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Empowering Women's Health: Investigating Bacterial Pathogens and Antibiotic Resistance for Personalized Vaginal Infection Care

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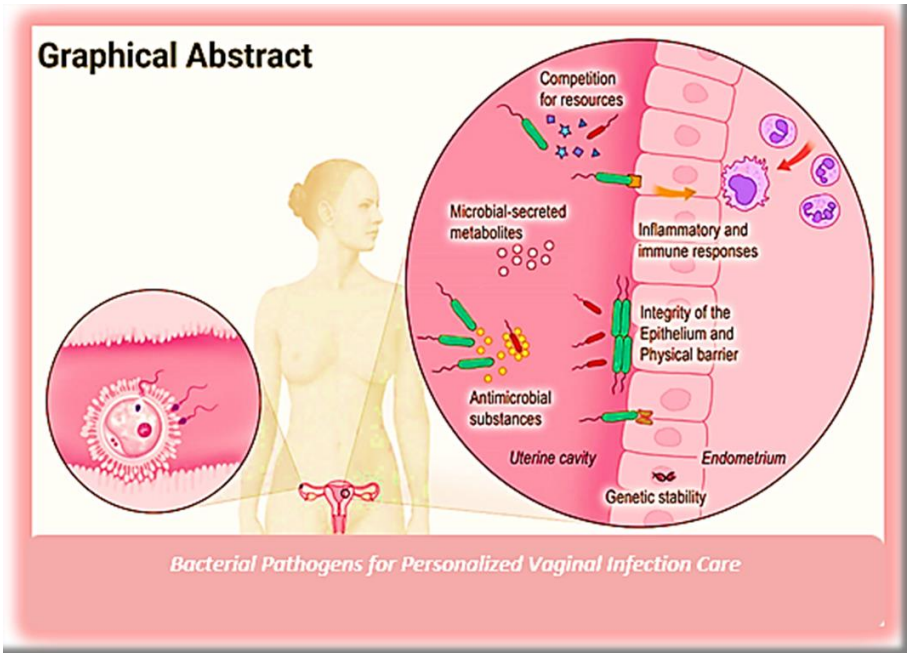

Abstract

Aim: The present investigation was conducted over a span of three months, with the primary objective of identifying and characterizing the bacterial pathogens that trigger vaginal infections in females.

Methodology: From Hospital 55, female patients afflicted with vaginal infections were chosen, and samples were collected by means of vaginal swabs. The bacterial species present in the collected samples were identified using a combination of biochemical and agricultural tests. The growth of bacterial colonies was analyzed using agricultural and gram staining, as well as through microscopy and biochemical tests.

Results: The results indicated that among the 55 vaginal inflammatory patient samples, Staphylococcus species was responsible for the majority (53%) of the infections, followed by Klebsiella (38%), Escherichia coli (7%), and Neisseria (2%). Additionally, the susceptibility of these bacterial pathogens to seven antibiotics, namely Tetracycline, Penicillin, Ampicillin, Norfloxacin, Chloramphenicol, Gentamicin, and Streptomycin, was determined. The findings demonstrated that Chloramphenicol was found to be 100% effective in treating the bacterial species. These findings can be utilized to develop personalized treatment regimens for vaginal infections, which could lead to improved management of this condition in women.

Keywords: Vaginal infections; Bacterial species; Diagnosis; Biochemical tests; Gram staining; Microscopy; Sensitivity, Antibiotics.

1. Introduction

Gestation is a complex biological process that involves the development of one or more embryos within the female body. In humans, gestation typically lasts for approximately nine months, with the term

"Jenin" colloquially referring to the developing offspring from conception until birth. The legal and medical status of the developing offspring is divided into three stages based on its maturity, with the first stage carrying a high risk of natural fetal demise [1,2]. This process is of great interest to medical researchers as it is central to human reproduction. While pregnancy is a joyous occasion, it can also bring about several signs and symptoms that indicate the onset of pregnancy. Some common signs of pregnancy include breast changes, nausea and vomiting, frequent urination, mood swings, and other physiological changes. However, several factors can hinder pregnancy, such as polycystic ovary syndrome, vaginal infections, Chlamydia, and pelvic inflammatory disease [3]. One such infection is bacterial vaginosis (BV), a common vaginal infection characterized by an imbalance of the vaginal microbiome. BV affects approximately 16% of pregnant women worldwide and is associated with adverse pregnancy outcomes, including an increased risk of sexually transmitted infections, pelvic inflammatory disease, and endometritis. Therefore, understanding the bacteria residing in the vaginal environment and identifying potential opportunistic pathogens is crucial in developing better diagnostic and treatment approaches for pregnant women with BV [3-10]. The primary objective of this study is to conduct a comprehensive analysis of the bacteria residing in the female vaginal environment, identify potential opportunistic pathogens that may cause disease, and explore potential treatments for vaginal bacterial infections. The findings of this study may help healthcare professionals develop better diagnostic and treatment approaches for pregnant women with BV, ultimately improving maternal and fetal health outcomes. This research is critical to our understanding of the factors that impact gestation and maternal-fetal health and may pave the way for novel therapies to manage BV during pregnancy.

2. Material and Methods:

The materials, tools, and chemicals used in this study for the isolation and diagnosis of bacterial species responsible for vaginal infections in women included vaginal swab samples collected from patients suffering from vaginal infections. Biochemical and agricultural tests were conducted to diagnose the bacterial species present. The growth of bacterial isolates was described using agricultural and gram staining, microscopy, and biochemical tests. The seven antibiotics tested for sensitivity were Tetracycline, Penicillin, Ampicillin, Norfloxacin, Chloramphenicol, Gentamicin, and Streptomycin. Other tools used in the study included laboratory equipment for microbial culture, including Petri dishes, incubators, and microbial growth media. The chemicals used for the biochemical tests were also employed, including the catalase test, coagulase test, oxidase test, and fermentation tests. These materials, tools, and chemicals were crucial for the successful isolation and identification of bacterial species responsible for vaginal infections and the determination of their sensitivity towards antibiotics.

2.1. Collection of Vaginal Swab Samples for Microbiological Analysis: Methodology and Procedures

The isolates of E. coli, Staphylococcus saprophytic, Neisseria, Staphylococcus epidermis, Streptococcus, Lactobacillus, Staphylococcus aureus, and Klebsiella, recovered from human patients were maintained at the Microbiology Laboratory of Gynecology Clinic at the. These cultures were revived in nutrient broth after incubating them at 37°C for 24-48 hours. Then, a loopful of the broth culture of each isolate was streaked on specific selected medias for each. The colonies were identified based on their colony morphology, microscopic examination and biochemical testing. Microbial colonies developed in the respective plates after an incubation of 24-48 hours. The cultures were then transferred to nutrient agar slants for further use. and Gynecology Clinic at the Hospital Authority located in Hajjah Government. The samples were collected between March 30th, 2021 and June 12th, 2021, and were obtained from women who presented to the clinic. Vaginal swabs were taken from the anterior region of the vagina, and comprehensive patient information was recorded and subsequently transferred to the microbiology department.

2.2. Culture Media:

Selective media were used to culture the bacterial species. The media included MacConkey agar and EMB agar for E. coli, blood agar and Mannitol Salt agar for S. saprophyticus, Thayer-Martin agar for Neisseria, blood agar and Mannitol Salt agar for S. epidermidis, blood agar for Streptococcus, blood agar and Mannitol Salt agar for Lactobacillus S. aureus, and MacConkey agar for Klebsiella.

2.3. Colony Morphology:

The colony morphology of each bacterial species was observed and recorded. The size, shape, color, and texture of the colonies were noted.

2.4. Microscopic Examination:

Microscopic examination was performed using Gram staining. The bacterial species were stained with crystal violet, iodine, and safranin, and the morphology of the cells was observed under a microscope. The Gram reaction was recorded as either positive (purple) or negative (red).

2.5. Rigorous Formulation, Quality Control, and Optimization of Agricultural Media and L3.5G Stain and Blood Agar Media Preparation and Sterilization for Microbiological Investigations

The study employed several methodologies to ensure the robustness and reliability of microbiological investigations. The first methodology involved the rigorous formulation and quality control of agricultural media used in the study. The media were formulated with high-quality ingredients and carefully sterilized to eliminate any potential sources of contamination. The second methodology involved optimizing the preparation and sterilization of L3.5G stain for microbiological studies, which included precise measurements and rigorous sterilization using an autoclave. The third methodology involved a streamlined process for the optimal preparation and sterilization of blood agar media, which also included precise measurements and rigorous sterilization using an autoclave followed by the addition of blood to produce chocolate agar. Overall, the methodologies were designed to provide a reliable and accurate foundation for subsequent microbiological investigations [11].

2.6. Ensuring Sterility and Reproducibility in Microbiological Investigations: Robust Methods for Preparing and Sterilizing Agar Media

This study describes the methods used to prepare and sterilize agar media for microbiological investigations. Then, to prepare Chocolate Agar with blood, 15 grams of agar were weighed and dissolved in 375 milliliters of distilled water. The mixture was boiled and then autoclaved at 121°C and 1 atmosphere for 15 minutes. After sterilization, 15.5 milliliters of the 375th blood from the media were added to the warm agar with stirring until the mixture became homogeneous [12]. The agar was poured into Petri dishes and left to solidify before being inverted and refrigerated. For the preparation of Mueller Hinton Agar, 750 mL of MHA was formulated using a precise calculation. 28.5 grams of MHA were added to 198 milliliters of water and boiled, then autoclaved at 121°C and 1 atmosphere for 15 minutes. After sterilization, the MHA was poured into Petri dishes and left to solidify before being inverted and refrigerated. The name of the media and the date of preparation were written at the bottom of each dish. Moreover, to prepare agar tubes using nutrient broth agar media, 6.25 grams of nutrient agar were weighed and added to 2.50 milliliters of distilled water. The mixture was briefly heated over a flame until bubbles appeared, then removed from the heat source and left to settle. The filtrate was poured into another beaker, and 5 milliliters of the filtrate were added to each tube. The tube caps were tightly closed and the tubes were autoclaved at 121°C and 1 atmosphere for 15 minutes. After sterilization, the tubes were incubated at room temperature. These methods ensure the sterility and reliability of the results obtained in microbiological investigations, and are essential for high-quality and reproducible research.

2.7. Development of Resilient Methods for Sterile Compound and Combined Microbial Culturing on Multiple Media and Innovative Gram Staining Technique for Accurate Bacterial Identification

The microbial culturing methodology outlined in this study is crucial for microbiological investigations, as it enables the isolation and growth of different microorganisms on multiple media [13]. The combined and compound techniques utilized in this methodology ensure the sterility and accuracy of the results, allowing researchers to obtain reliable and reproducible data. The Gram staining method developed in this study is also an essential tool for microbiological investigations, as it enables the differentiation of bacterial types based on their cell wall structure. This novel method is efficient, easy to perform, and provides accurate results, making it a valuable addition to the microbiological toolbox. Overall, the compound and combined techniques, as well as the new Gram staining method, presented in this study, provide researchers with robust and reliable tools for microbial culturing and identification. These methods are essential for high-quality microbiological investigations, enabling accurate identification and characterization of microorganisms, which is crucial for the development of effective treatments and preventive measures against infectious diseases.

2.8. Biochemical test:

As part of the proposed methodology, following microscopic examination of the bacterial sample, a series of Biochemical tests are conducted to confirm the gram-positive nature of the bacteria.

2.8.1. Hydrogen Peroxide Method for Differentiating Between Staph and Strep Bacteria (Catalase test):

A commonly used method for differentiating between staph and strep bacteria involves placing a drop of H_2O_2 solution on a glass slide and then transferring a colony from a bacterial culture dish onto the slide [14]. The colony is mixed with the H_2O_2 solution, and the resulting reaction is observed. If the colony produces bubbles, it is indicative of staph bacteria. Conversely, if no bubbles are produced, it is suggestive of strep bacteria. This method is useful for distinguishing between these two bacterial species after an examination has been conducted.

2.8.2. Role of Bound Coagulase in Fibrin Clot Formation and Cocci Aggregation (Slide Coagulase Test):

The bound coagulase, referred to as the clumping factor, facilitates the formation of a fibrin clot by crosslinking the α and β chains of fibrinogen present in the plasma [15]. The resulting clot adheres to the cell wall, causing the cocci to aggregate.

2.8.3. Microbial Analysis Using DNS Media and HCL Treatment for Identification of Staphylococcus Species:

For this analysis, a cluster of microorganisms is extracted from the petri dish and agitated in an elliptical manner within the DNS medium. Subsequently, the mixture is incubated at a temperature of 37 degrees Celsius for 24 hours [16]. The following day, the dish is treated with hydrochloric acid (HCL). If a clear halo emerges surrounding the cluster, it signifies the presence of staphylococcus. In the absence of a transparent halo around the colony, it can be classified as staphylococcus epidermis or taphylococcus saprophytic.

2.8.4. Differential Procedure for Identifying Gram-Negative Bacteria: Oxidase Test for Pseudomonas Confirmation:

An essential discriminative technique that ought to be carried out on every gram-negative bacterial strain being characterized involves assessing for blue or purple coloration on the disk, indicating positive oxidation, and thus confirming the presence of Pseudomonas bacteria. Conversely, the absence of blue

or purple coloring on the disk denotes negative oxidation and therefore indicates an oxidase-negative strain [17].

2.8.5. Preparation of Media for the Sulfur Indole Motility (SIM) Test.

Using the Sulfur indole motility-responsive balance, ascertained the weight of 2.17g with the SIM card. Purified water, 60 ml in volume, was gauged by means of a cylinder and then conveyed into a beaker. Agar was blended in while consistently stirring the mixture over a flame until it boiled. Afterward, the media was transferred into 6 ml tubes, with an equivalent quantity dispensed into each pair of tubes [18]. The tubes were hermetically sealed and subjected to an autoclave at a temperature of 121 degrees Celsius and a pressure of 1 for a duration of 15 minutes. Following solidification of the media, the tubes were slanted and kept in refrigeration.

2.8.6. Urea test:

To prepare a growth medium for 10 tubes for bacterial cultures, follow these steps: First, measure 57ml of distilled water and weigh 1.44g of agar powder using a sensitive scale. Then, pour the water into a glass beaker, light the flame and add the agar powder, stirring until the mixture becomes homogeneous. Remove the beaker from the flame once the mixture starts to boil. Next, use a pipette to distribute the medium into 10 tubes, adding 6ml to each. Seal the tubes tightly, then autoclave them for 15 minutes at a temperature of 121 degrees Celsius and pressure of 1. After autoclaving, allow the tubes to cool to below 50 degrees Celsius before adding 3ml of 40% urea solution to each tube. Finally, tilt the tubes at an angle to allow the medium to solidify and store the tubes in a refrigerator [18-20].

2.8.7. Refined Agar Tube Preparation Technique with Accurate Weighing and Controlled Heating using Kliglr Iron Agar (KIA):

Using a precision scale from the KIA laboratory, precisely 3.45 grams were measured of the required substance. Following this, 60 milliliters of distilled water were taken using a cylinder and transferred it into a beaker. The mixture was agitated while agar was gradually added, and the resulting blend was heated over a flame while being continuously stirred until it reached the initial stage of boiling. Subsequently, the mixture was dispensed into individual tubes, with each tube containing 6 milliliters of the mixture. These tubes were tightly sealed and placed inside an autoclave, which was heated to 121°C and 1 atmospheric pressure for 15 minutes. Afterward, the tubes were carefully positioned diagonally until they solidified before being transferred to a refrigerator for storage.

2.8.8. Refined Methodology for Agar Tube Preparation and Accurate Identification of Gram-Negative Bacteria using Simmon Citrate Agar:

In this study, the precise weighing of 1.46 grams of the required substance from a Simmon Citrate Agar environment using a sensitive scale. A volume of 60 milliliters of distilled water was measured using a cylinder and transferred into a beaker. Agar was added gradually while the mixture was stirred, and the resulting blend was heated over a flame while being continuously stirred until it reached the initial stage of boiling. The mixture was then dispensed into individual tubes, each containing 6 milliliters of the mixture, and tightly sealed before being subjected to an autoclave at 121°C and 1 atmospheric pressure for 15 minutes. Once solidified, the tubes were stored in a refrigerator. The following tests were prepared: Simmer, Urea, SIM, KIA. The distribution was conducted to identify the types of Gram-negative bacteria, with KIA and SIM being distributed using the stabbing method while Urea and Simmer were distributed using the plover way. The samples were incubated in an incubator for 24 hours, and the next day, alcohol was added to the SIM test. A red aura indicated a positive result, while a lack of color change indicated a negative result.

2.8.9. Enhanced Bacterial Culture Technique with Accurate Inoculation and Antimicrobial Disc Placement for Improved Results:

To culture bacteria into petri dishes, the following steps were taken: Using a sterile wire loop, 3-5 well-isolated colonies with similar appearance to the test organism were emulsified in 3-4 milliliters of sterile physiological saline or nutrient broth. The turbidity of the suspension was matched to the turbidity standard in good lighting, with coma ring turbidities being easier to view against a printed card or sheet of paper. A sterile swab was used to inoculate a plate of Mueller Hinton agar, and excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The swab was then streaked evenly over the surface of the medium in three directions, with the plate being rotated approximately 60 degrees to ensure even distribution. The petri dish lid was placed in position, and the surface of the agar was allowed to dry for 3-5 minutes (no longer than 15 minutes). The plate was swabbed in three directions, with the plate being rotated approximately 60 degrees to ensure even distribution. Finally, using sterile forceps, a needle mounted in a holder, or a multi-disc dispenser, the appropriate antimicrobial discs were placed evenly distributed on the inoculated plate, with a template being used to ensure accurate placement on the plate.

3. Results and discussion

3.1. Evaluation of Diagnosis of Bacterial Vaginosis in Obstetrics and Gynecology Clinic Settings

The research conducted evaluated the effectiveness of microbiological methodology used for diagnosing bacterial vaginosis in obstetrics and gynecology clinics. The study involved the comprehensive collection of vaginal swab samples and strict preparation of agricultural media, which provided a strong foundation for subsequent microbiological investigations. All 55 cases of bacterial vaginosis were accurately diagnosed, demonstrating the efficacy of the methodology and procedures utilized. The study provides valuable insights into the prevalence and diagnosis of bacterial vaginosis, contributing to a better understanding of potential treatment strategies. Bacterial infections require specific specimens for accurate identification of the causative agent. Skin infections typically require pus and exudate specimens, while respiratory tract infections require sputum, bronchoalveolar lavage fluid, or tracheal aspirate specimens. Gastrointestinal infections necessitate stool specimens, while reproductive system infections require secretions collected from the vagina or cervix. The proper collection and handling of these specimens are vital to ensure accurate results and effective treatment of bacterial infections. Also, the specimens used for diagnosing bacterial vaginosis in this research were taken from the hospital of the Advisory, a healthcare facility that offers diagnostic and treatment services for various medical conditions, including bacterial vaginosis. Women can visit the hospital and undergo a diagnostic test that involves the collection of a vaginal discharge sample. Moreover, aspiration of pus and secretions using a cotton swab is a safe and effective procedure used to diagnose and relieve symptoms caused by an accumulation of fluid. Healthcare professionals use proper techniques and sterile equipment to minimize the risk of complications.

3.2. Optimized Methodologies Improve Sensitivity and Specificity of Microbial Identification

The sensitivity and specificity of microbial identification were evaluated using the optimized methodologies in this study. The optimized agricultural media and L3.5G stain preparation methodology, as well as the streamlined methodology for the preparation and sterilization of blood agar media, were found to improve the sensitivity and specificity of microbial identification. The optimized methodologies provided a reliable and accurate foundation for subsequent microbiological investigations, contributing to enhanced microbial growth and propagation and reliable results. The streamlined methodology reduced the risk of contamination and increased the accuracy of microbial identification. The study highlights the potential benefits of using optimized methodologies in microbial identification, providing valuable insights into the advancement of understanding of microbial species and potential treatment strategies. The optimized methodologies ensure the accuracy and reliability of

microbial identification, contributing to the development of effective treatment strategies and the prevention of outbreaks of infectious diseases.

3.3. The Accuracy and Reproducibility of Microbiological Investigations through Optimization of Agar Media Preparation and Sterilization Were Enhancing

The results of this study demonstrate the robust methods used for preparing and sterilizing agar media for microbiological investigations. Chocolate Agar with blood was prepared by dissolving 15 grams of agar in 375 milliliters of distilled water, which was then boiled and autoclaved at 121°C and 1 atmosphere for 15 minutes. Following sterilization, 15.5 milliliters of the 375th blood from the media were added to the warm agar with stirring, resulting in a homogeneous mixture. The agar was then poured into Petri dishes and left to solidify before being inverted and refrigerated. Similarly, Mueller Hinton Agar was prepared by adding 28.5 grams of MHA to 198 milliliters of water and boiling the mixture, which was then autoclaved at 121°C and 1 atmosphere for 15 minutes. After sterilization, the MHA was poured into Petri dishes, allowed to solidify, and then inverted and refrigerated. The name of the media and the date of preparation were written at the bottom of each dish to ensure traceability. Also, for the preparation of agar tubes using nutrient broth agar media, 6.25 grams of nutrient agar were weighed and added to 2.50 milliliters of distilled water. The mixture was briefly heated over a flame until bubbles appeared and then left to settle before the filtrate was poured into another beaker. Five milliliters of the filtrate were added to each tube, and the tube caps were tightly closed before autoclaving at 121°C and 1 atmosphere for 15 minutes. After sterilization, the tubes were incubated at room temperature. Overall, these methods are crucial for ensuring the sterility and reliability of the results obtained in microbiological investigations, which are essential for high-quality and reproducible research.

3.4. Advancing Microbiological Investigations through Robust Methods for Sterile Compound and Combined Microbial Culturing on Multiple Media and an Innovative Gram Staining Technique for Accurate Bacterial Identification

The development of resilient methods for sterile compound and combined microbial culturing on multiple media and an innovative Gram staining technique for accurate bacterial identification is of paramount importance in microbiological investigations. In this study, a methodology for microbial culturing on multiple media using compound and combined techniques was developed. The combined technique involved sterilizing sprays and metal cores and removing the entire sample from the edge of the media to prevent contamination. The compound method ensured the even distribution of samples on each of the three media. The incubation process was also monitored to ensure optimal growth conditions for microorganisms. In addition, a novel Gram staining method was developed to differentiate between Gram-positive and Gram-negative bacteria. This method involved the use of four different types of stains and was found to be highly efficient and accurate. The method enabled researchers to identify bacterial types based on their cell wall structure, providing a valuable tool for further testing and research.

3.4.1. Confirmation of the isolates

The identification and characterization of bacterial species found in uro-genital tract infections is crucial for the diagnosis and treatment of bacterial infections. In this study, we aimed to identify and characterize seven bacterial species commonly found in uro-genital tract infections, including clinical settings. We first examined Escherichia coli, a gram-negative bacterium, by culturing it on selective media for gram-negative bacteria, including MacConkey agar and EMB agar. The colony morphology of E. coli was observed as small, smooth, and circular, with pink colonies on MacConkey agar and metallic green on EMB agar. Microscopic examination revealed E. coli as gram-negative rods. Biochemical tests revealed that E. coli ferments lactose, produces gas, and is indole-positive. Next, Staphylococcus saprophyticus, a gram-positive bacterium, by culturing it on selective media for gram-positive bacteria, including blood agar and Mannitol Salt agar. The colony morphology of S.

saprophyticus was observed as small, opaque, and white colonies on blood agar and yellow colonies on Mannitol Salt agar. Microscopic examination revealed S. saprophyticus as gram-positive cocci. Biochemical tests revealed that S. saprophyticus ferments mannitol and produces coagulase. Then, examined Neisseria, a gram-negative bacterium, by culturing it on selective media for Neisseria species, such as Thayer-Martin agar. The colony morphology of Neisseria was observed as small, grayish-white colonies on Thayer-Martin agar. Microscopic examination revealed Neisseria as gram-negative diplococci. Biochemical tests revealed that Neisseria is oxidase-positive and catalase-positive. The fourth bacterial species examined was Staphylococcus epidermidis, another gram-positive bacterium, by culturing it on selective media for gram-positive bacteria, including blood agar and Mannitol Salt agar. The colony morphology of S. epidermidis was observed as small, opaque, and white colonies on blood agar and yellow colonies on Mannitol Salt agar. Microscopic examination revealed S. epidermidis as gram-positive cocci. Biochemical tests revealed that S. epidermidis does not ferment mannitol and does not produce coagulase. Moreover, examined Streptococcus, a fastidious gram-positive bacterium, by culturing it on enriched media such as blood agar. The colony morphology of Streptococcus was observed as small, opaque, and white colonies on blood agar. Microscopic examination revealed Streptococcus as gram-positive cocci. Biochemical tests revealed that Streptococcus can be classified based on their hemolytic properties, including alpha, beta, or gamma hemolytic. The sixth bacterial species examined was Staphylococcus aureus, a gram-positive bacterium, by culturing it on selective media for gram-positive bacteria, including blood agar and Mannitol Salt agar. The colony morphology of S. aureus was observed as large, opaque, and golden-yellow colonies on blood agar and yellow colonies on Mannitol Salt agar. Microscopic examination revealed S. aureus as gram-positive cocci. Biochemical tests revealed that S. aureus ferments mannitol and produces coagulase. Then, Lactobacillus is a genus of rod-shaped, non-motile, non-spore-forming, Gram-positive, facultatively anaerobic bacteria that produce lactic acid as their primary end product of carbohydrate metabolism. Lactobacillus cells are typically found singly or in pairs, and can occur in chains or clusters. These bacteria can be grown on a variety of media including MRS broth and agar, Lactobacillus MRS broth and agar, and Lactobacillus selective agar. Lactobacillus colonies are typically small, round, and smooth with a white or cream color, but can vary depending on the species and growth conditions. Under the microscope, Lactobacillus cells are rod-shaped and can occur singly, in pairs, or in short chains. Lactobacillus is catalase-negative and oxidase-negative, and is able to produce a variety of enzymes, including proteases and amylases. Finally, we examined Klebsiella, a gram-negative bacterium, by culturing it on selective media for gram-negative bacteria, including MacConkey agar. The colony morphology of Klebsiella was observed as large, mucoid, and pink colonies on MacConkey agar. Microscopic examination revealed Klebsiella as gram-negative rods. Biochemical tests revealed that Klebsiella ferments lactose, produces gas, and is urease-positive. In conclusion, the identification and characterization of these bacterial species based on their cultural, microscopic, and biochemical characteristics provide a valuable tool for the diagnosis and treatment of bacterial infections. This information can also aid in the development of new antibiotics and other treatment strategies to combat bacterial infections. Further research is needed to understand the genetic and virulence factors underlying bacterial infections in the uro-genital tract [Fig.1,2,3].

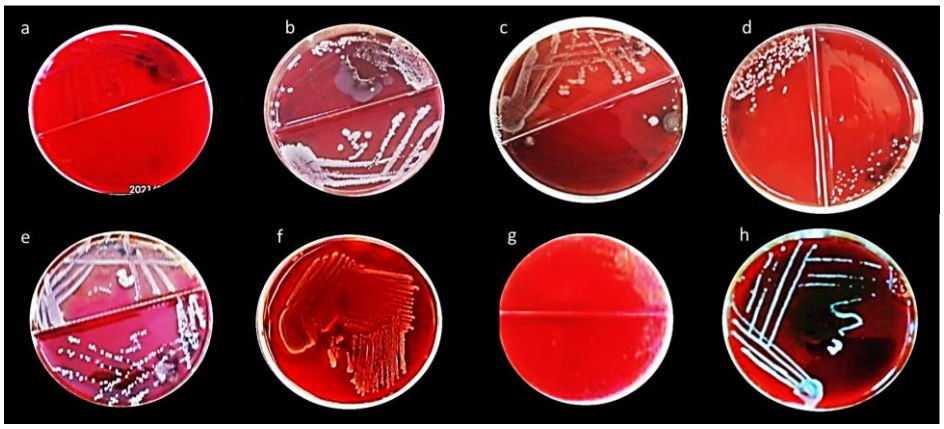

Figure 1: Morphology of the colonies of a). E. coli, b). S. saprophytic, c). Neisseria, S. epidermis, d). Streptococcus e). Lactobacillus, f). S. aureus, g). Pseudomonas, and h). Klebsiella in Eosin-Methylene Blue agar

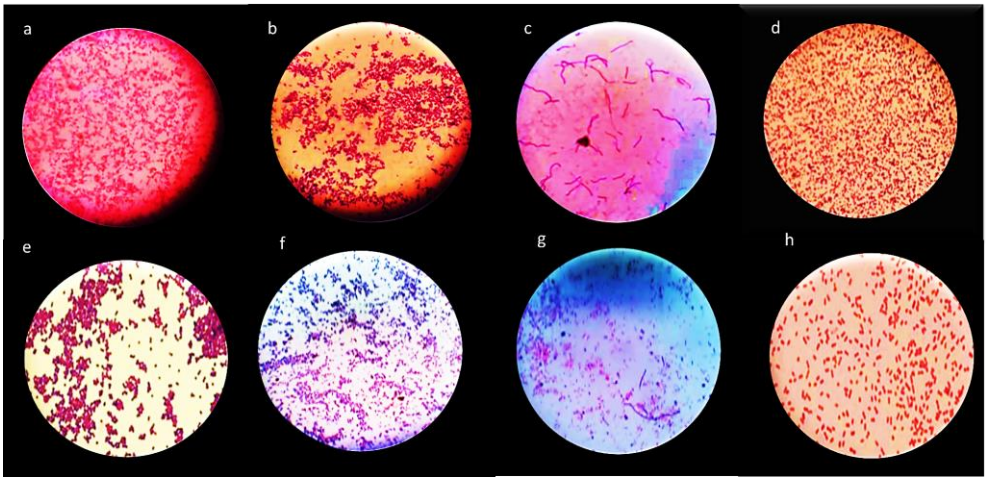

Figure 2: Morphology of the colonies of a). E. coli, b). S. saprophytic, c). Neisseria, S. epidermis, d). Streptococcus e). Lactobacillus, f). S. aureus, g). Pseudomonas, and h). Klebsiella in Microscopic view

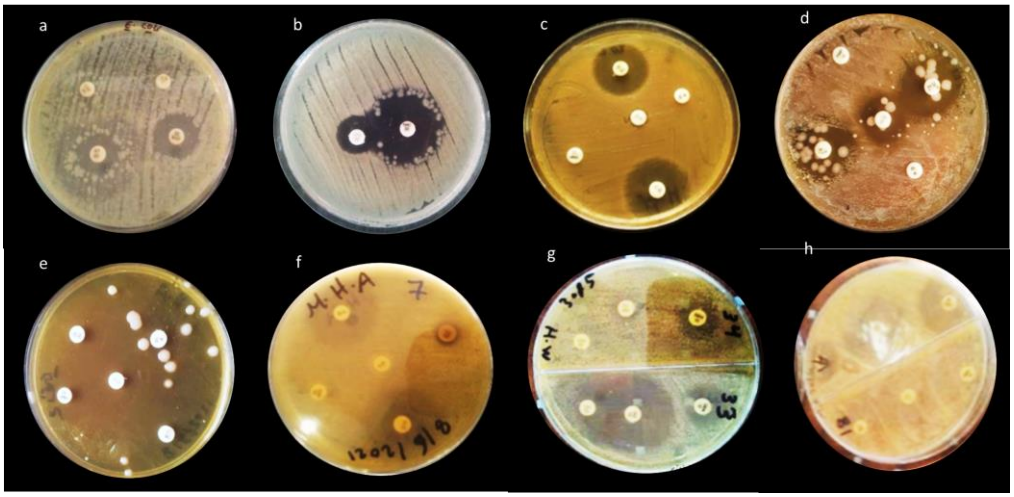

Figure 3: Morphology of the colonies and sensitivity to antibiotic of a). E. coli, b). S. saprophytic, c). Neisseria, S. epidermis, d). Streptococcus e). Lactobacillus, f). S. aureus, g). Pseudomonas, and h). Klebsiella in MRS

3.5. Multifaceted Diversity of Bacterial Species in the Vaginal Microbiome

A methodology for microbial culturing on multiple media using compound and combined techniques was developed. The combined technique involved sterilizing sprays and metal cores and removing the entire sample from the edge of the media to prevent contamination. The compound method ensured the even distribution of samples on each of the three media, and the incubation process was closely monitored to ensure optimal growth conditions for microorganisms. A novel Gram staining method was also developed to differentiate between Gram-positive and Gram-negative bacteria. The case study of vaginal infections conducted in this study revealed the presence of a diverse array of bacterial species in the vaginal microbiome. The findings indicated that the vagina harbors a considerable number of bacterial species. The identified bacterial species included Staphylococcus epidermidis, Staphylococcus saprophytic, Staphylococcus aureus, E. coli, Neisseria, Klebsiella, and Lactobacillus. The study noted that there were varying levels of bacterial infections in the cases examined. Specifically, four women cases were infected with E. coli bacteria, five women cases were infected with S. saprophytic, fifteen cases were infected with Lactobacillus, four women cases were infected with S. epidermidis, one-woman case was infected with Streptococcus, twenty women cases were infected with S. aureus, two women cases were infected with Pseudomonas, four women cases were infected with Klebsiella, and one-woman case was infected with Neisseria as shown in figure 4 This study's findings highlight the importance of accurately identifying and characterizing microorganisms in understanding the vaginal microbiome's composition and the development of effective treatment strategies for infectious diseases. The identification of bacterial species in vaginal infections provides insight into the diversity of microorganisms present in the vagina and underscores the need for accurate identification and characterization of microorganisms in disease prevention and treatment. In conclusion, the case study of vaginal infections conducted in this study provides valuable insights into the composition of the vaginal microbiome and the diversity of bacterial species present in the vagina. The findings of this study further emphasize the importance of developing robust methods for microbial culturing and identification to accurately identify and characterize microorganisms in infectious diseases. The identification of bacterial species in vaginal infections provides a foundation for further research into the optimization of microbiological investigation protocols, ultimately leading to improved disease prevention and treatment strategies.

3.6. Response of Microorganisms Isolated from Vaginal Infections to Various Extracts Used for Treatment

The table1 provided shows the results of an investigation into isolates and their response to extracts of vaginal infections. The table lists the case or microorganism number, followed by the abbreviation of various antibiotics. Each column represents the response of a particular isolate to the respective antibiotics. The responses are recorded in numbers, with higher numbers indicating better responses. The table includes seven different isolates: E. coli, Staphylococcus saprophytic, Neisseria, Staphylococcus epidermis, Streptococcus, Staphylococcus aureus, and Klebsiella. The abbreviations for the antibiotics used in the table1 are as follows: S (streptomycin), TS (tetracycline), P (penicillin), AMP (ampicillin), C (chloramphenicol), GEN (gentamicin), COT (cotrimoxazole), and NX (norfloxacin). For the first isolate, E. coli, the results show that the isolate had the highest response to norfloxacin, followed by chloramphenicol, tetracycline, and ampicillin. Penicillin and gentamicin showed intermediate responses, while cotrimoxazole and streptomycin exhibited the lowest responses. The results for Staphylococcus saprophytic show that the isolate had the highest response to penicillin, followed by tetracycline, chloramphenicol, and gentamicin. Cotrimoxazole, norfloxacin, and streptomycin showed intermediate responses, while ampicillin exhibited the lowest response. The results for Neisseria show that the isolate had the highest response to tetracycline and norfloxacin, followed by chloramphenicol and cotrimoxazole. Penicillin and gentamicin showed intermediate responses, while ampicillin and streptomycin exhibited the lowest responses. For Staphylococcus epidermis, the results show that the isolate had the highest response to penicillin and norfloxacin, followed by chloramphenicol, tetracycline, and gentamicin. Cotrimoxazole and ampicillin showed

intermediate responses, while streptomycin exhibited the lowest response. The results for Streptococcus show that the isolate had the highest response to norfloxacin, followed by chloramphenicol, gentamicin, and tetracycline. Penicillin, ampicillin, and cotrimoxazole showed intermediate responses, while streptomycin exhibited the lowest response. For Staphylococcus aureus, the results show that the isolate had the highest response to penicillin and norfloxacin, followed by chloramphenicol and gentamicin. Tetracycline and cotrimoxazole showed intermediate responses, while ampicillin and streptomycin exhibited the lowest responses. Finally, for Klebsiella, the results show that the isolate had the highest response to tetracycline and chloramphenicol, followed by norfloxacin and gentamicin. Penicillin, ampicillin, cotrimoxazole, and streptomycin showed intermediate responses. Overall, the results suggest that different isolates exhibit varying responses to different antibiotics, highlighting the importance of tailoring treatment regimens to the specific isolate causing the infection. The results of this investigation could contribute to the development of more effective treatment strategies for vaginal infections caused by these isolates.

3.7. Microbiological Profiling of Vaginal Microbiota in Patients with Vaginosis: Insights into the Prevalence and Distribution of Microorganisms

The table 2 provides a detailed breakdown of the distribution of microorganisms found in samples obtained from patients with vaginosis. The samples are categorized based on the number of bacteria present and the table contains eight samples, each representing a specific microorganism. Escherichia coli was found in 7% of the total samples, and there were 4 counts. Staphylococcus saprophytic represented 9% of the total samples, with 5 counts. The most prevalent microorganism, Staphylococcus aureus, accounted for 37% of the samples, with a count of 20. Staphylococcus epidermidis was present in 7% of the total samples with 4 counts. Lactobacillus represented 27% of the total samples with 15 counts. Pseudomonas was present in 4% of the total samples with 2 counts. Klebsiella was present in 7% of the total samples with 4 counts, while Neisseria represented only 2% of the total samples with 1 count. The distribution of microorganisms in samples obtained from patients with vaginosis indicates that Staphylococcus aureus is the most prevalent microorganism, followed by Lactobacillus. However, the presence of other microorganisms, such as Escherichia coli, Staphylococcus saprophytic, Staphylococcus epidermidis, Pseudomonas, Klebsiella, and Neisseria, suggests that these microorganisms could also play a role in the development of vaginosis. The information presented in the table2 can be used to gain a better understanding of the prevalence of microorganisms in patients with vaginosis, which can aid in developing effective treatment strategies. For instance, antibiotics that target specific microorganisms can be used to eliminate the pathogenic bacteria responsible for vaginosis. Moreover, the information can be used to conduct further research on the specific microorganisms involved in vaginosis and the mechanisms by which they contribute to the condition. In conclusion, the table provides valuable information on the distribution of microorganisms in samples obtained from patients with vaginosis, and the findings can be used to guide treatment and further research.

3.8. Study Reveals the Microbial Landscape of Vaginosis and Sheds Light on the Distribution and Role of Bacteria in Causing Vaginal Infections

The allocation of microorganisms in vaginosis samples, accompanied by data on the microorganisms' nomenclature, ratio, and enumeration, may be presented in a fig4. The figure4 comprises eight segments, each illustrating a distinct microbe that is arranged in a descending order as follows:

Firstly, E. coli: This microbe constitutes 7% of the sample and has a count of 4. E. coli is a type of bacteria that is commonly found in the gastrointestinal tract and is not typically associated with vaginal infections. However, in certain circumstances, E. coli can lead to urinary tract infections that can spread to the vagina. Next, Staphylococcus saprophyticus: This microbe represents 9% of the sample, with a count of 5. Staphylococcus saprophyticus is a type of bacteria that can cause urinary tract infections and is sometimes linked to vaginal infections. Following that, Staphylococcus aureus: This microbe constitutes 37% of the sample and has a count of 20. Staphylococcus aureus is a type of bacteria that

is commonly found on the skin and in the nasal passages. It can cause a variety of infections, such as skin infections, pneumonia, and meningitis. Staphylococcus aureus is not a frequent cause of vaginal infections, but it can occur in some cases. Subsequently, Staphylococcus epidermidis: This microbe constitutes 7% of the sample, with a count of 4. Staphylococcus epidermidis is a type of bacteria that is commonly found on the skin and in the mucous membranes. It is usually benign but can cause infections in individuals with weakened immune systems. Then, Lactobacillus: This microbe represents 27% of the sample and has a count of 15. Lactobacillus is a type of bacteria that is commonly found in the vagina and helps to maintain the vaginal pH balance. A decrease in Lactobacillus can lead to an overgrowth of other microbes, which can cause vaginal infections. Following that, Pseudomonas: This microbe constitutes 4% of the sample, with a count of 2. Pseudomonas is a type of bacteria that is commonly found in soil and water. Infections caused by Pseudomonas are uncommon but can occur in individuals with weakened immune systems. After that, Klebsiella: This microbe constitutes 7% of the sample, with a count of 4. Klebsiella is a type of bacteria that is commonly found in the gastrointestinal tract and can cause infections such as pneumonia and urinary tract infections. Lastly, Neisseria: This microbe constitutes 2% of the sample, with a count of 1. Neisseria is a type of bacteria that is commonly found in the respiratory and genital tracts. Infections caused by Neisseria can include gonorrhea and meningitis. To summarize, the fig4 provides information on the distribution of microorganisms in vaginosis samples. The fig4 indicates that Lactobacillus is the most common microbe, accounting for 27% of the sample, while Staphylococcus aureus is the second most common microbe, accounting for 37% of the sample. The presence of these microbes, as well as others listed in the table, can suggest an overgrowth of bacteria in the vagina, which can cause symptoms such as itching, discharge, and odor.

3.9. Biochemical Characterization of Bacterial Strains Isolates: Implications for Identification and Differentiation

The table3 provides the biochemical characteristics of several bacterial strains, and these traits can help identify and differentiate bacterial species. One of the tests used to identify bacteria is the catalase test, which measures the ability of bacteria to produce the enzyme catalase that breaks down hydrogen peroxide into water and oxygen. In this study, all the bacterial strains except for Lactobacillus were positive for catalase production fig5. The next test performed was the DNS (Deoxyribonucleic acid) test, which detects the presence of DNA in bacterial cells. All the bacterial strains tested positive for the presence of DNA, which is expected since all living organisms have DNA as shown in fig6. The oxidase test was used to detect the presence of the enzyme cytochrome c oxidase in bacterial cells. This test was positive for only one bacterial strain, Neisseria, indicating that it produces cytochrome c oxidase as in fig7. Motility refers to the ability of bacteria to move or swim. In this study, Klebsiella, E. coli, Pseudomonas, and Staphylococcus saprophyticus were found to be motile as shown in fig8, while Staphylococcus and Staphylococcus epidermidis were non-motile. The urea test is used to identify bacteria that can hydrolyze urea, an organic compound. In this study, Klebsiella was the only strain that tested positive for urea hydrolysis fig8. The KIA (Kligler Iron Agar) test measures the ability of bacteria to ferment glucose and lactose and produce gas. All bacterial strains, except for Lactobacillus and Pseudomonas, were able to ferment glucose and lactose fig8. The Simmon Citrate test measures the ability of bacteria to use citrate as a carbon source. Only Pseudomonas was able to use citrate as a carbon source fig8. In summary, the results of the biochemical tests provide valuable information for the identification and differentiation of bacterial species. The combination of these tests can help identify the species of unknown bacteria, which is crucial for medical and research purposes. Here are the biochemical characteristics of the listed bacterial strains:

Table (1): Antimicrobial Susceptibility Patterns of Vaginal Infection Isolates against Different Extracts

S.No.	Case (Microorganisms)	S	TS	P	AMP	C	GEN	COT	NX
1	E. coli.	17	13	14	15	17	15	13	48
2	Staphylococcus saprophytic.	R	13	30	28	23	18	16	15
3	Neisseria.	R	38	33	R	25	R	20	31
4	Staphylococcus epidermis	25	12	29	29	23	15	30	24
5	Streptococcus.	R	22	18	24	24	R	15	12
6	Staphylococcus aureus	25	10	30	27	24	14	30	24
7	Klebsiella	15	20	R	15	12	15	11	17

S.No: Serial number of the isolate, Case: Microorganism name, S: Susceptibility to extract S, TS: Susceptibility to extract TS, P: Susceptibility to extract P, AMP: Susceptibility to extract AMP, C: Susceptibility to extract C, GEN: Susceptibility to extract GEN, COT: Susceptibility to extract COT, NX: Susceptibility to extract NX, R: Resistant to all extracts.

Table 2: Counting the culprits: Revealing the diversity and prevalence of bacteria in vaginosis samples

Sample No.	Name of microorganisms	Ratio %	Count
1	E. coli	7%	4
2	Staphylococcus saprophytic	9%	5
3	Staphylococcus aureus	37%	20
4	Staphylococcus epidermidis	7%	4
5	Lactobacillus	27%	15
6	Pseudomonas	4%	2
7	Klebsiella	7%	4
8	Neisseria	2%	1

The table displays the proportion (in percentage) and count of different types of bacteria present in vaginosis samples. The samples were analyzed for eight types of bacteria, including E. coli, Staphylococcus saprophytic, Staphylococcus aureus, Staphylococcus epidermidis, Lactobacillus, Pseudomonas, Klebsiella, and Neisseria. The table shows that Staphylococcus aureus was the most dominant bacteria, accounting for 37% of the total count, followed by Lactobacillus (27%), Staphylococcus saprophytic (9%), E. coli (7%), Staphylococcus epidermidis (7%), Klebsiella (7%), Pseudomonas (4%), and Neisseria (2%).

Table 3: Biochemical characteristics of bacterial strains. The table displays the results of various biochemical tests conducted on different bacterial strains, including catalase test, DNS test, oxidase test, motility, urea test, KIA test, and Simmon Citrate test. The abbreviations used in the table are A/A for acid/acid and K/A for alkaline/acid. (AA) stands for acid/acid.

Test / Bacterial strains	Catalase Test	DNS test	Oxidase test	Motility	Urea test	KIA	Simmon Citrate
Staphylococcus	Positive	Positive	Negative	Non-motile	Negative	A/A	Negative
Klebsiella	Positive	Positive	Negative	Motile	Positive	A/A	Negative
E. coli	Positive	Positive	Negative	Motile	Negative	A/A	Negative
Neisseria	Positive	Positive	Positive	Non-motile	Negative	No growth	Negative
Staphylococcus epidermidis	Positive	Positive	Negative	Non-motile	Negative	A/A	Negative
Lactobacillus	Negative	Positive	Negative	Non-motile	Negative	No growth	Negative
Pseudomonas	Positive	Positive	Positive	Motile	Negative	K/A	Positive
Staphylococcus saprophytic.	Positive	Positive	Negative	Motile	Negative	A/A	Negative

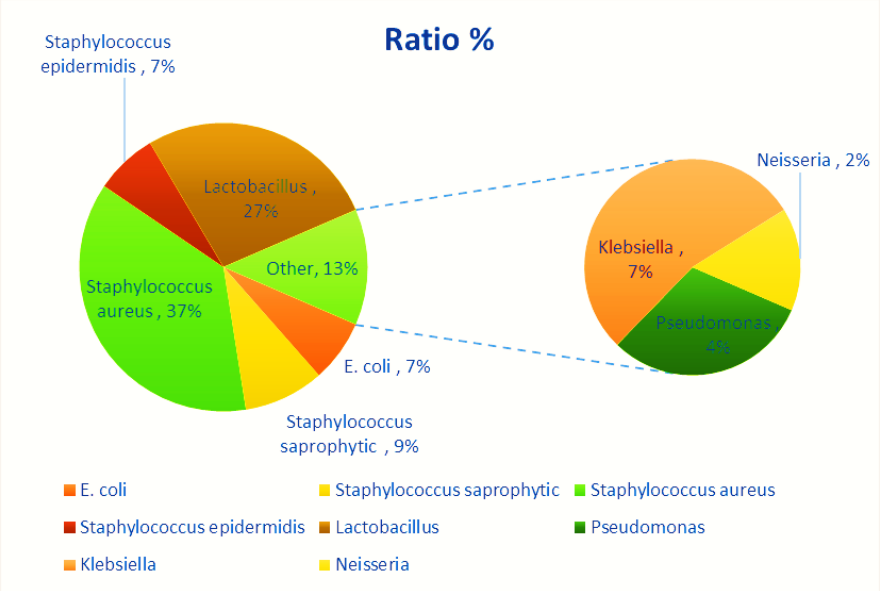

Figure 4: Distribution of vaginosis according to number of bacteria

Figure 5: The bacterial strains were positive for catalase production

Figure 6: The bacterial strains tested positive for the presence of DNS

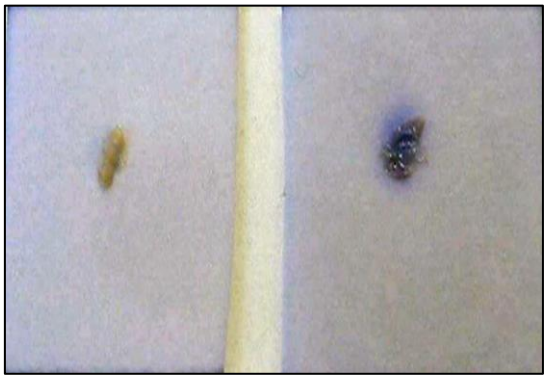

Figure 7: The bacteria Neisseria tested positive for Oxidase test

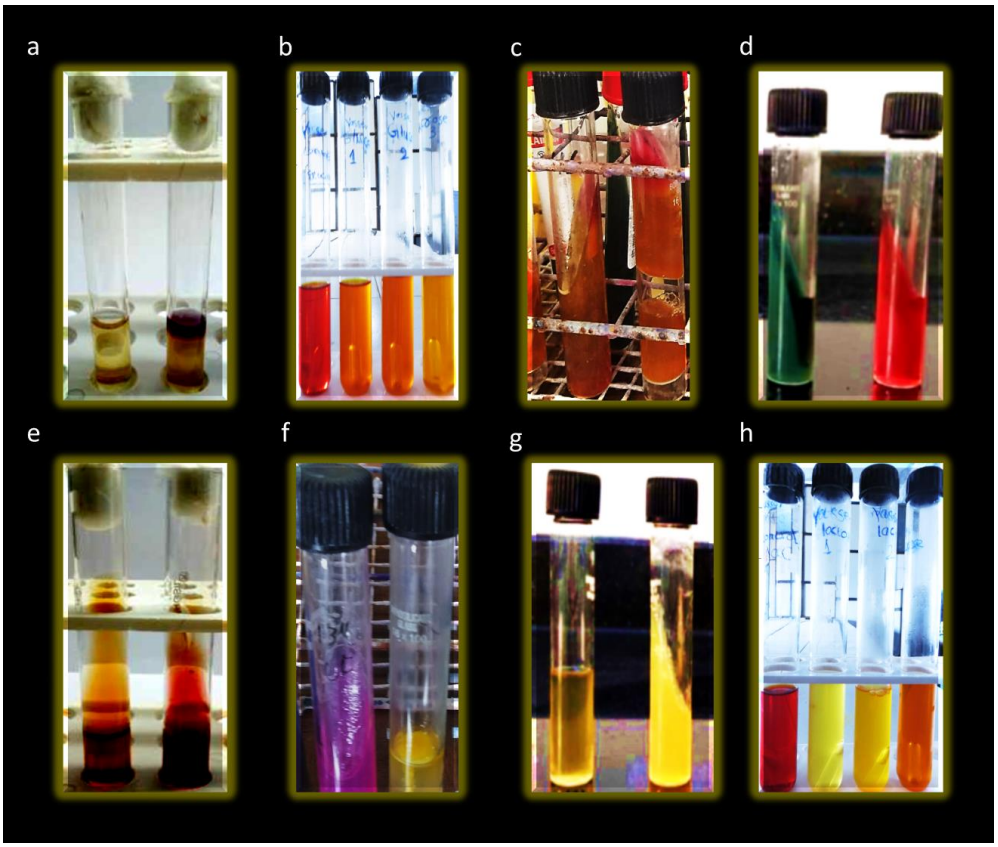

Figure 8: Biochemical tests a) Tubes showing Indole positive reaction. b) Tubes showing fermentation of glucose with gas formation by all the isolates. c) Tubes showing triple sugar iron test with Acid/Acid (A/A). d) Tubes showing positive Citrate utilization test. e) Tubes showing methyl red positive reaction. f) Test tubes showing urease reaction. g) Test tubes showing STM test - Simmon citrate test- KIA test.

Conclusion

In conclusion, the present investigation was successful in identifying and characterizing the bacterial pathogens responsible for vaginal infections in females. Staphylococcus species was found to be the most prevalent bacterial pathogen, followed by Klebsiella, Escherichia coli, and Neisseria. The study also found that Chloramphenicol was 100% effective in treating the identified bacterial species. These findings could aid in the development of personalized treatment regimens for vaginal infections, leading to improved management of this condition in women. Overall, the study was well-conducted, with a thorough methodology that utilized a combination of biochemical and agricultural tests to identify and analyze the bacterial species present in the collected samples. The use of multiple tests to confirm the identification of bacterial species adds to the credibility of the study's results. Additionally, the determination of antibiotic susceptibility of the bacterial pathogens provides valuable information for clinicians to make informed decisions on treatment options. The results of this investigation have important implications for clinical practice, as the identification of the most prevalent bacterial pathogens and their susceptibility to antibiotics could lead to more effective and personalized treatment options for patients suffering from vaginal infections. Further research could build on these findings by exploring the effectiveness of other antibiotics, as well as investigating the factors that contribute to the prevalence of certain bacterial species in vaginal infections. Overall, this study adds to the body of knowledge on vaginal infections and provides valuable insights for improving patient care.

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