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Correlation of Vaginal Microbiome and Bacterial Vaginosis in Pregnant Nigerian Women Attending Ante-Natal Care in Selected Hospitals in Enugu State, Nigeria

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ABSTRACT

Bacterial vaginosis (BV) is one of the most frequently reported vaginal infections among pregnant women and has been associated with several adverse maternal and neonatal health outcomes. The condition is primarily linked to disturbances in the normal vaginal microbiota, particularly the depletion of protective Lactobacillus species that normally help maintain a healthy vaginal environment. This study was conducted to investigate the composition of the vaginal microbiome, assess the prevalence of bacterial vaginosis, and evaluate the antibiotic susceptibility patterns of bacterial isolates obtained from pregnant women attending antenatal clinics in selected hospitals in Enugu. A total of sixty (60) high vaginal swab samples were collected from pregnant women receiving antenatal care, with thirty (30) samples obtained from Palms Medical Consultants and thirty (30) from St. Michael's Hospital. Prior to sample collection, participants were provided with structured questionnaires designed to gather demographic information as well as data related to clinical symptoms, medical history, and potential risk factors associated with vaginal infections.

The samples were inoculated on blood agar, sabouraud dextrose agar, Eosin methylene blue agar and de Man Rogosa and Sharpe media and isolates were characterized by standard microbiological methods. Selected isolates were further characterized by molecular tests; Polymerase chain reaction, Plasmid and Plasmid curing. Antibiotics susceptibility tests were done by Kirby bauer diffusion method. A total of 60 (100%) HVS samples were positive with Lactobacillus species; isolates responsible for bacterial vaginosis were not isolated, but other pathogenic organisms found were Escherichia coli (30%), Candida albicans (25%), Enterobacter aerogenes (20%), Staphylococcus aureus (16.7%) and Streptococcus viridans (8.3%). Lactobacillus species, Streptococcus viridans and Enterobacter aerogenes showed high susceptibility values to test agents at 76%-95%, 75.5%-97%, 75%-97% respectively. E.coli and Staphylococcus aureus showed multiple resistance to test agents.

The PCR were positive at 15000bp for 16SrRNA gene, showing Limosilactobacillus fermenter strain and TMPC 3451 and Lactobacillus helveticus strain IMAU 30124. The isolates had high molecular weight of 15000bp. The detection of ~15kb plasmid in the isolates indicates the presence of accessory genes that offer a competitive edge against other microorganisms in the vagina. The loss of plasmid after curing with 10% SDS indicates that the strain carried extrachromsomal elements. The detection of pathogenic isolates draws attention to maternal and neonatal risks. There is a high level of resistance to frequently used antimicrobials between E. coli and Staphylococcus aureus and it calls for concern.

Keywords: *Bacterial vaginosis, Vaginal microbiome, Amsel's criteria, Polymerase chain detection, Plasmid profiling.*

Introduction

The vaginal microbiome refers to the community of microorganisms that inhabit the vaginal environment, predominantly composed of bacterial species. This microbial community plays an essential role in maintaining vaginal health by protecting against pathogenic infections and helping to regulate the vaginal pH. During pregnancy, the vaginal microbiome has been recognized as an important factor influencing maternal health outcomes and the development of certain reproductive tract diseases. The stability and composition of this microbial ecosystem are therefore critical for maintaining a healthy pregnancy.

Vaginal microbial communities are often classified into community state types (CSTs) based on the dominant bacterial species present. These CSTs are typically characterized by the predominance of specific *Lactobacillus* species, including Lactobacillus crispatus (CST I), Lactobacillus gasseri (CST II), Lactobacillus iners (CST III), and Lactobacillus jensenii (CST V). In contrast, CST IV is characterized by a diverse mixture of strict and facultative anaerobic bacteria. Favorable pregnancy outcomes are commonly associated with a vaginal microbiome dominated by *Lactobacillus* species and relatively low bacterial diversity. These beneficial bacteria contribute to reproductive health by producing lactic acid and other antimicrobial substances that inhibit the growth of pathogenic organisms.

Bacterial vaginosis (BV) was initially described as infection caused by *Haemophilus vaginalis*. However, it is now recognized as a polymicrobial condition characterized by an imbalance in the vaginal microbial community. In BV, the normal vaginal flora dominated by *Lactobacillus* species is gradually reduced or completely replaced by anaerobic microorganisms. These microorganisms commonly include *Gardnerella vaginalis*, *Prevotella* species, *Bacteroides* species, and *Mobiluncus* species, as well as other organisms such as *Ureaplasma* and *Mycoplasma* species. This shift in microbial composition disrupts the natural protective environment of the vagina and contributes to the development of bacterial vaginosis.

Bacterial vaginosis is recognized as one of the leading causes of abnormal vaginal discharge among women of reproductive age and is frequently encountered in sexually transmitted infection (STI) clinics. The condition has also been strongly associated with several adverse pregnancy outcomes, including preterm birth, low birth weight infants, premature rupture of fetal membranes, and late miscarriage. These complications highlight the importance of early detection and proper management of BV, particularly during pregnancy.

The diagnosis of bacterial vaginosis is commonly based on four clinical criteria, which include:

(1) a vaginal pH greater than 4.5,

(2) the presence of thin and homogeneous vaginal discharge,

(3) a positive whiff test characterized by a fishy odor when potassium hydroxide is added to vaginal fluid, and

(4) the detection of clue cells during microscopic examination of wet smears.

In addition to these clinical indicators, laboratory diagnosis of BV may also be performed using the Nugent Gram stain method [27]. The Nugent scoring system evaluates bacterial morphotypes observed in Gram-stained vaginal smears and is widely used to determine the state of the vaginal microbiota. According to this system, a score of 0–3 indicates normal vaginal flora dominated by *Lactobacillus* species, scores of 4–6 represent intermediate flora, while scores of 7–10 are indicative of bacterial vaginosis.

The relationship between the vaginal microbiome and bacterial vaginosis is primarily associated with the reduction of beneficial *Lactobacillus* species, which normally help maintain vaginal acidity and inhibit pathogenic microorganisms. When these protective bacteria decline, anaerobic organisms may proliferate, resulting in microbial imbalance and the development of BV. Therefore, restoring the dominance of *Lactobacillus* species through probiotic therapy, improved hygiene practices, and healthy lifestyle interventions may provide an effective strategy for the prevention and management of bacterial vaginosis [18].

Statement of the Problem

Bacterial vaginosis is a prevalent vaginal infection among women of reproductive age and is of particular concern during pregnancy due to its association to maternal and neonatal outcomes. In Nigeria, despite increasing awareness of maternal health challenges, there remains limited research on how bacterial vaginosis influences with the alteration in the vaginal microbiome throughout pregnancy. Several studies have shown that shifts in the vaginal microbiota may expose pregnant women to bacterial vaginosis, which has been linked to complications.

The statement of the problem for this study is to bridge the knowledge gap by examining the correlation between bacterial vaginosis and the vaginal microbiome in pregnant women.

Aim of the Study

The aim of the study was to determine the vaginal microbiome, analyze infection with bacterial vaginosis and determine the antibiotic susceptibility profile of isolates in pregnant women attending ante-natal care in selected hospitals in Enugu.

Objectives of the Study

1. To determine prevalence of microorganisms from vagina of pregnant women attending ante-natal care in selected hospitals in Enugu.
2. To characterize isolates by general microbiological methods and molecular methods like Polymerase Chain Reaction (PCR), Plasmid profiling and Plasmid curing.
3. To determine bacterial vaginosis by Amsel's criteria and Nugent score in pregnant women
4. To determine the antibiotic susceptibility patterns of isolates

Materials and Methods

Media and Reagents

Laboratory media used during this study are from Rajasthan, India.

Study Population

The study was carried out in pregnant women attending ante-natal care in selected hospitals in Enugu. The hospitals comprised of Palms Medical Consultants and St. Micheal's Hospital. A total of 30 out-patients were tested from Palms Medical Consultants and St. Micheal's hospital.

Ethical Approval

Ethical approval for the study was obtained from Administrative officers of Palms Medical Consultants, and St. Micheal's Hospital, Enugu.

Pre-experimental Activity

Pre-experimental activity involved sharing of structured questionnaires comprising of signs and symptoms, lifestyle and hygiene practices, sexual history, medical and obstetric history.

Sample Collection

Vaginal swabs were collected from sixty (60) pregnant women comprising of thirty (30) samples from each hospital. Two swab sticks were used by each patient and sample collection were aided by the nurses. The samples were collected from 15th January to 28th February 2025. After each phase, samples were transported immediately to Microbiology Lab, Enugu State University of Science and Technology.

Amsel's Criteria Test

Whiff (Amine) Test

A small amount of vaginal discharge was smeared onto a clean glass slide, after which two drops of 10% potassium hydroxide (KOH) solution were added. The slide was gently mixed and immediately assessed for odor. The release of a characteristic fishy or amine-like smell following the addition of KOH was recorded as a positive whiff test, which is suggestive of bacterial vaginosis.

pH Test

The pH test was performed to determine the acidity of the vaginal specimen. Under normal physiological conditions, the vaginal environment is slightly acidic, with a pH typically ranging from 3.8 to 4.5, which helps inhibit the growth of pathogenic bacteria. In this study, a sample of vaginal discharge was applied to pH indicator (litmus) paper to determine its acidity. A pH value greater than 4.5 was considered abnormal and indicative of possible bacterial vaginosis.

Clue Cell Test

Clue cells are vaginal epithelial cells whose surfaces are densely coated with bacteria and are considered a key microscopic indicator of bacterial vaginosis. For this examination, a smear of vaginal discharge was prepared on a clean microscopic slide, and a drop of normal saline (sodium chloride solution) was added. The preparation was then examined under a light microscope to detect the presence of clue cells. The observation of clue cells, together with other clinical findings such as abnormal discharge, supports the diagnosis of bacterial vaginosis. Generally, the presence of two positive diagnostic criteria in addition to abnormal discharge is considered sufficient for diagnosis; however, if abnormal discharge is absent, all three diagnostic criteria must be present.

Isolation of Bacterial Organisms

For the isolation of bacterial organisms, each vaginal swab was immersed in 1 mL of sterile normal saline and gently agitated to release the sample into the solution. From this suspension, 0.1 mL was aseptically transferred using a sterile syringe and inoculated onto de Man Rogosa Sharpe (MRS) agar, blood agar, and Eosin Methylene Blue (EMB) agar plates using the pour plate technique. The EMB agar plates were incubated aerobically at 37°C for 24 hours, while blood agar plates were incubated at 37°C for 48 hours. MRS agar plates were incubated at 37°C for 48 hours in a 5% CO_2 environment using a candle jar to facilitate the growth of *Lactobacillus* species. After incubation, distinct bacterial colonies were sub-cultured onto nutrient agar plates and incubated at 37°C for 24 hours to obtain pure cultures. Colonies obtained from MRS agar were further sub-cultured onto fresh MRS plates and incubated at 37°C for 48 hours for purification.

Isolation of Fungal Organisms

For fungal isolation, 0.1 mL of the sample suspension was inoculated onto Sabouraud Dextrose Agar (SDA) plates and incubated at 35°C for 5 days. Emerging fungal colonies were purified by sub-culturing onto fresh SDA plates and further incubated at 35°C for 3 days. The purified fungal isolates were subsequently preserved on SDA agar slants at 4°C for further identification and analysis.

Characterization and Identification of Bacterial Isolates

Gram Staining

Gram staining of bacterial isolates was performed according to the method described by [68]. A drop of physiological saline was placed on a clean microscope slide, and a loopful of bacterial culture was emulsified in the saline to form a thin smear. The smear was allowed to air dry and then heat-fixed by passing the slide briefly through a Bunsen burner flame. The slide was stained with crystal violet for 1 minute, rinsed gently with water, and then decolorized with acetone-alcohol. After washing, the smear was counterstained with safranin for 1 minute, followed by a final rinse with water. The slide was air-dried and examined under a microscope using the appropriate objective lens. Gram-positive bacteria appeared purple or blue, while Gram-negative bacteria appeared pink or red.

Biochemical Tests

Several biochemical tests were conducted to identify the bacterial isolates using standard procedures as described by [31].

Catalase Test

The catalase test was performed to determine the ability of bacterial isolates to produce the enzyme catalase. A small portion of a bacterial colony was placed on a clean, dry glass slide using a sterile inoculating loop. A drop of 3% hydrogen peroxide (H_2O_2) was then added to the smear and gently mixed. The immediate production of oxygen bubbles within 15 seconds indicated a positive catalase reaction, whereas the absence of bubbling indicated a negative result.

Coagulase Test

For the coagulase test, two separate sterile glass slides were prepared and labeled as test and control. A drop of physiological saline was placed on each slide. Using a sterile inoculating loop, a portion of the bacterial colony was emulsified in each drop of saline to form a suspension. A drop of human plasma was added to the suspension on the test slide and mixed gently, while no plasma was added to the control slide. The formation of visible clumping or agglutination within 10 minutes was considered a positive coagulase test, indicating the presence of the coagulase enzyme.

Citrate Utilization Test

The citrate utilization test was carried out using Simmons citrate agar slants. A loopful of the test organism was obtained from a nutrient agar culture and inoculated onto the surface of the citrate agar slant using a sterile inoculating wire. The inoculated slants were incubated at 37°C for 24 hours. A color change from green to blue in the medium indicated a positive citrate utilization test, demonstrating the organism's ability to utilize citrate as a sole carbon source.

Indole Test

The indole test was conducted to determine the ability of the organism to produce indole from tryptophan. A loopful of the bacterial isolate was inoculated into 5 mL of peptone water and incubated at 37°C for 24–48 hours. After incubation, 2 drops of xylene followed by 3 drops of Kovac's indole reagent were added to the culture broth. The formation of a red or pink ring on the surface of the broth indicated a positive indole test, while the absence of color change indicated a negative result.

Oxidase Test

The oxidase test was carried out to determine the presence of the cytochrome oxidase enzyme in the bacterial isolates. Filter papers were impregnated with tetramethyl-p-phenylenediamine dihydrochloride reagent and lightly moistened with sterile distilled water. Using a sterile wooden applicator stick, a small amount of the bacterial colony was transferred onto the prepared filter paper. The development of a dark purple coloration within a few seconds indicated a positive oxidase reaction, whereas the absence of color change indicated a negative result.

Methyl Red Test

The methyl red test was performed to determine the ability of bacterial isolates to produce stable acid end products from glucose fermentation. A loopful of the test organism was inoculated into a tube containing glucose phosphate broth and incubated at 37°C for 24 hours. After incubation, approximately 2.5 mL of the culture broth was transferred into a sterile test tube, and a few drops of methyl red reagent were added. The development of a red coloration indicated a positive result, showing strong acid production, while the absence of color change or the appearance of a yellow color indicated a negative result.

Sugar Fermentation Test

Carbohydrate fermentation tests were conducted using 2% solutions of glucose, fructose, lactose, sucrose, and mannitol. Each sugar solution was sterilized using a membrane filtration method and dispensed into test tubes containing 5 mL of peptone water. A colony of the test organism was inoculated into each tube, and Durham tubes were inverted in the medium to detect gas production. The inoculated tubes were incubated at 37°C for 48 hours. A yellow color change in the medium indicated acid production, while yellow coloration accompanied by gas accumulation in the Durham tube indicated both acid and gas production. A red coloration indicated that fermentation of the sugar had not occurred.

Preparation of Inoculum

The McFarland turbidity standard was prepared by mixing 1 mL of barium chloride ($BaCl_2$) with 9 mL of sulfuric acid (H_2SO_4) to produce a suspension equivalent to the turbidity of bacterial cultures used for antimicrobial testing. A loopful of each pure bacterial isolate, including *Escherichia coli*, *Lactobacillus* species, *Staphylococcus aureus*, *Streptococcus viridans*, and *Enterobacter aerogenes*, was inoculated into 5 mL of sterile nutrient broth and incubated at 28°C for 24 hours. After incubation, the turbidity of each culture was adjusted to match the 0.5 McFarland standard, ensuring a uniform bacterial concentration for subsequent analyses.

Antibiotic Susceptibility Testing

Antibiotic susceptibility of the bacterial isolates was determined using the Kirby–Bauer disc diffusion method. A 0.1 mL aliquot of each bacterial suspension adjusted to 0.5 McFarland turbidity standard was aseptically inoculated onto sterile Mueller–Hinton agar plates and evenly spread to obtain a uniform lawn of bacterial growth. Commercial antibiotic discs were then carefully placed on the surface of the inoculated agar using sterile forceps. The plates were incubated at 37 °C for 24 hours.

A total of ten antimicrobial agents were used in this study: Ciprofloxacin (5 µg), Clindamycin (10 µg), Erythromycin (10 µg), Ofloxacin (10 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Ampicillin (30 µg), Cephalexin (30 µg), Cloxacillin (10 µg), and Levofloxacin (5 µg). After incubation, the zones of inhibition surrounding each antibiotic disc were measured in millimeters to determine the susceptibility of the isolates to the respective antimicrobial agents.

Molecular Identification

Selected *Lactobacillus* isolates were further characterized using Polymerase Chain Reaction (PCR) techniques to confirm their molecular identity.

DNA Extraction for Polymerase Chain Reaction (PCR)

Genomic DNA extraction was performed using ZR Bashing™ Lysis Tubes according to the manufacturer's protocol. Briefly, 2 mL of bacterial broth culture was transferred into a ZR Bashing™ Lysis Tube. Subsequently, 750 µL of lysis solution was added, and the mixture was processed at maximum speed for 5 minutes to ensure effective cell disruption. The tube was then centrifuged in a microcentrifuge at >10,000 × g for 1 minute.

Approximately 400 µL of the resulting supernatant was transferred into a Zymo-Spin™ IV Spin Filter (orange top) placed in a collection tube and centrifuged at 7,000 × g for 1 minute. Following this step, 1,200 µL of bacterial DNA binding buffer was added to the filtrate in the collection tube. From this mixture, 800 µL was transferred to a Zymo-Spin™ IIC column placed in a new collection tube and centrifuged at 10,000 × g for 1 minute.

The flow-through was discarded, and 200 µL of DNA Pre-Wash Buffer was added to the column and centrifuged at 10,000 × g for 1 minute. This was followed by the addition of 500 µL of Bacterial DNA Wash Buffer, and the column was centrifuged again at 10,000 × g for 1 minute. Finally, the Zymo-Spin™ IIC column was transferred to a clean 1.5 mL microcentrifuge tube, and 100 µL of DNA Elution Buffer was added directly to the column matrix. The column was centrifuged at 10,000 × g for 30 seconds to obtain the purified genomic DNA, which was subsequently used for PCR analysis.

Agarose Gel Electrophoresis for DNA and PCR Products

Agarose gel electrophoresis was performed to visualize DNA and PCR amplification products. For genomic DNA analysis, 1 g of agarose was dissolved in 100 mL of 1× Tris–Acetate–EDTA (TAE) buffer, while 2 g of agarose was used for PCR product analysis. The agarose powder was mixed with the buffer in a heat-resistant flask and heated in a microwave oven for 1–3 minutes until the agarose was completely dissolved.

The molten agarose solution was allowed to cool to approximately 50°C for about 5 minutes, after which 10 µL of EZ Vision DNA stain was added. This stain binds to DNA and enables visualization under ultraviolet (UV) light. The stained agarose solution was then poured into a gel casting tray fitted with a comb to create wells. The gel was allowed to solidify either at 4°C for 10–15 minutes or at room temperature for 20–30 minutes. Once solidified, the gel was placed in an electrophoresis chamber containing 1× TAE buffer, and the DNA samples were loaded into the wells for separation and visualization.

DNA Sequencing

The PCR-amplified fragments were sequenced using an Applied Biosystems Genetic Analyzer 3130xl following the manufacturer's protocol. Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit. The generated sequence data were edited and analyzed using Edit software and MEGA X for subsequent genetic and phylogenetic analysis.

Plasmid Analysis

Plasmid DNA extraction was carried out using the Zyppy™ Plasmid Miniprep Kit according to the manufacturer's instructions. Briefly, 1.5 mL of bacterial culture was centrifuged at 14,000 rpm for 30 seconds, and the supernatant was discarded.

The cell pellet was resuspended in 100 µL of 7× Lysis Buffer (Blue) and mixed gently by inverting the tube 4–6 times until the solution changed from opaque to clear blue, indicating complete cell lysis.

Next, 350 µL of cold Neutralization Buffer was added and mixed thoroughly. Successful neutralization was indicated by a yellow coloration and the formation of a yellowish precipitate. The sample was further inverted 2–3 times and centrifuged at 11,000–16,000 × g for 2–4 minutes. The supernatant containing plasmid DNA was carefully transferred to a Zymo-Spin™ IIN column placed in a collection tube and centrifuged for 15 seconds. The flow-through was discarded, and the column was returned to the collection tube.

Subsequently, 200 µL of Endo-Wash Buffer was added to the column and centrifuged for 30 seconds, followed by the addition of 400 µL of Zyppy™ Wash Buffer, which was centrifuged for 1 minute. The column was then transferred into a clean 1.5 mL microcentrifuge tube, and 30 µL of Zyppy™ Elution Buffer was added. After centrifugation for 30 seconds, purified plasmid DNA was obtained and stored for further molecular analysis.

Statistical Analysis

The collected data were organized and analyzed using appropriate statistical methods. Descriptive statistics, including frequencies and percentages, were calculated to summarize the study variables and demographic characteristics of the participants. The Chi-square (χ^2) test was employed to examine associations between categorical variables and to determine statistical significance among the observed parameters. All statistical analyses were conducted at a 95% confidence level, and a p-value of ≤ 0.05 was considered indicative of statistical significance.

Results

Occurrence of Clue Cells from HVS Sample

Out of 60 HVS samples, 60(100%) were positive with epithelial cells. Clue cells were absent in the samples.

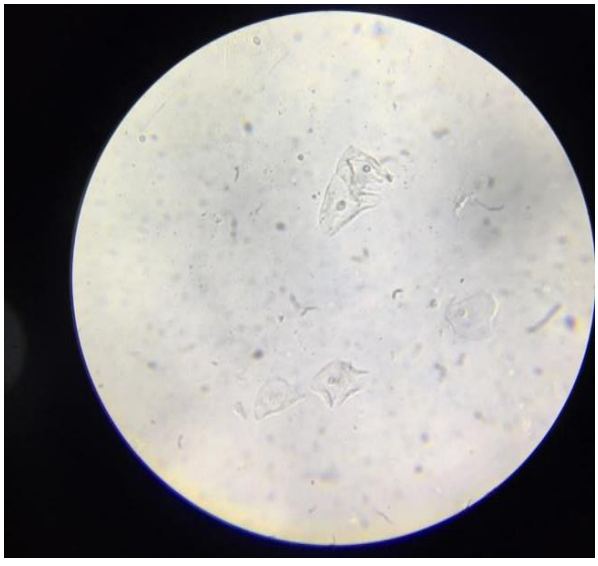


Plate 1: Photomicrograph shows large, flat, polygonal vaginal epithelial cells from HVS sample.

Growth and Morphology of Isolates on Different Media

Isolates showed varied cultural morphology on different media.

Table 1: Growth and Morphology of Isolates on Different Media

Media	Growth Morphology	Organism Isolated
Blood Agar	Round, smooth, golden yellow colonies	*Staphylococcus aureus*
Blood Agar	White colonies with greenish discoloration due to partial hemolysis(alpha hemolysis)	*Streptococcus viridans*
MRS Agar	Small, white, smooth colonies	*Lactobacillus spp*
EMB Agar	Dark purple round colonies	*Escherichia coli*
EMB Agar	Mucoid pink colonies	*Enterobacter aerogenes*
SDA	Creamy, white, smooth colonies	*Candida albicans*

Prevalence of Isolates from High Vaginal Swab Samples

Lactobacillus species were present in all the isolates while *Escherichia coli, Staphylococcus aureus, Streptococcus viridans* and *Candida albicans* varied in occurrence.

Table 2: Prevalence of Isolates from High Vaginal Swab Samples

Total Number of Samples(n=60)	Prevalence of Isolates	Percentage occurrence (%)
60	*Lactobacillus spp*	60(100%)
60	*Escherichia coli*	18(30%)
60	*Staphylococcus aureus*	10(16.7%)
60	*Streptococcus viridans*	5(8.3%)
60	*Candida albicans*	15(25%)
60	*Enterobacter aerogenes*	12(20%)

Table 3: Mixed Isolates from the Positive Population

Mixed Isolates from the Samples	Number of Samples
Lactobacillus spp *Staphylococcus aureus* *Streptococcus viridans* *Escherichia coli*	1
Staphylococcus aureus *Candida albicans*	1
Lactobacillus spp *Staphylococcus aureus* *Streptococcus viridans*	1
Escherichia coli *Enterobcater aerogenes*	1
Lactobacillus spp *Escherichia coli* *Enterobacter aerogenes*	1
Staphylococcus aureus *Streptococcus viridians*	1
Lactobacillus spp *Escherichia coli*	1
Streptococcus viridians *Candida albicans*	1
Streptococcus viridans *Escherichia coli* *Lactobacillus spp*	1
Escherichia coli *Streptococcus viridans*	1
Staphylococcus aureus *Candida albicans*	1
Candida albicans *Escherichia coli*	1

Identification and Characterization of Bacterial Isolates

The bacterial isolates shows varied inference for biochemical tests and sugar fermentation test for each isolate.

Table 4: Identification and Characterization of Bacterial Isolates

Biochemical Test	*Escherichia coli* Inference	*Lactobacillus spp* Inference	*Staphylococcus aureus* Inference	*Streptococcus viridians* Inference	*Enterobacter aerogenes* Inference
Catalase Test	+ve	-ve	+ve	+ve	+ve
Oxidase Test	-ve	-ve	-ve	-ve	-ve
Citrate Test	-ve	-ve	+ve	+ve	+ve
Coagulase Test	-ve	-ve	+ve	-ve	-ve
Indole Test	+ve	-ve	-ve	-ve	-ve
Methyl Red Test	+ve	+ve	+ve	+ve	+ve
Sugar Fermentation Test					
Glucose	AG	AG	A	A	AG
Fructose	AG	AG	A	A	AG
Maltose	AG	AG	A	A	AG
Lactose	AG	AG	A	A	AG
Gram staining	-ve	+ve	+ve	+ve	-ve
Microscopy	Short rod	Long rod	cocci	Rod shape	Short rod

Identification and Characterization of Fungal Isolate

The fungal isolate also showed varied inference for each biochemical tests.

Table 5: Identification and Characterization of Fungal Isolate

Biochemical Test	*Candida albicans* Inference
Catalase Test	+ve
Urease Test	-ve
Sugar Fermentation Test	
Glucose	AG
Fructose	AG
Maltose	AG
Lactose	-
Microscopy	Pseudohyphae

Key: *+ Positive, – Negative, A= Acid and AG= Acid/Gas*

Prevalence of Genital symptoms and Clinical signs in Pregnant women

Pre-experiment activity involved in sharing questionnaires comprising of signs and information obtained from the respondents in grouping them into symptomatic population. 33.3% were symptomatic. Symptoms varied amongst the respondents.

Table 6: Prevalence of Genital symptoms and Clinical signs in Pregnant women (n=60)

Criteria symptoms	Number of symptomatic persons (n=60)	*E.coli* positive culture (n=18)	*Enterobacter aerogenes* positive culture (n=12)	*Staphylococcus aureus* positive culture (n=10)	*Streptococcus viridans* positive culture (n=5)	*Candida albicans* positive culture (n=15)
Offensive vaginal odor	20 (33.3%)	2(11.1%)[a]	3(25%)[a]	-	-	-
Burning sensation	15 (25%)	6(33.3)	5(41.7%)	2(30%)	2(40%)	15(100%)[ab]
Itching of vagina	15 (25%)	5(27.8%)	4(33.3%)	2(20%)	1(20%)	15(100%)[ab]
Dysuria	21 (35%)	18(100%)[ab]	8(66.7%)	2(20%)	2(40%)	-
Cloudy urine	18 (30%)	16(88.9%)[ab]	3(25%)	8(80%)[ab]	1(20%)	-

[a] *indicates not significantly associated at p > 0.05*
[ab] *indicates Significantly associated at p < 0.05*

Analysis of Bacterial vaginosis by Amsel's Criteria

The Amsel's criteria showed that the pregnant population of women were not positive for bacterial vaginosis but the presence of homogeneous discharge and change in pH could be due to the presence of other isolates.

Table 7: Analysis of Bacterial Vaginosis by Amsel's Criteria

Amsel's criteria	*Lactobacillus spp* positive culture (n=60)	*Escherichia coli* positive culture (18)	*Enterobacter aerogenes* positive culture (12)	*Staphylococcus aureus* positive culture (n=10)	*Streptococcus viridians* positive culture (n=5)	*Candida albicans* positive culture (n=15)	*Gadnerella vaginalis*	*Mobiluncus spp*
Homogeneous discharge	-	2(11.1%)	3(25%)	2(20%)	2(20%)	15(100%)[ab]	-	-
Whiff test	-	-	-	-	-	-	-	-
pH test	-	18(100%)[ab]	12(100%)[ab]	10(20%)[ab]	5(100%)[ab]	15(100%)[ab]	-	-
Clue cells	-	-	-	-	-	-	-	-

[ab] *indicates significantly associated at p < 0.05*

Analysis of Bacterial vaginosis by Nugent Score

Lactobacillus morphotypes were positive at 100% and were large positive rods. The samples showed normal flora at 0-3 nugent range showing absence of bacterial vaginosis.

Table 8: Analysis of Bacterial vaginosis by Nugent score

Nugent score (n=60)	Number positive for *Lactobacillus* morphotypes	Large Gram positive rods	Number positive for *Gardnerella vaginalis*	Small Gram-negative rods	Number positive for *Mobiluncus spp*	Curved Gram-negative rods
0-3 (Normal flora)	60 (100%)	60 (100%)	-	-	-	-
4-6 (Intermediate flora)	-	-	-	-	-	-
7-10 (Bacterial vaginosis)	-	-	-	-	-	-

Pattern of Antimicrobial Susceptibility of Lactobacillus species

Most of the *Lactobacillus* species were sensitive to the test agents

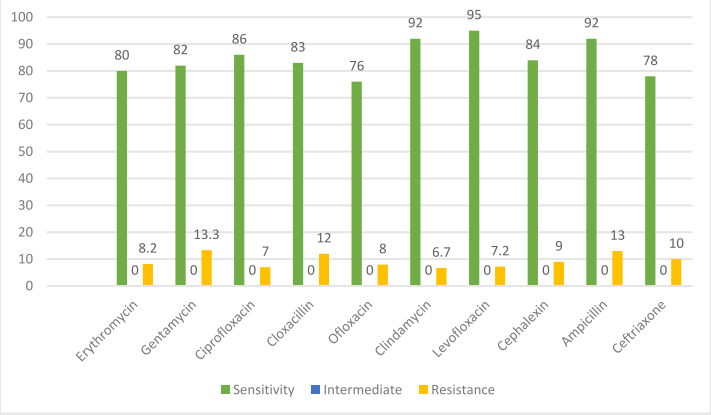


Fig 1: Antimicrobial Susceptibility Pattern of Lactobacillus species

Pattern of Antimicrobial Susceptibility of *Escherichia coli*

Escherichia coli showed varied antimicrobial susceptibility pattern to the test agents with ofloxacin and clindamycin being highly resistant at 76% and 80% respectively.

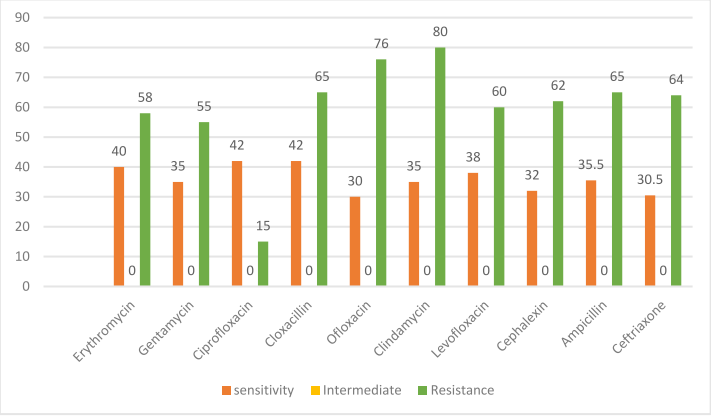


Fig 2: Antimicrobial Susceptibility Pattern of Escherichia coli

Pattern of Antimicrobial Susceptibility of *Staphylococcus aureus*

Staphylococcus aureus showed multiple resistance to most of the test agents with range from 60.5 to 85%.

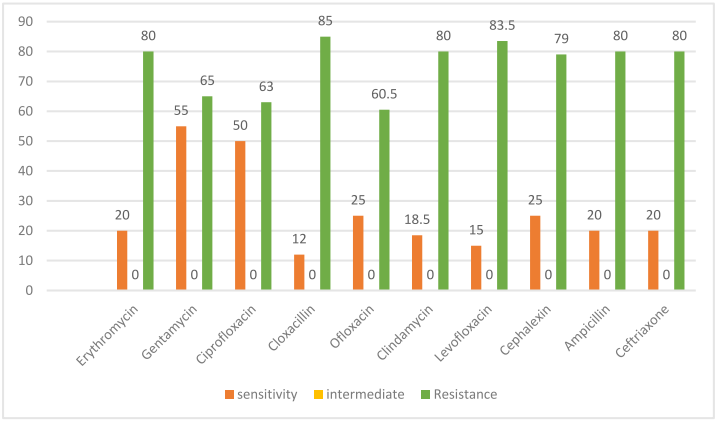


Fig 3: Antimicrobial Susceptibility Pattern of Staphylococcus aureus

Pattern of Antimicrobial Susceptibility of *Streptococcus viridians*

From the study, *Streptococcus viridians* were sensitive at 75.5% to 97% to most of the test agents.

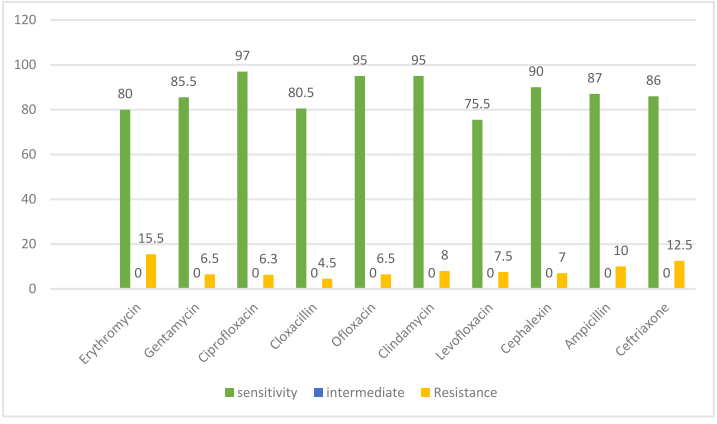


Fig 4: Antimicrobial Susceptibility Pattern of Streptococcus viridans

Pattern of Antimicrobial Susceptibility of *Enterobacter aerogenes*

From the study, *Enterobacter aerogenes* were sensitive to most of the test agents.

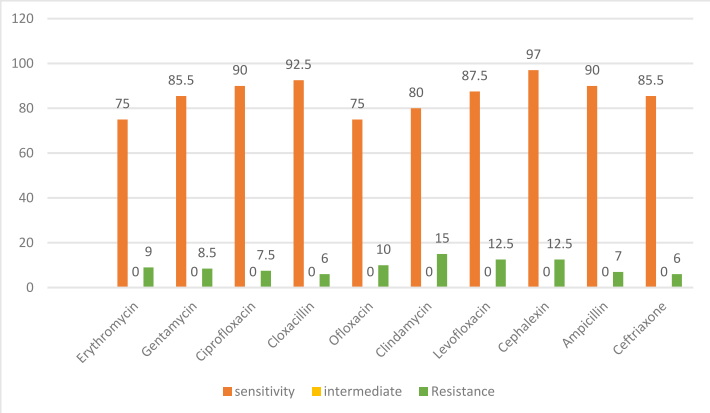


Fig 5: Antimicrobial Susceptibility Pattern of Enterobacter aerogenes

The Amplified PCR Positive Products

The amplified PCR products were positive at 15000bp for 16SrRNA gene showing *Limosilactobacillus fermenters* strain TMPC 34351 and *Lactobacillus helveticus* strain IMAU30124

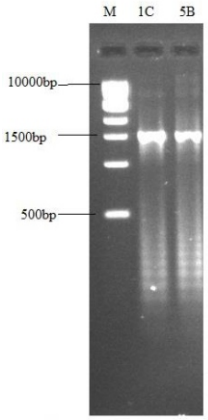


Fig 6: Agarose gel electrophoresis of PCR amplified products lane 1 and 2 shows positive amplification for 1500bp for 16SrRNA gene

Lane 1C: *Limosilactobacillus fermenters strain TMPC 34351*
Lane 5B: *Lactobacillus helveticus strain IMAU30124*

Phylogenetic Analysis of HVS isolates

Lactobacillus helveticus strain IMAU30124 with NCBI accession number FJ49690.1 showed pairwise similarity at 98.92% with KR858825.1 *Lactobacills helveticus* strain IMAU11172 at 88.88% while *Limosilactobacillus fermentum* strain ADHM 14 showed pairwise similarity with *Limosilactobacillus fermentum* strain TMPC 281047

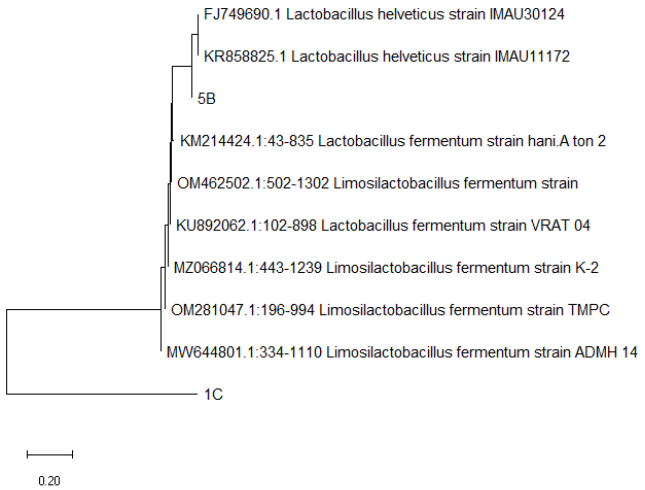
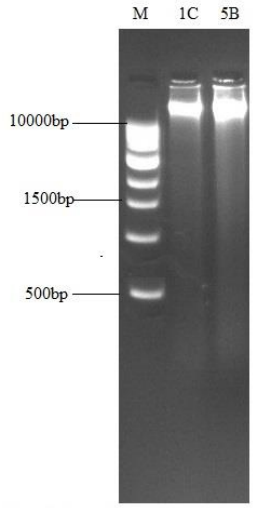


Fig 7: Phylogenetic analysis of HVS isolates

Plasmid Profile of Isolates

From the study, the isolates had high molecular weight plasmids of 15000bp.



Gel electrophoresis showing profiling of high molecular weight Plasmid above 10kbp. Lane M is a 1kbp DNA ladder

Fig 8: Gel electrophoresis profile of plasmid DNA (>10 kbp).

Plasmid Curing of the Isolates

From the study, the two isolates lost their plasmids when treated with 10% SDS.

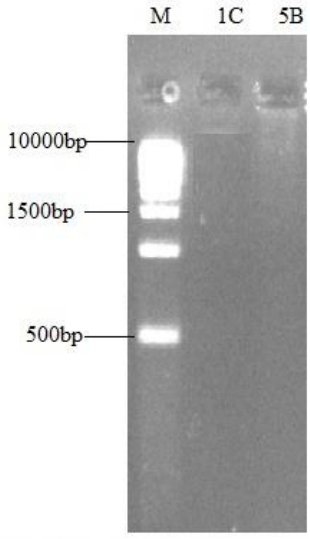


Fig 9: Gel electrophoresis of cured plasmids using 10% SDS.

Discussion

Occurrence of Clue Cells from High Vaginal Swab Samples

In the study, out of 60 HVS samples, epithelial cells were seen in abundance and there was absence of clue cells showing absence of bacterial vaginosis (Plate 1). Epithelial cells in the HVS are normal vaginal lining cells and they are usually polygonal, flat and large. This is in line with the works of [44], which indicates that epithelial cells are vital for protection against pathogens in pregnant women. The epithelial cells possess intracellular and extracellular pathogen recognition receptors, secrets chemokines and cytokines that initiates, regulates, link innate and adaptive immune responses and produce intracellular and secreted antimicrobial factors that kill invading microbes.

Growth and Morphology of Isolates on Different Media

In the study, the growth morphology of *Staphylococcus aureus* on blood agar were round, smooth, golden yellow colonies due

to pigment formation. Colonies exhibited beta hemolysis, forming clear zones around colonies due to complete lysis of red blood cells and it is in line with [41]. *Streptococcus viridians* on blood agar formed small, dome-shaped white colonies surrounded by alpha hemolysis, which appeared as greenish discoloration of the medium due to partial hemolysis and it's supported by [6]. *Escherichia coli* fermented lactose vigorously on Eosin Methylene blue agar(EMB), producing dark purple round colonies due to acid production precipitating the dyes which is in line with [7]. *Enterobacter aerogenes* on EMB agar were large mucoid pink colonies. They formed pink colonies but without the metallic sheen seen in E.coli. This is in accordance with [12] and he also populated that it helps in differentiating them from E.coli.

Lactobacillus species cultured on de Man, Rogosa, Sharpe (MRS) agar were typically small, smooth, white to cream colonies. MRS medium enhances their growth under microaerophilic/ anaerobic conditions and it is supported by [16]. *Candida albicans* were creamy, white, smooth colonies on SDA. SDA supports fungal growth due to its acidic pH and high dextrose content and this is in accordance with [20]. (Table 4.2).

Prevalence of Isolates from HVS Samples

From the study, out of 60 (sixty) high vaginal swab samples collected from pregnant women, *Lactobacillus species* was the most predominant isolate, being present in all samples at 100% (Table 4.3). This agrees with existing literature of [22] where *Lactobacillus species* are reported as the dominant normal flora of the vagina, playing a protective role by producing lactic acid, hydrogen peroxide and bacteriocins that help an acidic vaginal environment and suppress the growth of pathogenic organisms. Among the pathogenic isolates, *E.coli* (30%) showed the highest prevalent followed by *Candida albicans* (25%), *Enterobacter aerogenes* (20%) *Staphylococcus aureus* (16.7%) and *Streptococcus viridans* (8.3%). The detection of *E.coli* is of clinical significance, as it is an opportunistic pathogen that may ascend into the urinary tract and cause urinary tract infections (UTI) in pregnancy, which is associated with complications such as preterm labor and low birth weight.

The occurrence of *Candida albicans* (25%) is consistent with previous reports that pregnancy predisposes women to vulvovaginal candidiasis due to hormonal changes, increased glycogen deposition and altered vaginal immunity [1],[14].

Enterobacter aerogenes (20%) and *Staphylococcus aureus* (16.7%) were less frequently isolated but still clinically relevant. *Staphylococcus aureus* colonization in the genital tract has been linked with the risk of puerperal sepsis and neonatal infections and it can be compared with the works of [34]. Similarly, *Streptococcus viridans* though part of the commensal flora, can act as an opportunistic pathogen under altered vaginal microecology. The variations in the prevalence of these isolates highlights the balance between normal protective flora (*Lactobacillus*) and potential pathogens. Disruptions of this balance can predispose pregnant women to infections that may adversely affect maternal and neonatal health.

The present study recorded a prevalence of *Streptococcus viridans* at 8.3%. *Streptococcus viridans* are generally low-virulence organisms, but they can act as opportunistic pathogens under conditions of altered vaginal microbiota or reduced immunity, such as during pregnancy. The detection of *Streptococcus viridans* in pregnant women is clinical significant, as these bacteria have been implicated in cases of neonatal infections, and occasionally endocarditis in susceptible hosts.

Several studies have reported the isolation of *Streptococcus viridans* from the vaginal tract with the potential to disrupt the protective role of *Lactobacillus*-dominated flora [1].

Mixed Isolates from the Positive Population

The occurrence of mixed microbial isolates in high vaginal swab samples indicates the presence of more than one microorganisms in the genital tract of the study population (Table 4.4). Mixed growth is clinically important as it suggests co-infection or secondary colonization resulting from an imbalance in the normal vaginal flora. In pregnancy, the vaginal environment is usually dominated by *Lactobacillus species*, which help maintain an acidic pH and prevent the overgrowth of pathogenic organisms. However, when this balance is disrupted, opportunistic pathogens such as *Staphylococcus aureus, E.coli, Streptococcus viridians, Enterobacter aerogenes* and *Candida albicans* may colonize and co-exist leading to mixed infections. The detection of mixed isolates among positive samples in this study may also be associated with poor hygiene practices, sexual activity, prolonged antibiotics use or underlying health conditions that compromise the host's immune defense. This is in line with the works of [5],[43] which indicates that different microbial colonization complicates treatment, as different organisms may exhibit varied antimicrobial resistance patterns, making empirical therapy less effective and necessitating targeted antimicrobial susceptibility testing.

Identification and Characterization of Bacterial and Fungal Isolates

In the study, the bacterial and fungal isolates showed varied inference for biochemical tests and sugar fermentation test for each isolate. They were identified based on cultural, Gram staining and biochemical tests. *Escherichia coli* are Gram-negative, rod shaped bacterium that grows well on Eosin Methylene blue (EMB) agar, producing dark purple colonies. Biochemically, they are catalase positive, oxidase negative, indole positive and Methy red positive, but citrate negative. Mircoscopically, it appears as short rods arranged singly or in pairs. This is according to [13].

Lactobacillus species are Gram-positive non-spore forming rods, which often occurs in chains. They grow on de Man, Rogosa, and Sharpe (MRS) agar, producing small white colonies. Biochemically, they are catalase negative, oxidase negative, indole negative and exhibit strong acid production from carbohydrate fermentation, often giving a positive methyl red reaction and this is supported by [3].

Staphylococcus aureus are Gram-positive coccus that appears in grape-like clusters under the microscope. On blood agar, it formed golden-yellow colonies with beta-hemlysis. Biochemically, *Staphylococcus aureus* were catalase positive, oxidase negative and coagulase positive, which differentiates it from coagulase-negative *Staphylococci* (*Staphylococcus epidermidis*). The coagulase test remains the golden standard for confirming *Staphylococcus aureus* and this is supported with previous reports by [4].

Streptococus viridans are Gram-positive cocci arranged in chains. On blood agar, they typically produce alpha hemolysis (green discoloration). Biochemically, they are catalase negative, oxidase negative, indole negative and Methyl red negative, coagulase negative and do not utilize citrate. This is in accordance with [24].

Enterobacter aerogenes are Gram-negative rod belonging to the Enterobacteriaceae family.

On EMB agar, the colonies appear mucoid and lactose fermenting pink. Biochemically, they are catalase positive, oxidase negative, citrate positive, indole negative and Methyl red negative. This is in agreement with [15].
Candida albicans was identified based on its creamy white colonies on Sabouraud dextrose agar, Gram-positive budding yeast morphology.further confirmation was supported by its ability to form chlamydospores on cornmeal agar and urease negative reaction, consistent with previous reports (28). These findings align with clinical diagnostic guidelines for candidiasis [38].

Prevalence of Genital Symptoms and Signs in Pregnant Women (n=60)
Genital symptoms and clinical signs are pre-requisite in determination of vaginal infection. In the study, pre-experimental activity were achieved by sharing questionnaires to the respondents. Out of the 60 respondents, they had varied results which comprised of 33.3% positive for offensive vaginal odor, 25% for burning sensation and itching of vagina, 30% for dysuria while 30% for cloudy urine (Table 4.6). The presence of these symptoms indicates vaginal infection which could be caused by varied organisms. This is suggestive of these symptoms being caused by the presence of other isolates which may be responsible for the observed symptoms.
Dysuria, cloudy urine, offensive odor were present in samples positive with *Escherichia coli*. Similarily, *E.coli* was significantly associated with dysuria and cloudy urine ($p < 0.05$), confirming its role as a uropathogen in pregnant women and it is supported by [38]. Offensive odor was not significantly associated with any of the isolates ($p > 0.05$). This suggests that vaginal odor may be multi-factoral, possibly linked to different bacterial flora or subjective perception, rather than attributable to a single organism. Similar findings were reported by [26] which noted that vaginal odor alone is not a reliable diagnostic indication of vaginal infection.
Burning sensation, cloudy urine, itching of vagina and Dysuria were present in samples positive with *Staphylococcus aureus*. *Staphylococcus aureus* also showed significant association with cloudy urine, which agrees with reports of [36] that it may contribute to genital infection.
Respondents HVS samples positive with *Candida albicans* were positive burning sensation and itching of vagina. Symptom-based associations showed that *Candida albicans* was strongly inked with burning sensation and vaginal itching ($p > 0.05$). This is supported with the work of [37] which aligns with established clinical evidence that vulvovaginal candidiasis is commonly characterized by vaginal itching and burning sensation.

Analysis of Bacterial Vaginosis by Amsel's Criteria
In the study, the pregnant population did not have bacteria vaginosis, as 3 out of the 4 positive results in Amsel's criteria is indicative of bacterial vaginosis. The presence of some of the criteria is suggestive of infection by other organisms. pH > 4.5 showed a statistically significant association ($p < 0.05$) with all organisms though non-specific. This indicates that while elevated pH is a useful screening tool, it cannot reliably predict the exact pathogen. However, homogeneous discharge was 100% associated with *Candida albicans* ($p < 0.05$) underscoring its diagnostic value in candidiasis. The statistical analysis strengthened the microbiological findings by demonstrating significant clinical correlations.

Analysis of Bacterial Vaginosis by Nugent Score
In the study, *Lactobacillus* morphotypes were present at 100% and were large positive rods. Out of 60 HVS samples analyzed by Gram staining according to Nugent scoring system, 100% were classified as having normal vaginal flora, characterized by a predominance of *Lactobacillus* morphotypes none for bacterial vaginosis and intermediate flora. According to [27], several studies have demonstrated the reliability of Nugent scoring in both pregnant and non-pregnant women. It has a high sensitivity and specificity for bacterial vaginosis. The distribution of Nugent scores in this study demonstrates the absence of bacterial vaginosis among the study population.

Pattern of Antimicrobial Susceptibility of *Lactobacillus* species
In the study, the *Lactobacillus* species were highly susceptible to the antimicrobial agents tested ranged from 76% to 95%. The broad sensitivity pattern observed highlights that *Lactobacillus species* generally remain susceptible to commonly used beta-lactam antibiotics; Ampicillin(92%), Cloxacillin(83%), Ceftriaxone(78%) as well as macrolides; Erythromycin(80%). This finding is in agreement with previous studies which reported that many *Lactobacillus* isolates exhibit preserved sensitivity to cell wall active agents and macrolides, making them reliable therapeutic options when treatment is indicated. [8].

Pattern of Antimicrobial Susceptibility of *Escherichia coli*
E.coli showed varied antimicrobial susceptibility pattern to the test agents with Ofloxacin and Clindamycin being resistant at 76% and 80% respectively. This findings is consistent with global reports that *E.coli* exhibits variable resistance, particularly due to extended-spectrum lactamase production and plasmid-mediated resistance genes. High levels of resistance are commonly reported against Ampicillin, Tetracyclines and Trimethoprin-sulfamethoxazole, while better activity is usually retained for carbapenems and aminoglycosides [19],[29].

Pattern of Antimicrobial Susceptibility of *Staphylococcus aureus*
Staphylococcus aureus showed multiple resistance at 60.5 to 85%, with sensitivity between 12% and 55%. This suggests a high burden of antimicrobial resistance, possibly including methicillin-resistant *Staphylococcus aureus* (MRSA). Such high resistance rates have been widely documented, particularly against Penicillin and some macrolides, while vancomycin, linezolid and newer agents generally retain efficacy, [21],[40]. The presence of resistant strains pose clinical challenges, as treatment options become limited, and highlights the importance of routine surveillance.

Pattern of Antimicrobial Susceptibility of *Streptococcus viridans*
Streptococcus viridans isolates demonstrated high susceptibility (75.5% to 97%) to the test agents. The strong sensitivity pattern is in line with earlier findings that *viridans* group *Streptococci* usually remain susceptible to beta-lactams e.g., Ampicillin (87%), Ceftriaxone(86%) and macrolides (Erythromycin(80%) though emerging resistance has been reported in some clinical contexts, [11].

Pattern of Antimicrobial Susceptibility of *Enterobacter aerogenes*

The findings showed that *Enterobacter aerogenes* isolates were sensitive to most of the test agents. *Enterobacter species* are opportunistic pathogens that harbor inducible AmpC beta-lactamases, which can confer resistance to third generation cephalosporins and penicillins, [23]. However, they typically retain susceptibility to carbapenems, fluoroquinolones and aminoglycosides unless multi-drug resistance mechanisms are acquired. The high sensitivity observed in this study may reflect a relatively low prevalence of resistant strains in the study population compared to global reports where multi-drug resistance is a growing concern.

The amplified PCR Positive Products

The molecular analysis in this study confirmed successful amplification of the 16S rRNA gene (1500bp) from the bacterial isolates, which enabled accurate species identification. The gel electrophoresis profile showed clear positive bands for *Limosilactobacillus fermentum* and *Lactobacillus helveticus*, consistent with the expected product sizes. The 16S rRNA gene is widely recognized as the gold standard for bacterial identification because of its high conservation and discriminatory power among closely related texa [10].

Pairwise similarity analysis further supported the taxonomic assignment, with *Lactobacillus helveticus* strain IMAU30124 showing 98,92% similarity to the reference strain, and *Limosilactobacillus fermentum* strains clustering with deposited sequences. These findings are in line with previous studies where 16S rRNA sequencing has reliably distinguished *Lactobacillus species* at the strain level, [32]. The isolates also exhibited high molecular weight bands (1500bp), suggesting intact genomic DNA suitable for further molecular characterization. This is important as DNA integrity is crucial for downstream applications such as phylogenetic analysis and gene detection.

Plasmid Profiling of Isolates

From the study, the isolates had high molecular weight of 15000bp. Plasmid profiling serves as an important epidemiological and molecular typing tool, allowing comparison between isolates and tracking of resistance dissemination in bacterial populations [2].

Plasmid profiling was carried out using agarose gel electrophoresis to determine the presence and molecular weight of plasmids. The bacterial isolates harbored high molecular weight plasmid of approximately 15,000 base pairs (15kbp), which migrated above the 10 kb band of the 1 kb DNA ladder. High molecular weight plasmids, particularly those larger than 10kb, have been associated with the carriage of multiple antibiotic resistance genes and conjugation machinery, thereby enabling horizontal transfer between bacterial species. The detection of ~15kb plasmids in the isolated studied suggests the possible presence of mobile genetic elements such as integrons and transposons. These elements provide a genetic platform for the acquisition and spread of antimicrobial resistance or virulence determinants and this is in accordance to [9],[33]. The presence of ~15 kb plasmids in the bacterial isolates indicates a strong potential for the carriage and dissemination of resistance and virulence genes. *Lactobacillus* species primarily indicates the presence of accessory genes that provide adequate advantage, enhancing the bacterium activity to survive in specific, often challenging environments.

Plasmid Curing of the Isolates

Plasmid curing demonstrated that the isolates had lost plasmids after treatment with 10% SDS indicating that the strains carried extrachromosomal elements. The ability to cure plasmids is significant in understanding the genetic basis of antibiotic resistance or probiotic functional traits. Studies have shown that plasmids often harbor resistance genes or metabolic traits, and their elimination provides insights into chromosomal versus plasmid-encoded functions [17].

Conclusion

From the study, Lactobacillus species were predominant in all the samples from the pregnant women and showed the absence of bacterial vaginosis. Other pathogenic and commensal organisms were isolated comprising of *Escherichia coli, Staphylococcus aureus, Streptococcus viridians, Enterobacter aerogenes.* The isolates, *E. coli* and *Staphylococcus aureus* were highly resistant to most common antibiotic which calls for concern. These findings emphasize the importance of combining classical and molecular methods for accurate microbial identification. Clinically, the detention of pathogenic isolates draws the attention to maternal and neonatal risks, while the presence of *Lactobacillus species* underscores their protective role in maintaining vaginal homeostasis.

Recommendation

It is recommended that routine screening of pregnant women should be encouraged to detect potential pathogens early and prevent complications such as preterm birth, urinary tract infection and neonatal sepsis. Public health interventions should promote the role of probiotics and healthy lifestyle practices in maintaining balanced vaginal microbiota. Awareness campaigns should also educate women on the importance of antenatal screening and vaginal health management during pregnancy.

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