعمار المهبلي مع بعض أنواع البكتيريا خلال الفترة الأخيرة من الحمل على صحة الأجنة المواليد الجدد مما يؤدي إلى ارتفاع معدلات المرضى والوفيات بين الأطفال حديثي الولادة. المسببات المرضية الشائعة لالتهاب المهبل الهوائي الميكروبيولوجي هي المجموعة Bالعقدية والمكورات المعوية البرازية والإشريكية القولونية والمكورات العنقودية الذهبية. التهاب المهبل الهوائي هو عدوى انتهازية داخلية تحدث بسبب اختلال الجراثيم المهبلية الطبيعية. قد يقلل التشخيص والعلاج المبكران أثناء الحمل من مخاطر ظهور الحمل السلبي.

أجريت الدراسة الحالية لتحديد مدى انتشار العدوى المهبلية وعزل مسببات الأمراض المرتبطة بالتهاب المهبل.

أجريت دراسة مقطعية بولاية الخرطوم، السودان خلال الفترة من مارس إلى سبتمبر 2022. تم الاتصال بالمرضى المتتاليين للمشاركة في الدراسة ، وتم الحصول على البيانات الاجتماعية والديموغرافية والسريرية من كل مشاركة بإجمالي 100 امرأة حامل باستخدام استبيانات تم اختبارها مسبقًا. . جمعت مسحة مهبلية عالية وزرعتها للكشف عن مسببات الأمراض البكتيرية الهوائية.

تم جمع ما مجموعه 100 مسحة مهبلية من النساء الحوامل والتي أظهرت 49% نمو إيجابي مكون من أنواع مختلفة من مسببات الأمراض بينما أظهر 51% عدم وجود نمو ، كانت النسب المئوية الإيجابية لزراعة المسحات المهبلية عالية تشمل أنواعًا مختلفة من مسببات الأمراض. كان 16 (16 %) هو الأعلى نموًا للإشريكية القولونية، 15 (15 %) كان عدوى العنقوديات الذهبية ثاني أعلى نمو ، 7 (7 %) كان عدوى المكورات المعوية البرازية ،3 (3%) كان عدوى الكلبسية الرئوية، 6(6%) كان عدوى فطريات المهبل و 2 (2%) كانت عدوى العقدية القاطعة للدر.

أظهرت نتائج البحث أن هناك علاقة معنوية بين نمو البكتيريا والأعراض التالية (الحمى ، التعب ، الإفرازات المهبلية ، تهيج المهبل) ، وكانت الأعراض الأكثر شيوعاً بين النساء الحوامل هي الإفرازات المهبلية 61 (61%) لوحظ النمو على 48 (78%) من النساء الحوامل المصابات بإفرازات مهبلية و 13 (22%) لا ينمو لديهن. كما أظهر التهيج المهبلي ارتباطًا معنويًا بنمو البكتيريا 44(83%) و 9 (17%) أظهروا عدم وجود نمو. و 46 (46%) يعانون من التعب أظهروا نسب نمو إيجابية 28 (60%). هنالك علاقة بين الافرازات المهبلية و نمو البكتريا ,للإشريكية القولونية و العنقوديات الذهبية اكثر البكتيريا وجودا في هذه الدراسة.

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AV: Aerobic Vaginitis.

AVF: Abnormal Vaginal microflora.

BV: Bacterial Vaginosis.

DIV: Desquamative Inflammatory Vaginitis.

EOD: Early Onset Disease.

GBS: Group B Streptococcus.

HVS: High Vaginal Swab.

IV: Inflammatory Vaginitis.

LOD: Late Onset Disease.

MMPs: Matrix metalloproteases.

PCR: Polymerase Chain Reaction.

PROM: Premature Rupture Of the Membranes.

TV: Trichomonas Vaginalis.

UPEC: Uropathogenic *Esherichia coli.*

UTI: Urinary Tract Infection.

VLBW: Very Low Birth Weight.

VVC: Vulvovaginal Candidiasis.

WHO: World Health Organization.

CHAPTER I

INTRODUCTION

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INTRODUCTION

1.1 Introduction:

The term vaginitis is the diagnosis given to women who present complaining of abnormal vaginal discharge with vulval burning, irritation or itching (Schorge *et al.*,2012). Inflammation of the vaginal mucosa called vaginitis, is one of the most frequent complaints in women attending gynaecological clinics accounting for 10 million office visits each year (Forbes *et al.,* 2007).The most frequently observed microorganisms in the female genital tract are *group B streptococcus, Escherichia coli, Ureaplasma urealyticum, Mycoplasma hominis, Chlamydia trachomatis*, and those responsible for bacterial vaginosis (Tempera and Furneri.,2010 ;Tibaldi *et al.*,2016). Vaginitis is the most common disease of female reproductive tract infections. It is reported that in 2019, about 14.7 million female patients among 20–64 years old have vaginitis in world wide (Lakshmi *et al*.,2019).

The vagina is a habitat where under normal conditions there is equilibrium among the different bacterial species in a commensalistic symbiosis with the host organism. The stabilization of this equilibrium is due to the presence of lactic acid bacteria, among these, probably lactobacilli through different amensalistic activities (production of substances toxic to competing populations; low pH, production of H_2O_2, competition with pathogens for adhesiveness and for nutrients of the vaginal mucosa) are able to protect the vagina from attack by both exogenous and endogenous pathogens (Tempera and Furneri .,2010 ; Fan *et al*.,2021)

Disruption of the vaginal ecosystem during aerobic vaginitis cause an increase in pH to 6, decrease in lactate concentration and an increase in leucocytes and pro-inflammatory cytokines concentration in the vaginal discharge. The common presenting features are yellowish vaginal discharge and dyspareunia with red inflammation of vagina. The increased local production of IL-1, IL-6 and IL-8 associated with aerobic vaginitis is responsible for the increased risk of preterm delivery, Premature rupture of the membranes (PROM) and chorioamnionitis during pregnancy (Donder *et al.,* 2002).

About 1.4-8% of non-pregnant women and 3-8% of pregnant women are diagnosed with aerobic vaginitis. These percentage could actually be higher, because AV is often misdiagnosed as an inflammatory form of bacterial vaginosis. It wasn't until 2002 that AV was recognized as a different condition.common symptoms of AV include sticky yellowish vaginal discharge with foul or rotten smell, redness and swelling around the opening of the vagina, vaginal itching and burning sensation (Donder *et al*.,2002 ; Han *et al*.,2019).

Previous studies mainly focused on early or late stage pregnancy, and it is hard to understand the incidence of vaginitis across the whole of pregnancy. The study of risk factors of vaginitis in pregnant women also has importent clinical significance. Many studies have explored the risk factors of vaginitis in non pregnant women, which may differ significantly from those of pregnant women , due to hormonal and physiological changes(Wang *et al*.,2017 ; Pek *et al*., 2021). Epidemiological studies have shown that older maternal age, multiple sexual partners, previous spontaneous miscarriages, and alteration of vaginal bacterial communities are among the risk factors for AV and BV (Kaambo *et al*.,2018).

Escherichia coli accounts for 80 to 90% of aerobic vaginitis infections on pregnant women (Schnarrb and Smaill., 2008). .*E. coli* can coexist alongside commensals and adhere to vaginal epithelium, adherence triggers a series of events that lead to uropathogenic *Escherichia coli* (UPEC) internalization into non-degradative vacuoles (Brannon *et al*.,2020).

Group B streptococcus (GBS) (*Streptococcus agalactiae*), a gram-positive coccus, is one of the major causes of maternal or neonatal severe infection and sepsis. Maternal infection associated with GBS includes acute chorioamnionitis, endometritis, and urinary tract infection. Neonatal GBS infection is characterized as early onset if occurring within 7 days of age or late onset otherwise, and involves bacteremia, pneumonia, or meningitis (Winn H. N.,2007 ; Hansen *et al*., 2004).

The rate of vaginal carriage of *S.aureus* has been reported to be 4% - 22% of the vaginal microbiota of pregnant women (Top *et al* 2012; Bourgeois *et al* 2010). *Enterococcus faecalis* causing life threatening infections in preterm infants (Hufnagel *et al*.,2007).

Listeriosisis an uncommon bacterial infection that is potentially fatal in the foetus, in new borns and immunocompromised adults. *Listeria monocytogenes* cause invasive listeriosis with central nervous system involvement (meningitis, meningoencephalitis) and bactaeremia with a high case fatality rate (20% to 30%). (Goulet *et al*.,2013). Pregnant women are more common by 20 times to get infection by *Listeria monocytogenes* than in the general population, Pregnant women account for 27% of all listerial infections,which can cause mild illness in mothers, but can be devastating to the fetus, in some cases leading to severe disease or fetal death (Janakiraman., 2008).

1.2 Rationale

Aerobic vaginitis is a new none classifiable pathology that is neither specific vaginitis nor bacterial vaginosis (Fan *et al* .,2012) . The association between bacterial vaginosis and preterm delivery has been extensively studied, while there is limited research on the clinical significance of abnormal vaginal colonization by aerobic bacteria(Daskalakis *et al* .,2006).The diversity of this microbiological peculiarity could also explain several therapeutic failures when patients were treated for infections identified as bacterial vaginosis. Good and early diagnosis of infected and even conducted a routine antenatal screen-and-treat program for asymptomatic vaginal infections in pregnancy can be efficient and prevent infection and safe fetus life, The aim of this study is to detect the aerobic vaginal microorganisms in pregnant women.

1.3Objectives

1.3.1 General Objective:

To detect aerobic bacterial pathogens causing bacterial vaginitis among pregnant women in third trimester, in Khartoum State, sudan.

1.3.2. Specific Objectives:

- To isolate and identify bacterial pathogens from vaginal swab using blood agar , CLED agar and biochemical tests.
- To determine the association between presence of bacteria and clinical symptoms of bacterial vaginitis in pregnant women.
- To investigate the presence of abnormal vaginal colonization among pregnant women according to Gravidity ,Age group ,use of contraceptive and history of abortion .

CHAPTER II
LITERATUREREVIEW

CHAPTER II

LITERATURE REVIEW

2.1 Cervicovaginal infections:

Cervicovaginal, is an infectious or non-infectious inflammation of the vaginal mucosa, which sometimes involves the vulva (external genitals) and cervix. This inflammation often causes itching, burning, irritation, discharge and discomfort (Razzak *et al*.,2011). The vagina is a highly nutrient chamber for microbes (Danielsson *et al*.,2011). As a result, the composition of the vaginal microbiota is affected by numerous host factors, including age, change in hormone level, other genital infections, as well as sexual and hygiene practices (El Aila *et al.*,2009 ; Ma *et al*.,2012).

Cervicovaginal infections have been associated with a series of pregnancy-related complications, including preterm delivery, premature rupture of the membranes (PROM), spontaneous abortion, intrauterine growth restriction, intrauterine death, neonatal infections, postpartum endometritis, and postoperative infections (Tibald *et al*.,2016).

The most bacterial agents causing vaginitis include *Staphylococcus aureus, Echerichia coli, Group B streptococci (GBS), Listeria monocytogenes, Klebsiella pneumoniae, Acinetobacter spp, Neisseria gonorrhoea*, and the bacteria that cause bacterial vaginosis in addition to Chlamydia. (Coddington.,2002).

Aerobic vaginitis has been described as a disruption of the normal vaginal microbiota with a reduction in hydrogen peroxide producing lactobacilli in a mainly facultatively anaerobic microbiota, accompanied by an increased vaginal PH, sings of inflammation with leukocyte infiltration (Donders *et al*.,2011 ; Tansaril *et al.*,2013).

In Vagina, there was an estrogen lacking state and the insusceptible traded off status because of diabetes or other related vaginal variables can prompt development of anomalous commensals which may thus prompt contaminations. Vaginal contaminations are a typical gynaecological issue in the investigation zone, The fundamental inclining factors including helpless cleanliness, low financial status , and early sexual action, for indicative vaginal contaminations (Gopal and Kalpana., 2013).

Aerobic Vaginitis differs distinctly from bacterial vaginosis in its clinical features and host response with Bacterial Vaginosis being a condition brought about by an imbalance and shift of the normal vaginal flora in favour of anaerobic bacteria with no sings of inflammation (Donders *et al*.,2002). The inability to diagnose subclinical ascending uterine infection may result in foetal infection with a neonatal mortality rate of 25%_90% due to congenital neonatal sepsis (K umari and Tempe., 2015).

2.2Aetiology of Aerobic Vaginitis:

In a healthy vaginal microenvironment, the balance and interaction between microbes are critical. This balance can transform into a disturbed state named ‘abnormal vaginal microflora’ (AVF) or ‘dysbiosis’ , resulting in conditions such as bacterial vaginosis, aerobic vaginitis or candidiasis (Macklaim *et al*.,2012 ; Wang *et al*.,2016).

The most frequently encountered bacteria are *Eschreichia coli, Staphylococcus aureus,* coagulase negative Staphylococci such as *S. epidermidis,* group B *Streptococcus* (*Streptococcus agalactiae*) and *Enterococcus faecalis (*Donders *et al.,* 2002 ; Fan *et al.,* 2013 ;Dermendjiev *et al.,* 2015).

2.3Epidemiology and Risk factors of Bacterial Vaginitis:

About 5 to 10% of women are affected by aerobic vaginitis (Tansarli *et al.,* 2013).Reports in pregnant women point to a prevalence of 8.3-10.8% (Zodzika *et al.,* 2011). In a study of 631 patients attending routine prenatal care from a vaginitis clinic, 7.9% has moderate to severe AV sing and symptoms and 6% had ‘full blown’BV (Donders *et al.,* 2002).

In a study of 3,000 women, 4.3% were found to have severe AV, also called Desquamative Inflammatory Vaginitis.Furthermore, 49.5% of the women with DIV were peri- or postmenopausal. A reported hypothesis is that a drop in estrogen my trigger the development of AV in the aforementioned menopausal women, as well as postpartum nursing women (Sobel *et al*.,2011).

In a more recent study of 215 women, 19.1% were found to have ‘common vaginitis’ caused by BV, vulvovaginal candidiasis(VVC), or tricomoniasis (TV), whereas 12.6% were found to have ‘inflammatory vaginitis’ (IV). Of the IVgroup , 77.8% were characterized as having DIV. In fact ,42.9% of the women with DIV were found to be GBS positive , a 5-fold increase over the healthy patients (17.7% positive) (Leclair *et al*.,2010).This study was similar to an earlier study that found 43% of DIV patients were GBS positive (Sobel.,1994).Aerobic vaginitis has been observed in 8-11% of pregnant women (Donders *et al* .,2011) and (Donders *et al*.,2009). Only very few studies have been carried out thus far to evaluate risk factor for the development of aerobic bacterial vaginitis , the risk factors for AV is similer to BA,The major risk factors associated with bacterial vaginosis includes higher number of lifetime sexual partners. Other epidemiological risk factors associated are high frequency of vaginal intercourse, history of pregnancy, and cigarette smoking (Bukusi *et al*., 2006).

Maternal infection is also an important modifiable cause of stillbirth, especially in low and middle income countries shows that severe maternal infection occur in 10.9 women per 1,000

live births and leads to disproportionately high rates of stillbirth and early neonatal death (WHO., 2020).

2.4 Comparison of clinical and microbiological characteristics of Aerobic vaginitis and Bacterial vaginosis:

The contribution of AV and BV to vaginal health and pregnancy outcome has been investigated for over a century, yet they remain incompletely understood (Rampersaud *et al*.,2012). In epidemiologic studies, it has been suggested that having multiple sexual partners, increased maternal age, previous spontaneous abortions, and altered vaginal bacterial communites (including decreased lactobacillus species and concurrent colonization with Candida species) are the risk factors for vaginal colonization with microbes associated with endogenous infection such as AV and BV(Rampersaud *et al*.,2012 ; Nardis *et al*.,2013).

Aerobic vaginitis was first characterized in 2002 (Donders *et al* 2002). As a vaginal condition distinct from BV , which may require different clinical management and have distinct clinical risks (Donders *et al* .,2011). AV is associated with increased vaginal pH and more genital inflammation, increased numbers of leukocytes visible in vaginal smears, with increased activity to pathogens termed "toxic leukocytes" (Donders *et al*.,2015).

Bacterial vaginosis is defined according to the presence of clinical symptoms and increased vaginal pH typically ≥4.5, existence of white adherent discharge that contains exfoliated epithelial cells with Gram- variable polymorphic rod-shaped bacteria attached to their surface (clue cells), and a fishy odor (Turovskiy *et al*.,2011).

AV microbiological common pathogens are Group *B Streptococcus, Enterococcus faecalis, Escherichia coli* and *Staphylococcus aureus*. BV is typically polymicrobial , characterized by the presence of mainly anaerobic microorganisms including *Gardnerella vaginalis, Prevotella* species, and *Mycoplasma hominis, Mobiluncus* species (Srinivasan *et al*.,2009 ;Rampersaud *et al*.,2012 ; Zarbo *et al* .,2013).

There is no generally accepted clinical strategy for treating AV. AV is treated with antibiotics with intrinsic activity against bacteria of fecal origin, in addition to ensuring minimal interference with vaginal Lactobacillus species (Han *et al*.,2015),treated with Kanamycin, Clindamycin. Fluoroquinolones such as Ciprofloxacin and Ofloxacin, have been used in treatment because they have little effect on the normal flora allowing for a rapid recovery from AV (Tempera and Furneri 2010) . Unlike BV, AV dose not respond well to metronidazole, which is commonly used for the treatment of T.vaginalis and BV (Donders .,2007). Clindamycin is therefore considered to be a better choice than metronidazole for pregnant women with an abnormal vaginal microbiota (Tempera and Furneri., 2010).

2.5 Pathogenesis:

The pathogenesis of aerobic vaginitis is not yet unrevelled, However it seems coincide with different factors such as:

2.5.1 Decrease in lactobacillus dominated microflora

The healthy microbiome of the vagina predominantly consists of lactobacilli. Lactobacilli can inhibit adherence of various urogenital pathogens , including group *B Streptococcus* and *S.aureus* (Zarate and Nader., 2006).Competitive exclusion through to vaginal epithelial cells, two other general strategies have been proposed to explain how lactobacilli inhibit growth of other microorganisms in the vaginal niche in permenopausal women competition for nutrients and production of antimicrobials, such as bacteriocins, hydrogen peroxidise and lactic acid (Boris and Barbes., 2000).Bacteriocin production by some isolates of lactobacilli inhibited the growth of *E.coli*, while its production in other isolates prevented growth of *S.aureus* (Razzak and Al-Greitty., 2011).

2.5.2The role of local estrogen levels

A vaginal smear of normal vaginal fluid contains epithelial cells. A lack of estrogen lead to shift of exfoliating epithelial cells towards intermediate, or in worse cases, parabasal cells (Gorodeski *et al*.,2005). Estrogen play an important role in AV treatment, in conjunction with other components supporting the role of locally lowered estrogen levels in AV pathogenesis (Donders *et al*.,2015).

2.5.3Imbalance in local immune modulation

The increase in pro inflammatory cytokines is directly related to endocrine influences were taken into account, as the menstrual cycle, contraceptive use and pregnancy also influence cytokine responses. High IL-8 levels and sialidase activity present in AV oppose the idea that microbial sialidases may interfere with the cytokine cascade leading to diminished IL-8 levels, as was suggested as a mechanism to explain its depression in BV (Marconi *et al*., 2013). While IL-6 is an essential trigger of the inflammation cascade (Scheller *et al*.,2011). IL-8 is a chemoattractant cytokine that attracts and activates neutrophils in infected regions (Marconi *et al*.,2013).

2.5.4 Production of sialidases

Some bacterial species or some strains within a species produce sialidases, which degrade host defence molecules such as IgA and which can remove sialic acid from mucosal epithelial cells and mucins. Removal of sialic acid from secretory IgA is the mechanism by which sialidase leads to IgA proteolytic , and consequently , a lowered local immune response (Lewis *et al*.,2012).

2.6*Streptococcus agalactiae*

Streptococcus agalactiae is also known as Group B *streptococcus (*GBS) because it was identified as Lancefield Group B in precipitin test among a group of beta-hemolytic streptococci. Like other groups of *Streptococci,* GBS is catalase-negative and and gram positive beta-hemolytic cocci. GBS readily grows on 5% sheep blood agar at 37C for 24_48 hours of incubation with or without 5% carbon dioxide. GBS usually appears as translucent to opaque, flat and glossy colonies which are 3-4 mm in size with or without narrow zone of beta hemolysis on 5% sheep blood agar (Forbes *et al.*,2007).

Streptococcal species of clinical importance are divided into six groups depended on pathogenic and clinical characteristics. *Streptococcus agalactiae* descents into the pyogenic group. The pyogenic streptococci are recognized on blood agar plates by the classical zone of β haemolysis surrounding colonies. Further classification of the B-haemolytic *streptococc*i is by serological typing of the polysaccharide capsule, a method developed by Rebecca Lancefield in the 1940s (Madigan *et al*., 2006 ; Greenwood *et al.,* 2012).

2.7 Pathogenesis of *Streptococcus agalactiae*

Streptococcus agalactiae is asymptomatic bacterium found in the genital and lower intestinal tracts, that's contribute to isolated between 10 to 30% of pregnant women (Campbell *et al*., 2000).

Moreover, it associated with significant maternal periportal infection including bacteraemia, endocarditis, chorioamnionitis, endometritis, UTI, arthritis, and responsible for serious bacterial illness and sometimes leading to deaths in nonpregnant women that have underlying diseases and in elderly adults (Raabe and Shane., 2019).

GBS can also pass through the cervix without causing serious cervicitis, and cross intact amniotic fluid causing amnionitis thereby infecting the foetus in the uterus (Dilrukshi *et al*., 2021).

Reports suggesting that nearly 50 % of the GBS transmitted from the carrier mother to the neonates at the deliver in which 1-3% of this rate develops severe GBS infections, such as neonatal pneumonia, sepsis, and meningitis (Brouwer *et al*., 2010) .

There are some virulence determinants for the GBS infectious process. The first step involves the binding of GBS to human epithelial surfaces such as vaginal epithelium, placental membranes, alveolar epithelium, pulmonary endothelium and blood brain barrier endothelium. The acidic condition of the vaginal mucosa also favors GBS adherence. The second virulence determinant is the penetration of host cellular barriers by a series of processes including intra

cellular invasion, induction of inflammation and direct cell lysing by secreting cytolytic enzymes (Doran and Nizet., 2004).

The effect of GBS on neonates divided into two types: Early onset disease (EOD) and Late onset disease (LOD) are two major syndromes of GBS that may cause sepsis and meningitis in neonates. EOD normally occurs within the first week of life or begins a few hours after birth that may cause severe infection among neonates. LOD occurs on or after 7 days of age and is not as common as EOD. In USA ,early onset diseases were found in 0.1% to 0.2% of live births with mortality rate up to 20% (Lee *et al*.,2007). Fatality rates of EOD and LOD were approximately 6.5% and 2.85% respectively (Balter and Schuchat., 2006).

2.8 *Listeria monocytogenes*:

Listeria monocytogenes is an intracellular, aerobic and facultative anaerobic, Gram positive bacterium, which was discovered accidentally in a prevalent infection in laboratory animals (Mateus *et al*.,2013). *L. monocytogenes* is primarily transmitted to humans orally via food, accounting for 99% of cases (Scallan *et al.*,2011). *L. monocytogenes* can develop in the refrigerator over a long time and withstand large-scale temperatures (-0.4- 45) (Pagliano *et al*.,2017). Its incidence rate is considerably low, and it is preventable and treatable However , it is associated with high hospitalization and mortality rates (20-50%) (Swaminathan and Gerner-Smidt., 2007).

For many years the genus *Listeria* only contained one species, *L. monocytogenes.* Currently however, there are six recognized species including *L. monocytogenes, L. innocua, L. welshimeri, L. seeligeri, L. ivanovii, and L. grayi*. (Ryser and Marth.,2007). According to the World Health Organization (WHO), the onset of *listeria* during pregnancy accounted for nearly 43% of total cases, and 14% occurred in late pregnancies (Wadhwa and Smith., 2017).

2.9 Pathogenesis of *Listeria monocytogenes*

Listeriosis is the disease caused by *L. monocytogenes* infections. Listeria is widely distributed in the environment and can also be found in the gastrointestinal tract of individuals who remain as asymptomatic carriers. This non-invasive listeriosis occurs in healthy adults but generally only amounts to gastrointestinal illness, fever, vomiting and diarrhoea, where the degree of severity is dependent on the characteristics of the host and the organism's environment. The most common invasive listeriosis infections occur in children, the elderly, pregnant women and their featus, among pregnant women it can cause still births and abortions (Wang *et al* .,2002).

Mothers infected with *Listeria* may be aware of reduced fetal movement and noticeable utrine contractions, or symptoms of threatened preterm birth, such as abdominal pain, vaginal

bleeding, or premature rupture of membrane. Maternal infection with LM could lead to chorioamnionitis or meconium-like amniotic fluid during delivery, the literature also reports that abnormalities of fetal digestive tract are discoverable through an ultrasound, including fetal ascites, gallbladder enlargement, intestinal echo enhancement, and small intestine widening, implying the likelihood for a fetal intrauterine infection. The newborn was born with moderate jaundice and dyspnea in this case (Hasbun *et al.*,2013).

2.10.***Escherichia coli***

E. coli is an enteric gram-negative bacterium that belongs to Enterobacteriaceae family, it is facultative anaerobes that ferment glucose, oxidase negative and reduce nitrate to nitrites. Naturally, it inhabits the intestinal tracts of humans and animals and is released into the environment through deposition of faecal material. Furthermore, *E. coli* inhabits the female genital tract as a normal flora (Devi *et al*., 2014 ; Lebea, and Davies., 2017).

According to Saez-Lopez *et al* (2016), *E. coli* is reported as one of the most common organisms found in the genital tract of non-pregnant (9–28%) and more prevalent in pregnant women (24–31%) .

2.11 Pathogenesis of *Escherichia coli*

Specific virulence determinants in uropathogenic strains of *E.coli* are associated with invasive infection and pyelonephritis in pregnancy by facilitating their adherence to uroepithelial cells, thereby preventing bacteria from urinary lavage and allowing for multiplication and tissue invasion (Schnarr and Smaill.,2008).

E.coli has also been more frequently reported in women who deliver preterm than those delivering full term (McDonald and chamber 2000 ; Carey and Klebanoff 2005). Some studies have reported an emerging trend of *E.coli* predominating in early neonatal sepsis following prophylaxis for GBS colonization during pregnancy (Baltimore *et al*.,2001 ; Cordero *et al* .,2004).

E. coli can be a harmless resident of the gastrointestinal tract. However, it also has the pathogenic capability to cause significant intestinal and extraintestinal diseases such as the urinary tract, bloodstream, and central nervous system. In addition, various pathogenic *E. coli* pathotypes studied in humans, animals, food, as well as the environment are responsible for many diseases and deaths in the world. The pathotypes possess different features that allow them to inhabit the intestinal mucosa and cause diseases in humans (Croxen *et al*., 2013).

Among pregnant women *E. coli* was reported as causes of preterm delivery, neonatal sepsis (Cools ., 2017 ; Patnaik *et al*., 2017).

2.12 *Staphylococcus aureus*

Is a Gram positive spherically shaped bacterium, frequently found in the upper respiratory tract and on the skin . It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need of oxygen (Masalha *et al.*,2001).

2.13 Pathogenesis of *Staphylococcus aureus*

Infections during pregnancy commonly occur as pathogens ascend from the vagina through the cervix to the gestational membranes, but hematogenous spread to the placenta also occurs. Infection and the subsequent inflammatory response result in release of cytokines, prostaglandins and matrix metalloproteases (MMPs). These mediators promote uterine contraction and metalloprotease-induced membrane damage, ultimately resulting in premature rupture of membranes and preterm birth (Goldenberg *et al.*,2000).

S.aureus account for more than 90% of late onset sepsis in neonates. Late-onset sepsis is noted to be four times higher in very low birth weight (VLBW) infants (Grass and Leone., 2013).

2.14 *Enterococcus faecalis*

Formerly classified as part of the group D *Streptococcus* system – is a Gram-positive, commensal bacterium inhabiting the gastrointestinal tracts of humans (Rayn and Ray., 2004 ; De Almedei *et al.*, 2018).

2.15 Pathogenesis of *Enterococcus faecalis*

Group D *enterococci* and non-enterococci are part of the genital tract, but are a common cause of urinary tract infections, subacute bacterial endocarditis, abdominal abcesses, and wound infections (Hufnagel *et al*.,2007 ; Al-Abbas 2012) . The predominance of asymptomatic genital tract infection in pregnant women has been reported in the literature (A kerele *et al*.,2002), with *enterococci* causing life threatening infections in preterm infants and other immuno-compromised patients (Hufnagel *et al*.,2007). Furthermore, in neonates, *E.faecalis* is associated with 6% mortality rate in early onset septicaemia (EOS) which increases to 15% in late-onset (LOS) infections, whilst in general it is implicated in 7% to 50% of fatal cases (Al-Abbas., 2012).

2.16 Clinical Feature of Vaginal Infection

Abnormal vaginal discharge differs in color and consistency(thin, thick, frothy, yellow, green , gray or white) compared with physiological (normal) discharge, and frequently is associated with other symptoms, including itching, fishy, and foul smelling (Syed and Braverman .,2004). Woman with aerobic vaginitis is usually present with a thinned reddish vaginal muocosa, sometimes with extensive erosions or ulcerations and abundant yellowish discharge (without the fishy amine odour, typical of bacterial vaginosis). The pH is usually high. Symptoms can

include burning, stinging and dyspareunia. The symptoms can last for long periods of time sometimes even years. Typically ,patients have been treated several times with antibiotic drugs without relief. In asymptomatic cases , there is microscopic evidence but no symptoms. The prevalence of asymptomatic cases is unknown (Donders *et al*.,2011).

2.17 Laboratory Diagnosis of Vaginal Infection

An unmoistened sterile speculum was insereted before examination of vagina. Three HVSs were collected in sterile saline solution to avoid dryness of samples. The first swab was used Gram's-staining (Mackie and McCartney .,2006). Second swab was used for the preparation of wet mount and KOH mount (Donders *et al*.,2002).

In 2002, Donders *et al*. Published guidelines to characterize the presence and severity of aerobic vaginitis. This was based on a similar Nugent scoring method used for bacterial vaginosis determination, which is based on a Gram stained microscopic evaluation that enumerates specific bacterial morphotypes. The presence and number of the different bacterial morphotypes, such as healthy Gram positive lactobacilli and anaerobic BV associated Gram negative and Gram variable rods, contribute to the overall Nugent score. A Nugent score of 0 to 3 indicates normal flora, 4 to 6 intermediate flora, and 7 to 10 bacterial vaginosis (Donders *et al.*,2002).

The determination of AV is also established by an AV score. the score is calculated with the use of high power field microscopy to evaluate the presence or absence of health lactobacilli, number of leukocytes, number of toxic leukocytes ,type of vaginal flora and parabasal epithelial cells. Here, the presence of the healthy Gram positive Lactobacilli is compared to the presence of aerobic or facuitative anaerobic Gram positive cocci (such as *Streptococci*,*Staphylococci*, or *Enterococc*i) and Gram negative bacilli(*E.coli* and *Klebsiella* species) (Donders *et al*.,2011).

Lactobacillary grade must be evaluated:

Grade I

Numerous pleiomorphic lactobacilli; no other bacteria

Grade IIa

Mixed flora , but predominantly lactobacilli

Grade IIb

Mixed flora ,but proportion of lactobacilli severely decreased because of an increased Number of bacteria

Grade III

Lactobacilli severely depressed or absent because of overgrowth of other bacteria

AV Score	*Lactobacillary grades*	*Number of leukocytes*	*Proportion of toxic leukocytes*	*Proportion of parabasal epitheliocytes*
0	I and IIa	≤ 10/hpf	None	None or ≤1%
1	IIb	≥10/hpf, ≤10 /epi	≤ 50% of leukocytes	≤10%
2	III	≥10/ epi	≥50% of Leukocytes	≥10%

The AV score is calculated according to what is described in the table.

AV score less than3: no signs of AV

AV score 3or 4: light AV

AV score 5or 6: moderate AV

AV score ≥ 7: severe AV

Although widely accepted as efficient in diagnosis Aerobic vaginitis , the above method is time consuming, only employs a microscopic examination and is often adapted according to available resources (Sangeetha *et al* .,2015).

The third swab was inoculated onto MacConkey's agar, blood agar, and chocolate agar (Forbes *et al.,* 2007).Culture Most Enterobacteriaceae grows on routine laboratory culture media such as Blood Agar 5 %, Chocolate and MacConkey Agar. Most *E. coli* species are lactose fermenter. The change in pH due to lactose fermentation can be used to differentiate between lactose-fermenting and non-lactose-fermenting strains. The lactose-positive *E. coli* colonies will appear red or pink on media such as MacConkey agar (Maab *et al*., 2018).

The aerobically incubated bacterial growth was identified by standard biochemical reactions (Allen *et al*.,2005).

In 2016, Rumyantseva *et al* evaluated the use of quantitative PCR for the diagnosis of aerobic vaginitis .using mathematical formulas, including the concentration of lactobacilli, aerobic and anaerobic microorganisms as variables, they able to accurately detect the cases of AV.

However, the authors recognized the limitation of the quantitative PCR for diagnosis AV, because inflammation and immaturity of the epithelial cells were not accounted for. A shortcoming of the study was its lack of sensitivity and specificity analysis (Rumyantsea *et al*.,2016).

Finally, for screening purposes, pH testing could be proposed as a proxy test for AV due to its high sensitivity of 90% (Donders *et al*.,2016).

2.18 Treatment of bacterial vaginitis

Treatment is not always easy and aims at correction the three key changes encountered in aerobic vaginitis: the presence of atrophy, inflammation and abnormal flora (Wang *et al*.,2016) Treatment of aerobic vaginitis may include antibacterial , non steroidal anti inflammatory and /or probiotic therapy and can be local or systemic treatment. AV dose not respond well to metronidazole , clindamycin is considered to be a better choice for treating pregnant women with an unconfirmed diagnosis of abnormal vaginal microbiota because it is able to eradicate both AV and BA associated bacteria (Sobel *et al*.,2011).

Topical application of kanamycin has also been reported (Tempera *et al*.,2004).Conventional treatment for AV consists of a course of broad spectrum antibiotics that target anaerobic and aerobic bacteria such as the aminoglycoside, kanamycin . And the quinolone, moxifloxacin both of which are proven to be effective (Zarbo *et al* .,2013).

2.19 Previous Studies

In Vietnamese over 15% of the women had AV in third trimester, of positive samples, *S.agalactiae* (6%), *Enterococcus faecalis* (4%) and *S.aureus* (4%) (Nguyen *et al*.,2022).In other study in india the prevalence rate of AV was (21.2%) the common bacteria were *S.aureus* (24.2%), *GBS* (21.2%),*E.coli* (15.2%) (Lakshmi *et al*.,2019). In recent study in china the common organisms were *E.coli* (28.6%) followed by *GBS* (24.2%) , Klebsiella spp (21.2) (Li *et al*.,2021). In other study in india the most common isolated organism is the *E.coli* amounting (34%) followed by *Enterococci* scoring (10%), then *Staphylococci* (8%) and Group B *Streptococcus* (5%) reported by (Sujata *et al* 2016).

E. coli was in one of the fourth miscarriage case accounting for 8 cases in total, in general (Cools.,2017) reported that the world overall pooled prevalence of cervicovaginal *E. coli* carriage rates in pregnant women was (35.6%) in Africa. In Sudan the prevalence rate was (2.3%) reported by (Abdelaziz *et al* .,2014), in Burkina the prevalence reported was (16.7%). In Kenya the prevalence was (40.1%), in Rwanda it was (20.0%), while in South Africa at 2012 the rate reported was (42.3%) (Cools., 2017).

According to the World Health Organization (WHO), the onset of *Listeria* during pregnancy accounted for nearly (43%) of total cases, and (14%) occurred in late pregnancies. (Wadhwa and Smith., 2017). Recently, published data for incidence index in pregnancy-related listeriosis accounts for (11%) of all listeriosis cases in Italy, (Mammina *et al*.,2013) (16%), and (17.7%) in Spain France respectively (Goulet *et al*.,2012).

In Africa *L.monocytogenes,* is generally reported to have a low prevalence rate but a high fatality rate. (Dufailu *et al*.,2021).

In East Africa, the prevalence rate of *L. monocytogenes* among pregnant women in northern Ethiopia was reported to be (8.5%) (Welekidan *et al*.,2019).

For *Streptococcus agalactiae* the prevalence in South Africa was higher than other studies by (Chukwu *et al*., 2015 ; Cools *et al*., (2016) (37%), sampling of different populations could contribute to differences in prevalence rates. (Mukesi.,2019)

A study performed at Thammasat Hospital, Thailand showed that the *GBS* colonization rate was (16%) amongst 406 pregnant women receiving antenatal care (Tor-Udom *et al*.,2006). In Egypt found that the *GBS* colonization rate was (25.3%) among 150 vaginal swabs collected from pregnant women (Shabayek *et al.*,2009).

In Sudan the prevalence *Streptococcus agalactiae* was (8%) reported by (Elhassen *et al*.,2018) , (4%) reported by (Ibrahim *et al*.,2018) , and(3.7%) reported by (Abdelaziz *et al* .,2014).

The prevalence of *S.aureus* in sudan was (6%) (Abdelaziz *et al* .,2014), Nigeria (9%) (Mohammed *et al.,* 2016), India (5.4%) (Ravishankar and Prakash .,2017)and also reported (18.6%) in Indonesia(Febriani *et al*., 2017).

In South Africa the prevalence of *Klebsiella* was (7.7%) (Cutland *et al*., 2012), in India was (7%) (Ravishankar and Prakash .,2017), and (3.3%) was reported in Indonesia (Febriani *et al*., 2017).

CHAPTER III
MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. Study Design:

The design of the study was a cross-sectional study.

3.2. Study Area:

The study was conducted at Khartoum state Hospitals at Omdurman Maternity Hospital and ALSaudi Hospital.

3.3 Study Duration:

The study was conducted during the period from March 2022 to September 2022.

3.4. Study Population:

Pregnant women in third trimester were enrolled in this study.

3.4.1. Inclusion Criteria:

Pregnant women in third trimester who volunteered, with symptoms of (Vaginal discharge, Vaginal irritation, fatigue).

3.4. 2 Exclusion Criteria:

Pregnant women in first and second trimester, pregnant women who have taken antibiotics within seven days at the time of data collection.

3.5 Ethical Consideration

The study was approved from Scientific and Ethical Committee of Sudan University of science and Technology and from Federal Ministry of Health. Verbal consent was obtained from all pregnant women in this study prior to sample collection after full explanation of research purpose.

3.6 Sampling Size:

One hundred samples were collected in this study n=100.

3.7 Methodology:

3.7. 1 Data Collection:

Demographic data and Presence of signs and symptoms were collected using structured data questionnaire. (Appendix I)

3.7.2 Specimen Collection:

High Vaginal Swab (HVS) was collected from pregnant women in this study.

3.8 Laboratory Investigation:

3.8.1 Isolation of Bacteria:

High vaginal swab samples was cultured on Blood agar and CLED agar and incubated overnight at 37^0C and pure isolated colonies of pathogenic bacteria was obtained.

3.8.2 Gram Stain:

Slide smear was prepared from growth colony and stained with Gram stain to differentiated gram positive from gram negative bacteria.

3.8.3Biochemical test for Gram Negative:

3.8.3.1 Motility Test

Procedure:

The tested organism was inoculated in semi solid media Stab once to depth of only 1/3 to1/2 inch in the middle of the tube. Be sure to keep the needle in the same line it entered as it is removed from the medium ,Incubate at 35 -37^{0}C and Observe for a diffuse zone of growth flaring out from the line of inoculation(Tille and Forbes., 2014).

Result:

Diffuse growth: Motile bacteria

Growth as stab line: Non motile bacteria (Bachoon *et al*.,2008).

3.8.3.2 KIA (Kligler Iron Agar):

Procedure:

The tested organism was inoculated in KIA medium, using a straight wire loop, agar butt was stabbed, the opening was closed, then the top slope was streaked (as a zigzag). The medium was incubated at 37°Cfor 24 hrs, and glucose fermentation, lactose fermentation, H2Sproduction, and gas production were looked for.

Result:

Fermentation:

Positive Test for Slant Reaction_ Yellow (acid)

Negative Test for Slant Reaction_ Red (alkaline)

Positive Test for Butt Reaction_ Yellow (acid)

Negative Test for Butt Reaction_ Red (alkaline)

Hydrogen sulfide production:

Positive test _Black color.

Negative test _No black color.

Gas production:

Positive test _ Bubbles, cracking and displacement of the medium or separation of the medium.

Negative test_ No bubbles, no separation , no displacement of the medium(Downes and Ito .,2001).

3.8.3.3 Indole Test:

Procedure:

A sterile loop was used to inoculate the tested organism into 2 ml peptone water, the tube was incubated at 37°Cfor 24 hrs. in the next day 0.5 ml of Kovac's reagent (4 (p) – dimethylaminobenzaldehyde) was added, it was shaken gently and examined for the red color in alcohol layer within 10 mints (MacWilliams .,2009).

Result:

Positive: Top red reagent layer

Negative: Absence of Layer (MacFaddin., 2000).

3.8.3.4 Urease Test

Procedure:

The test organism was inoculated into the slope surface of Christensen's urea medium with phenol red as an indicator using sterile straight wire, the medium was incubated at 37 °C for 24 hrs, change in color of the indicator to purple-pink means a positive result.

Result:

Positive: deep pink colour

Negative: Failure to develop deep pink colour. (Bailey and Scott)

3.8.3.5 Citrate Utilization Test

Procedure:

The tested organism was inoculated into 2 ml of Simmon citrate medium with bromothymol blue as an indicator using sterile straight wire. Then the medium was incubated at 37°C for 24 hrs, change in color of the indicator from light green to blue color or streaking of growth mean a positive result.

Result:

Positive: Change of medium to green colour

Negative: RemainBlue colour of medium. (Bailey and Scott).

3.8.4 Biochemical for Gram Positive:

3.8.4.1 Catalase Test

Procedure (tube method):

About 4-5 drops of 3% of hydrogen peroxide are added to a test tube,Using a wooden applicator stick, a small amount of tested organism from a well isolated 18-24 hours colony was collected and placed into the test tube, thenThe tube is places against a dark background and observed for immediate bubbles (Karen .,2016).

Result:

Positive: Bubble formation.

Negative: Absence of Bubble formation. (Karen., 2016).

1.8.4.2 Coagulase Test

Procedure:

Slide Test:

About 10 micro liter of deionized water or physiological saline was added to a slide,Several colonies form a tested organism was collected with an inoculating loop and are emulsified into the water to obtain a smooth milk colored suspension then A drop of a rabbit or human plasma is added to the slide, and the clumping is observed immediately.

Result:

Positive: Coagulation of plasma

Negative: No agglutination (Berke and Tilton .,1986).

3.8.4.3 Bacitracin SusceptibilityTest:

Procedure:

Using an inoculating loop, streak two or three suspected colonies of tested organism onto a blood agar plate,Using heated forceps, bacitracin disk was placed in the first quadrant(area of heaviest growth). Gently tap the disk to ensure adequate content with the agar surface,Incubated the plate for 18 to 24 hours at 35-37^{0}C in ambient air for staphylococci and in 5% to 10% carbon dioxide (CO2) for streptococci differentiation. Look for a zone of inhibition around the disk (Tille and Forbes., 2014).

Result:

Positive: Any inhibition zone greater than 10 mm susceptible

Negative: No zone of inhibition resistance.(Harley, 2005)

3.8.4.4 Bile Esculin Test:

Procedure:

The test organism was inoculated into the slope surface of bile esculin medium with using sterile straight wire, the medium was incubated at 37 °C for 24 hrs, change in color of the indicator to black means a positive result (Tille and Forbes .,2014).

Result:

Positive: Growth and blackening of agar slant.

Negative: No growth or growth and no blackening .(Forbes et al., 2007)

1.8.4.5 Deoxyribonuclease Test:

Procedure:

A sterile loop was used to inoculate the tested organism into DNase agar, the plate was incubated at 37°Cfor 24 hrs. in the next day 1 N HCL was added, Allow the reagent to absorb into the plate and Observe for clear zone around the colonies within 5 minutes.

Result:

Positive: clear zone around inoculation line.

Negative: No clear zone around inoculation line (Tille and Forbes 2014).

3.9. Statistical Analysis

Data and result obtained was analyzed using statistical package for social sciences (SPSS) version 22, Quantitative variables was expressed as mean ± standard deviation (SD) ,and qualitative variables was expressed as numbers and percentages. Students t test was used for quantitative and chi square test was used for qualitative variables. P value ≤ 0.05 was considered as significant for all study results.

CHAPTER IV
RESULT AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1Results

The current study evaluated the percentages of HVS culture regarding positive growth and negative growth. A total of hundred high vaginal swabs were collected from pregnant the mean of their age was 25.8 years , The different age groups showed different percentage of positive and negative growth on HVS culture The highest growth was obtained in age group (15-25) which was27 (56%) , In age groups (26-35) and (36-43) showed positive growth percentages of 20 (55%) and 2 (12%) respectively Table 4.1. Most of pregnant women were university graduate 45 (45%), 27 (27%) were secondary School, 19 (19%) were Primary School and only 9(9%) were illiterates Figure 4.1. In this study 53(53%) of pregnant women lived in urban and 47 (47%) lived in rural area the growth observed mainly on rural area pregnant women in percentage 26(55%) Table 4.1 and Figure 4.2. A total of hundred high vaginal swabs were collected and cultured which 49% showed positive growth constituted of various type of pathogens while 51% showed no growth the percentages of positive HVS culture were include different type of pathogens 16(16%) was *E. Coli* highest growth,15 (15%) was *S aureus* second highest growth, 7 (7%) was *E.faecalis*, 3(3%) was *Klebsiella* ,6 (6%) was *Candida* and 2 (2%) was *S.agalactiae* Figure 4.5.

According to gravidity 38 (38%) had multi –pregnancy, 24 (24%) had second- pregnancy and 38(38%) had prime-pregnancy, The result showed that the growth mainly observed in multi-gravida in percentage 22(57%) and *S.agalactiae* observed in multi-gravida only. In secundi-gravida the growth observed in 10(41%) and 14(59%) had no growth. In primi-gravida 17(44%) showed growth and 21(56%) showed no growth. Figure 4.2 and Table 4.1.

The result of the research showed that there was a significant association between growth of bacteria and following symptoms (fatigue, vaginal discharge, vaginal irritation), The most frequent symptoms among pregnant women were vaginal discharge 61 (61%) the growth observed on 48 (78%) from pregnant women with vaginal discharge and 13(22%) had no growth. also vaginal irritation 53(53%) showed significant association with bacterial growth 44(83%) and 9(17%) showed no growth and 46 (46%) had fatigue showed positive growth percentages 28(60%) as showed in Table 4.2.

In this study we observed the association between uses of intrauterine device and vaginal infection (mainly *S.agalactiae*), 83 (83%) of pregnant women not use any type of contraceptive ,Only 17(17%) of pregnant women used contraceptive,11(64%) of them showed positive growth, 7 of them used intrauterine device the growth observed in 5 of them 2

*S.agalactiae,*one *E.coli,* one *E.faecalis* and one *candida.* The other 10 pregnant women used different types of contraceptives 4 of them used familia, 3 used implant and 3 used combined pillis. Figure 4.3.

In this study also we observed the significant association between the history of abortion and bleeding with vaginal infection,Thirty-two (32%) of pregnant women in this study had a history of abortion the growth observed in 28(87%) and 4(13%) had no growth.and 13(13%) had chronic disease 9(69%) from pregnant women had diabetes and 4(31%) of them had hypertension, 7(53%) showed growth and 6(47%) had no growth Table1.1.

The study showed the association between urinary tract infection and vaginal infection 39 (39%) had UTI 27 (69%) from pregnant women with UTI had positive growth, and 12 (31%) had no growth. Total of 50 (50%) with cervical infection 37 (74%) from pregnant women with cervical infection showed growth and 13 (26%) had no growth Table 4.2 and Table 4.3.

Table 4.1 Frequency of Variables and Growth

Variables		Frequency of Variables%	Frequency of Growth %
Age Group	(15-25)	48%	27 (56 %)
	(26-35)	36%	20 (55%)
	(36-43)	16%	2 (12%)
Residence	Urban	53%	23(45%)
	Rural	47%	26(55%)
Gravidity	prime	38%	17(44%)
	Second	24%	10(41%)
	Multi	38%	22(57%)
Contraceptive use		17%	11(64%)
History of abortion		32%	28(87%)
Chronic Disease		13%	9 (69%)
Fatigue		46%	28(60%)
Vaginal Discharge		61%	48(78%)
Vaginal Irritation		53%	44(83%)
Cervical Infection		50%	37(74%)
UTI		39%	27(69%)

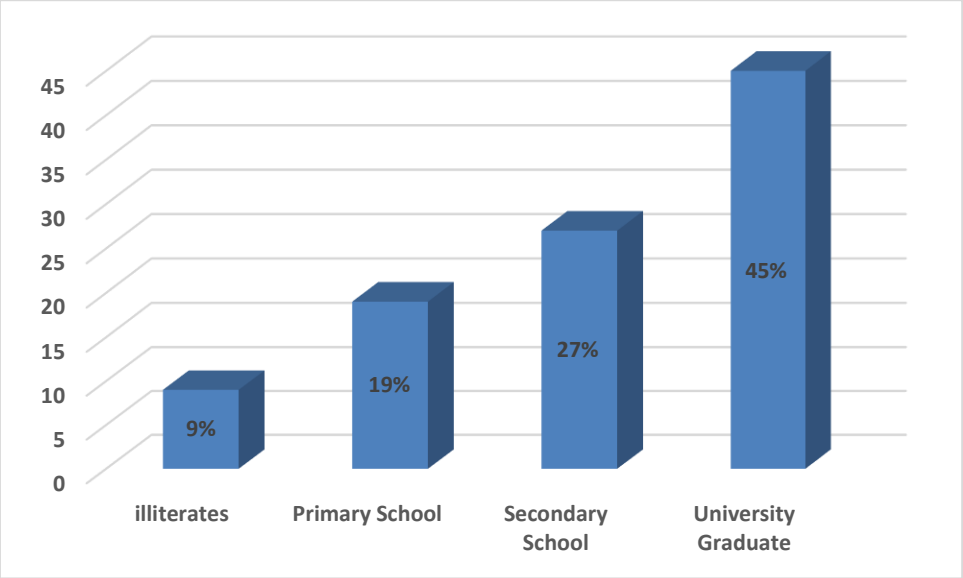

Figure 4.1 Distribution of Pregnant Women according to Education level

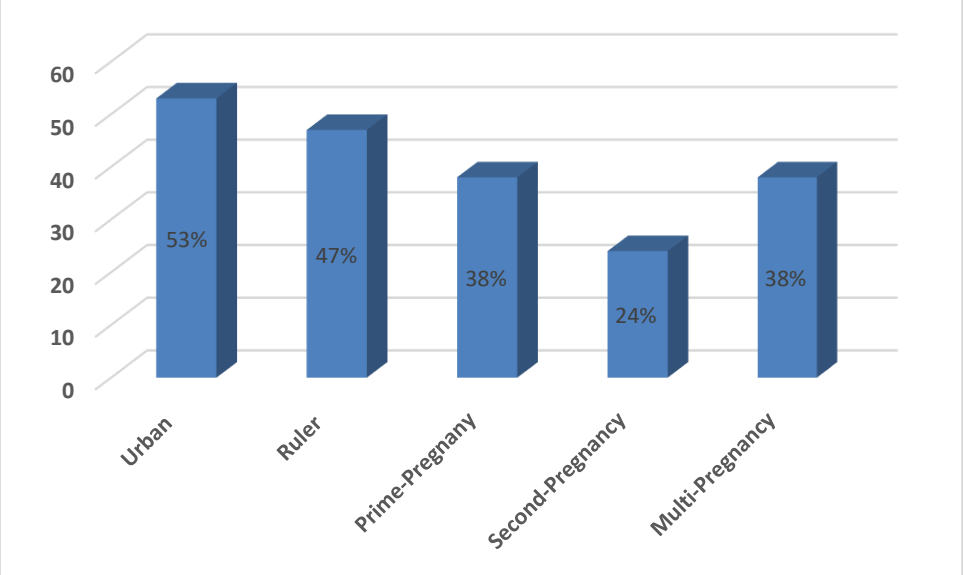

Figure4.2 Distribution according to Residence and Number of Pregnancy

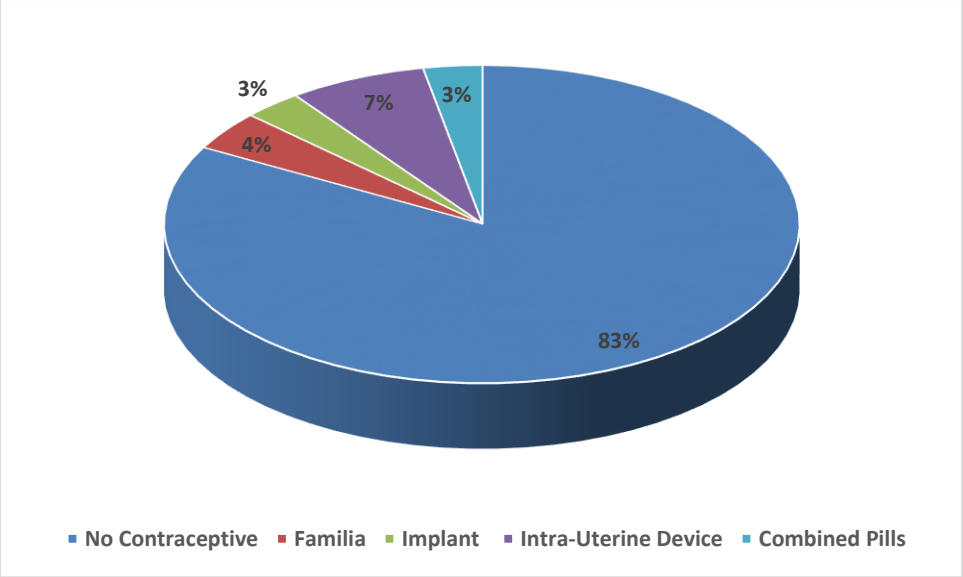

Figure4.3 Distribution of pregnant women according to Type of Contraceptive

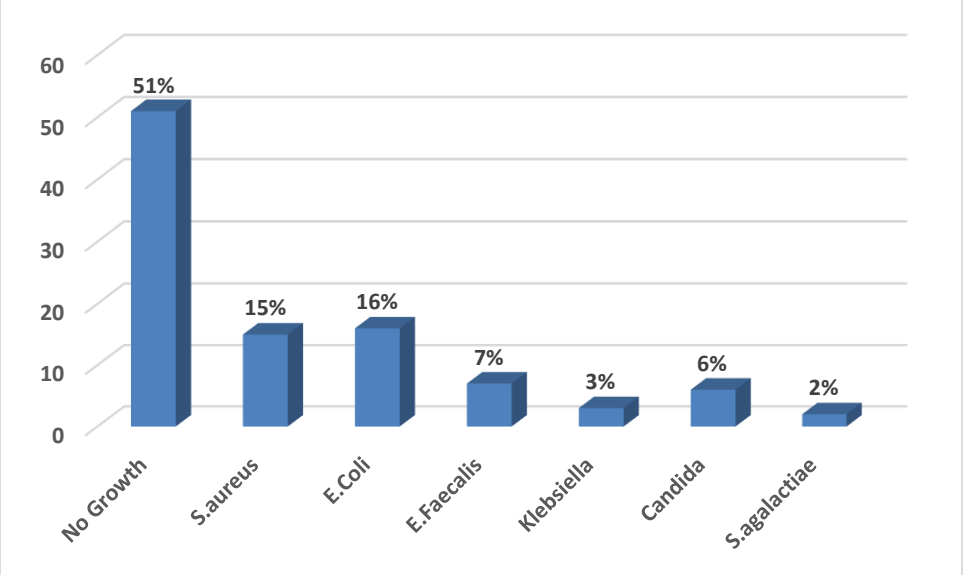

Figure 4.4 Percentages of Isolated Pathogens from vaginal swab

Table 4.2 Association between Symptoms and Bacteria Growth Result

	Fatigue		**Vaginal Discharge**		**Vaginal Irritation**		**Cervical Infection**	
	Yes	No	Yes	No	Yes	No	Yes	No
Growth	28	21	48	1	44	5	37	12
No Growth	18	33	13	38	9	42	13	38
Total	46	54	61	39	53	47	50	50
P Value	0.1		0.02		0.00		0.00	

Table 4.3 Association between disease UTI, history of abortion , using of contraceptive and Bacteria Growth Result

	UTI		**History of Abortion**		**Using of Contraceptive**	
	Yes	No	Yes	No	Yes	No
Growth	27	22	28	21	11	38
No Growth	12	39	4	47	6	45
Total	39	61	32	68	17	83
P Value	0.001		0.00		0.15	

4.2 Discussion

Vaginal infections with bacterial vaginosis, candidiasis , trichomoniasis and bacterial vaginitis are global health problem for women. Pregnant women who are carriers vaginal infection have 50_60% potential capacity for vertical transmission of the microorganism, to their newborns, develop invasive infection (sharmial *et al* 2011).

Recenty, greater attention has been paid to bacterial vaginitis, to date there was contradictory data about bacterial vaginosis and other vaginal infection, this study aimed to detect Aerobic bacterial species in pregnant women in Khartoum state.

This study enrolled 100 pregnant women in Khartoum state, the mean of their age was 25.8 years, most of pregnant women in this study were university graduate 45 (45%) and (38%) had multi pregnancy. The most frequents symptoms among pregnant women was vaginal discharge 61 (61%) and vaginal irritation 53 , this result was similar to Prasad *et al* (2021) shawed that vaginal discharge and dysuria was the most common symptoms followed itching and UTI, in contrast The result of other study showed that also the same association with other symptoms they present with pruritus, lower abdominal pain and pain during coitus and this about 10% range up to 66% as mentioned by Rao *et al* (2004).

The frequent risk factor for bacterial vaginitis among pregnant women in this study was the contraceptives use 17 (17%) and abortion 32(32%) , this was similar to Epidemiological studies have shown that older maternal age, multiple sexual partners, previous spontaneous miscarriages, and alteration of vaginal bacterial communities are among the risk factors for AV and BV as mentioned by Kaambo *et al* (2018). However Bukusi *et al* (2006) study showed that high frequency of vaginal intercourse, history of pregnancy, cigarette smoking and Maternal infection are the main risk factors of aerobic vaginitis. The variation observed can be due to epidemiological variations and environmental nature.

In current finding, *E.coli* was the most common pathogen related to vaginitis in HVS culture, showed 16 (16%) of prevalence, Also previously study reported that the world overall pooled prevalence of cervicovaginal *E. coli* carriage rates in pregnant women was 35.6% in Africa, in Burkina the prevalence reported was 16.7%. In Kenya the prevalence was 40.1%, in Rwanda it was 20.0%, while in South Africa at 2012 the rate reported was 42.3% as mentioned by Cools (2017). The lowest prevalence showed in india by Lakshmi *et al* (2019) (15.2) and in Sudan the prevalence rate was 2.3% reported by Abdelaziz *et al* (2014).

S.agalactiae was the leading cause of neonatal disease in developed countries until it was control by Intrapartum antimiotic prophylaxis. However, invasive *S.agalactiae* is not frequent reported from developing countries including Ethiopia. This study revealed low frequency of *S.agalactiae* 2(2%) this result was nearby other results in Sudan the prevalence *Streptococcus agalactiae* was (8%) reported by Elhassen *et al*(2018), (4%) reported by Ibrahim *et al*(2018) and(3.7%) reported by Abdelaziz *et al* (2014). compared to other study done in South Africa was higher than other studies by Chukwu *et al* (2015) ; Cools *et al* (2016) (37%).

Another potential pathogen detected in this study was *S.aureus* with the prevelance of (15%,)The finding was similar to the prevalence of *S.aureus* in Indonesia (18.6%) Febriani *et al* (2017).In contrast, countries such as Sudan (6%) Abdelaziz *et al* (2014), Nigeria (9%) (Mohammed *et al.,* 2016), India (5.4%) Ravishankar and Prakash (2017) reported a lower prevalence of *S.aureus*.

The prevalence of *Klebsiella* in this study was (3%) is consistent with a study conducted in Indonesia (3.3%) Febriani *et al* (2017).On the other hand a higher prevalence of *Klebsiella* was reported In South Africa (7.7%) Cutland *et al* (2012), in India(7%)Ravishankar and Prakash (2017). In this study the percentage of *E.faecalis* was (7%) this is higher than study reported by Nguyen *et al*(2022) (4%) and lower than Sujata *et al* (2016) with percentage (10%).

Fortunately, there was no growth of *Listeria monocytogenes* in this study compared to other studies Recently, published data for incidence index in pregnancy-related listeriosis accounts for 11% of all listeriosis cases in Italy, Mammina *et al* (2013) 16%, and 17.7% in Spain France respectively Goulet *et al* (2012).

In Africa *L.monocytogenes,* is generally reported to have a low prevalence rate but a high fatality rate Dufailu *et al* (2021).In East Africa, the prevalence rate of *L. monocytogenes* among pregnant women in northern Ethiopia was reported to be 8.5%. Welekidan *et al* (2019). The variation observed can be due to the laboratory methods used for the diagnosis and screening is not performed in the majority of health care centers.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

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5.1Conclusion

Bacterial culture and its sensitivity in vaginal discharge should be done not only because of its troublesome symptoms but for its complications like subfertility, preterm delivery, ectopic pregnancy and its increased susceptibility for HIV and oncogenic virus. *E.coli* and *Staphylococcus* were the predominant bacteria found in present study.

There was association between vaginal discharge and bacterial growth, *E.coli* and *Staphylococcus* were the predominant bacteria found in present study,There was no growth of *Listeria monocytogenes*.

5.2Recommendations

From the finding of this study, we recommended that:

- Health education programs through different media to educate pregnant women about the difference between normal and abnormal vaginal discharge and when to consult their doctor.
- There should also be regular screening for bacterial vaginitis during the routine clinic visits by the health worker to enable early detection and treatment.
- Further studies on the pattern of complications of bacterial vaginitis in pregnant women with abnormal vaginal discharge are needed to determine future strategies for prevention and treatment of bacterial vaginitis in pregnant women.

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APPENDIXS

Appendix I

SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF GRADUATED STUDIES

Questionnaire

Detection of Aerobic bacterial pathogens causing bacterial vaginitis among pregnant women in third trimester, in Khartoum state,Sudan

Demographic data

ID: ……..... Tel number:.....

Age: .....Age Group:.....

Education illiterate ☐ Primary ☐

Secondary ☐ Graduate ☐

Residence: Rural ( ) Urban ( )

Gravidity Prima ( ) Second ( ) Multi ( )

Any complications as a newborn or prenatal care Yes ( ) No ( )

Specify……

Have you had any cervical infection before Yes ( ) No ( )

Frequent headaches or fatigue Yes ( ) No ( )

History of UTI Yes ( ) No ( ) History of abortion Yes ( ) No ( )

contraceptive use Yes ( ) No ( )

Specify…….

Broad spectrum antibiotics use Yes ( ) No ( )

Lower abdominal pain Yes ( ) No ( )

Abnormal vaginal discharge Yes ( ) No ( )

Vaginal itching Yes ( ) No ( )

Duration of Disease……

Any chronic Disease .....

AppendixII

1. **Equipments and instruments:**

- Light microscope.
- Incubator 37^0C.
- Hot air oven.
- Sensitive balance.
- Refrigerator.
- Bunsen burner.
- Bacteriological loops.
- Straight wire.
- Wooden stick.
- Cotton.
- Autoclave.
- Filter paper.
- Physiological saline.
- Distilled water.

2. **Glass were**:

- Petri dishes.
- Flasks 100,500, 250 ml.
- Measuring cylinder 500 and 1000 ml.
- Test tubes.

3. **Media and reagent:**

- Blood agar.
- CLED agar.
- Kligler Iron Agar.
- Christensen's urea medium.
- Simmon citrate medium.
- Pepton water.
- Kovac's reagent.
- Semi solid media.
- DNA media.
- Nutrient agar.
- Catalase reagent.
- Gram stain set.

Appendix III

Preparation of media:

CLED Agar:

pH (at 25^0C) =7.3+/-0.2

Formula:

Ingredients Gms/litter lactose 10.0, pancreatic digest of gelatin 4.0, pancreatic digest of casein 4.0, beef extract 3.0, L-cystine 0.128, bromothymol blue 0.02, agar 15.0.

Directions:

Suspend 36 grams in 1000ml distilled water. Heat to boiling to dissolve the medium completely sterilize by autoclaving at 15 Ibs pressure (121^0C)for 15 minutes. Cool to 45^0C-50^0C, mix well pour into sterile petri plates.

Blood Agar:

pH (at 25^0C) =7.4+/-0.2.

Formula:

0.5% peptone, 0.3 %beef extract /yeast extract, 1.5% agar, 0.5% NaCl, Distilled water.

Directions:

Suspend 28 grams of nutrient agar in 1000ml distilled water. Heat to boiling to dissolve the medium completely sterilize by autoclaving at 15 Ibs pressure (121^0C) for 15 minutes. When the agar has cool to 45^0C-50^0C add 5% sterile defibrinated blood that has been warmed to room temperature, mix well pour into sterile petri plates

Kligler Iron Agar:

pH (at 25^0C) =7.4+/-0.2

Formula:

ingredients Gms/litter peptic digest of animal tissue 15.0 Beef extract 3.00 Yeast extract 3.00 proteose peptone 5.00 Lactose 10.00 Dextrose 1.00

Directions:

Suspend 38.0 grams in 1000ml distilled water. Heat to boiling to dissolve the medium completely sterilize by autoclaving at 15 Ibs pressure (121^0C) for 15 minutes. Cool to 45^0C-50^0C, mix well pour into sterile tubes.

Urea agar base:

pH (at 25^0C) =7.3+/-0.1

Formula:

Ingredient Gms /litter peptic digest of animal tissue 1.50 Dextrose 1.00 S odium chloride 5.00 Monopotassium phosphate 2.00 Phenol red 0.012 Agar 15.00.

Directions:

Suspend 24.51 grams in 1000ml distilled water. Heat to boiling to dissolve the medium completely sterilize by autoclaving at 15 Ibs pressure (121^0C) for 15 minutes. Cool to 50^0C and aseptically add 50 ml sterile 40% urea solution, mix well pour into sterile tubes.

Simmons citrate agar:

pH (at 25^0C) =6.8+/-0.2

Formula:

Ingredients Gms/ litter Magnesium sulphate 0.02 Ammonium dihydrogen phosphate 1.00 Dipotassium phosphate 1.00 Sodium citrate 2.00 Sodium chloride 5.00 Bromothymol blue 0.08 Agar 15.00.

Directions:

Suspend 24.28 grams in 1000ml distilled water. Heat to boiling to dissolve the medium completely sterilize by autoclaving at 15 Ibs pressure (121^0C) for 15 minutes. Cool to 45^0C-50^0C, mix well pour into sterile tubes.

Motility Test:

pH (at 25^0C) =7.2+/-0.2.

Formula:

Ingredients Gms/ Litter Tyrptose 10.00 Sodium chloride 5.00 Agar 5.00.

Directions:

Suspend 20 grams in 1000ml distilled water. Heat to boiling to dissolve the medium completely sterilize by autoclaving at 15 Ibs pressure (121^0C) for 15 minutes. Cool to 45^0C-50^0C, mix well pour into sterile tubes.

Bile Esculin Agar:

pH (at 25^0C) =6.6+/-0.2

Formula:

Ingredients Gms/ litter Peptic digest of animal tissue 5.00 Beef extract 3.00 Esculin 1.00 Bile salts 40.0 Ferric citrate 0.50 Agar15.00.

Directions:

Suspend 64.5 grams in 1000ml distilled water. Heat to boiling to dissolve the medium completely sterilize by autoclaving at 15 Ibs pressure (121^0C) for 15 minutes. Cool to 45^0C-50^0C, mix well pour into sterile tubes.

DNase Test:

pH (at 25^0C) =7.3+/-0.2.

Formula:

Ingredients Gms/ Litter Tryptone 15.00 Soya peptone 5.00 Deoxyribonuclic acid 2.00 Sodium chloride 5.000 Agar 15.00.

Directions:

Suspend 42 grams in 1000ml distilled water. Heat to boiling to dissolve the medium completely sterilize by autoclaving at 15 Ibs pressure (121^0C) for 15 minutes. Cool to 45^0C-50^0C, mix well pour into sterile petriplates.

Prepare Crystal Violet Stain:

Dissolve 2 g crystal violet in 20ml of 95% ethyl alcohol.

Dissolve 0.8 g ammonium oxalate monohydrate in 80 ml deionized water.

Mix the crystal violet and ammonium oxalate monohydrate solutions to make the crystal violet stain. Filter the stain if necessary.

Prepare the staining solution:

Add 20 mg safranin powder to 100 ml beake.

Pour 20ml distilled water in the beaker and make 0.1% safranin staining solution by constant stirring. Filter both the staining solutions to avoid particles.

Acetone alcohol:

To make one liter:

Acetone ……500 ml

Ethanol or methanol absolute…….475 ml

Distilled water……..25 ml