**ISOLATION AND SENSITIVITY OF BACTERIA ISOLATE FROM VAGINAL DISCHARGE TO ANTIBIOTICS**

**BY**

**GIDEON PLANGNAN DAGIN**

**U14/NAS/MCB/054**

**DEPARTMENT OF MICROBIOLOGY,**

**FACULTY OF NATURAL AND APPLIED SCIENCES**

**GODFREY OKOYE UNIVERSITY UGWOMU NIKE,**

**ENUGU STATE.**

**JULY, 2018**

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**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,**

**FACULTY OF NATURAL AND APPLIED SCIENCES**

**GODFREY OKOYE UNIVERSITY UGWOMU NIKE,**

**ENUGU STATE**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE(B.Sc) DEGREE IN MICROBIOLOGY**

**SUPERVISOR**

**PROF. E. A. EZE**

**JULY, 2018**

**APPROVAL**

This project has been presented to and approved by Godfrey Okoye University, Enugu. In partial fulfillment of yhe requirement for the award of bachelor of Science (B.Sc), in microbiology from the department of Microbiology

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Head of Departmen.

**DEDICATION**

I dedicate this work to God Almighty, who has given me strength to keep going going.

And to my parents Mr and Mrs Gideon Dagin.

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I am eternally greatfull to God Almighty for granting me the previlage to undertake this programme and bringing me to a successful end.

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**ABSTRACT**

Normal vaginal flora contains a wide range of microorganisms. Bacterial vaginosis BV is the main reason of vaginal discharge. Many gram positive and gram negative rods i.e. *E.coli, Klebsiella*, *Proteus, Acinetobacter* and *Pseudomonas* spp. are major contributors in bacterial vaginosis. Aim: The present study was conducted to elucidate the frequency of various bacteria in high vaginal swabs and sensitivity pattern of bacteria to antibiotics that are currently used Material and Methods used are a total of 6 High vaginal swabs (HVS) which were collected from patients presenting with symptoms of vaginal discharge. Swabs were inoculated on blood and Chocolate agar. After overnight incubation plates were examined for growth, colonial morphology, final confirmation was done on the basis of biochemical testing. Antibiotic sensitivity testing was done by (modified Kirby-Bauer’s) disc diffusion method using amikacin(30μg), ampicillin(10μg), amoxicillin(10μg),) clavulanic acid, imipenem(10μg), ciprofloxacin(10μg), and cefixime(5μg). After overnight incubation plates were examined to read the susceptibility zone. Results showed that Highly sensitive antibiotics against bacteria were imipenem (27mm), and Ciprofloxacin (28mm) whereas least affective antibiotics against gram negative rods were penicillins, amikacin due to indiscriminate use of antibiotics. In conclusion, high prevalence of gynecological infections demands that the patients who have *vaginosis* must be investigated regularly and carefully through culture and identification of causative bacteria. Emergence of antibiotic resistance must be controlled in order to avoid improper use, frequent abuse, insufficient dosages, trouble-free availability of antibiotics and treatment schedule must be designed subsequent to proper laboratory investigations.

**CHAPTER ONE**

**1.1 INTRODUCTION**

**1.2 Background of the Study**

Antimicrobial resistance is a global concern, particularly pressing in developing nations where infectious diseases, poverty and malnutrition are endemic. Infections caused by resistant bacteria have been shown to be more frequently associated with increased morbidity and mortality than those caused by susceptible pathogens. In areas of concentrated use, such as hospitals, antimicrobial resistance lead to hospital stays, increased health care costs and in extreme cases untreatable infections. The lack of clinical microbiology laboratories to identify the specific etiologic agents and their antimicrobial susceptibility testing has increased empirical therapy which in turn leads to emergence of AMR. Moreover, self-antibiotic prescription, lack of access to local antibiogram data and poor awareness of prescriber about AMR were the leading local factors for AMR development in Ethiopia (Abera*et al*., 2014).

Studies have shown that besides the temporal changes in profile of infecting microorganisms and pattern of resistance over time, antimicrobial resistance profile of bacteria varies among population because of difference in geography, local antimicrobial prescribing practices and prevalence of resistant bacterial strains. Such differences are never stable and may change rapidly especially in places where misuse of antibiotics are common particularly in developing countries. A systematic review in Ethiopia has also indicated a trend towards an increasing resistance rates among pathogens such as *Escherichia coli*, P*roteus, Klebsiella, Pseudomonas,Citrobacter* and A*cenotobacter* to commonly prescribed antibiotics, including Ampicillin, Amoxicillin, Amikasin, Imipenem, Cefixime and Ciprofloxacin (Moges *et al.,*2014). Thus, up to date information on microbial resistance is needed at local level to guide the rational use of the existing antimicrobials.

The adult human vagina is a complex biota containing a profusion of microorganisms. These can be either unicellular or multicellular and are present everywhere in nature. They include bacteria, fungi, archaea, protists, some microscopic plants such as green algae and animals such as planktons and palanarian. On account of their nature, viruses may or may not be included. Bacteria and yeast form normal flora of this ecosystem, which is normally found on the skin and every opening of the body such as mouth, ears, rectum and vagina. Even a neonate carries specific flora of his/ her mother and soon develops own floral community. This flora persists till death of the individual. An adult human carries normal flora consisting of more than 200 bacterial species. Normally these are harmless and are involved in benefiting their hosts. Yet some are parasitic in nature, living at the expense of their host, and some are even pathogenic.These pathogenic microbes, after getting a chance, invade their hosts and lead to opportunistic infection. These diseases caused by normalflora are termed endogenous diseases *(Khan et at.,*2002).

Resistance of bacteria to antimicrobial agents is an imminent threat to patient management all over the world. This issue has plagued policy makers and clinicians everywhere but there seems to be no simple way of circumventing the problem. Rapidly rising antibiotic resistance is a challenge to comprehensive patient care in all branches of medical science. The interaction between various clinical bacteria and the antimicrobial agents is a complex issue involving the prokaryotic adaptive mechanisms and genetic changes. This complex interaction must be studied in depth in order to achieve a sustainable and effective solution to the looming threat of antibiotic resistance. Earlier, the problem of antibiotic resistance was primarily a concern for not so comical infections. But now, even community acquired infections are caused by organisms with high levels of antibiotic resistance. As a report had demonstrated, such multi-drug resistant community acquired infections can be a cause of significant.

Earlier, such drug resistant organisms were said to infect mainly patients with identifiable risk factors or profound immune suppression. But now, reports are showing such infections in seemingly normal healthy persons. Also, such drug-resistant infections may complicate the newly emerging infectious diseases. For example, influenza epidemics are sometimes reported to be complicated by superadded infection with drug-resistant bacteria (Hageman *et al*., 2004). The issue of drug resistance in clinical bacteria is such a vital threat that the UN held a special assembly in 2016 to address only this issue. In that assembly, the issue was said to be of as much importance as climate change and it was deemed to require a global response (Farr, 1994) and non-pregnant women attending the University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria”.

**1.3 Antibiotic Sensitivity**

Antibiotic sensitivity is a term used to describe the susceptibility of bacteria to antibiotics. Antibiotic sensitivity testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection  *in vivo*. Testing for antibiotic sensitivity is often done by the Kirby-Bauer method while other methods include the Stokes method, E-test (also based on antibiotic diffusion) and Agar and Broth dilution methods (for Minimum Inhibitory Concentration determination). Muller Hinton agar is most frequently used in this antibiotic susceptibility test. Our study was aimed at the isolation, identification and antibiotic sensitivity testing of URINARY TRACT INFECTION (UTI) causing bacteria

**1.4 AIM**

To isolate, identify and check antibiotic sensitivity of bacteria implicated in URINARY TRACT INFECTION (UTI)s.

**1.5 OBJECTIVES OF THE STUDY**

* To isolate bacteria from the urinary tract of women of child-bearing age using vaginal swab.
* To identify the isolated bacteria.
* To determine the antibiotic sensitivity of bacteria isolated.

**CHAPTER TWO**

**2.1 LITERATURE REVIEW**

**2.2 Origin of antibiotic resistance**

Antibiotic resistance was reported to occur when a drug loses its ability to inhibit bacterial growth effectively. Bacteria become ‘resistant’ and continue to multiply in the presence of therapeutic levels of the antibiotics. Bacteria, which replicate even in the presence of the antibiotics, are called resistant bacteria.

Antibiotics are usually effective against them, but when the microbes become less sensitive or resistant, it requires a higher than the normal concentration of the same drug to have an effect. The emergence of antimicrobial resistance was observed shortly after the introduction of new antimicrobial compounds. Antibiotic resistance can occur as a natural selection process where nature empowers all bacteria with some degree of low-level resistance. For example, one study confirmed that ampicillin and tetracycline that were commonly used in yesteryears, but now have no longer role in treating non-cholera diarrhea disease in Thailand (Hoge *et al*.,1998). At the same time, another study conducted in Bangladesh showed the effectiveness of the same drugs in treating them effectively (Rahman *et al*., 2017). In fact, resistance was documented even before the beginning of the usage of the antibiotics in fighting the infection (Abraham and Chain, 1940). Non-judicial use of antibiotic is responsible for making microbes resistant. Since the introduction of sulfonamides in 1937, the development of specific mechanisms of resistance had provoked their therapeutic use. However, sulfonamide resistance was reported in the 1930s, which reveals the same mechanism of resistance that still operates even now, more than 80 years later). Within six years of the production of the aminoglycosides, aminoglycoside-resistant strains *of Staphylococcus aureus* was developed (Gootz , 1990). Introduced in 1961, Methicillin was the first of the semisynthetic penicillinase-resistant penicillin to target strains of penicillinase-producing Staphylococcus aureus. However, resistance to methicillin was reported soon after its initiation (Br Med , 1961). Further, although fluoroquinolones were introduced for the treatment of Gram-negative bacterial diseases in the 1980s, fluoroquinolones resistance later revealed that these drugs were also used to treat Gram-positive infections. Most recently, the clinical isolates of Vancomycin-resistant *Staphylococcus aureus* (VRSA) were found in 2002, after 44 years of Vancomycin introduction to the market. Antibiotics used in agriculture are often the same or similar to antibiotic compounds used clinically (McEwen and FeDorka-Cray , 2002), this over-usage could also invite drug resistance. The food chain can be considered the main route of transmission of antibiotic-resistant bacteria between animal and human populations (Witte , 1998). In some developed countries, animals receive antibiotics in their food, water, or parenterally which may be responsible for carrying microbe resistance to that specific antibiotic (McEwen and Fe Dorka-Cray, 2002). For example, the use of antibiotics in cattle feed as growth promoters increase antibiotic resistance (Levy , 1993). Recent evidence suggests that poultry or pork might be a possible source of quinolone resistant-*Escherichia coli* in the rural villages in Barcelona, where one-fourth of children were found to be fecal carriers of these organisms. However, these kids were never exposed to quinolones (Garan, *et al*.,1998)

**2.3 Development of antibiotic resistance**

Antibiotics fight to eliminate bacteria. Hence, bacteria tend to have a natural process that encourages resistance. The resistance process occurs via gene level mutations. (Laxminarayan and Brown, 2001). Antibiotics induce selective pressure and the genes act in association with selective pressure (Levy, 1993). Bacteria possess the quality to directly transfer genetic material between each other by transferring plasmids, which signifies that natural selection is not the only mechanism by which resistance evolves. Broad spectrum antibiotics are prescribed in hospitals as a solution for nosocomial infections; however, it increases resistance (Lowey, 2003).

Antibiotics can generally eliminate the majority of bacteria in a colony. However, there may exist a different colony of bacteria that are genetically mutated which can lead to resistance (Alanis, 2005). The level of antibiotic-resistant infections was found to be strongly correlated with the degree of antibiotic consumption (Goossens, *et al*., 2005). Development of resistance may also likely to occur if users fail to take their full course of prescribed antibiotic treatment. The bacteria subsequently remain untouched gaining more strength against the antibiotics (Levy, 1993). Bacteria may collect multiple resistance traits over time and can become resistant to multiple classes of antibiotics (Avon, *et al*., 2001). For example, resistance was found in *Staphylococci* from the chromosomal mutations, ineffective transport of aminoglycosides into the bacteria as well as enzyme modification (Lowey, 2003). A single antibiotic may not only select resistance to one particular drug. Resistance can occur with other structurally related compounds of the same class. For example, resistance to tetracycline may incur resistance to oxytetracycline, chlortetracycline, doxycycline, and minocycline (Chopra and Roberts, 2001). Antimicrobials possessed resistance genes that defend their antimicrobial products and these genes developed antibiotic resistance even long ago before the antibiotic started working for treatment purpose (Chadwick and Goode, 1997).

**2.4 Colonization and Transmission of urinary tract infection (UTI)**

Urinary tract is regularly flushed with sterile urine and its acidity, there it makes difficulty for microbes to access and establish here but anterior urethra is inhabited by relatively constant flora such as *Staphylococcus epidermidis, Enterococcus faecalis* and some Alpha-hemolytic streptococci (Pubus and Enderdonk, 1999)

Occasionally, some enteric bacteria like *E. coli, Proteusand* corynebacteria may be found there. Vagina is an available space for certain microbes. It is colonized with Corynebacteria, Staphylococci, Streptococci*, E. coli*, and Doderlein’s bacillus *(Lactobacillus acidophilus).*

. During reproductive life vaginal epithelium contains glycogen which is metabolized by *Lactobacillus acidophilus*. The lactic acid and other products of metabolism inhibit colonization of offending agents in that area and allow only *Lactobacillus acidophilus* and some other lactic acid producing species to grow(Noskan *et at.,* 2005)

Oral contraceptives, steroids, and antibiotics disrupt either the normal flora or naturally acidic Phof the urinary tract. Reduction in lactic acid leads to high pH of vaginal epithelium which encouragesother offending agents to grow and results in embarrassing vaginal odour (sometimes described as smelling “fishy”), abnormal discharge (often thin and white-grey in colour) and discomfort (normally irritation or soreness in and around vagina). Vaginal discharge is a term given to the biological fluids contained within or expelled from vagina. Normally this discharge demonstrates various phases of menstrual cycle but it may also be due to vaginal infections. That is why vaginal infection is checked by the presence or absence of these offending microorganisms in a vaginal discharge. The three major kinds of vaginitis are vaginal candidiasis*,* bacterial vaginosis(BV) and Trichomoniasis. At a time, a woman may have any vaginal co-infections. Untreated vaginal infection may lead to any complication especially in pregnant women (Momoh et tal., 2007)

**2.5 Consequence of antibiotic resistance**

Antibiotic resistant organisms are known as superbugs. These are not only a laboratory concern but have become a global threat responsible for high death tolls and life-threatening infections (Lipp, *et al.,* 2002). Consequences of these infections are aggravated enormously in volatile situations such as civil unrest, violence, famine and natural disaster (Fact Sheet, 2015). World Health Organization (WHO) (Fact Sheet, 2015) has warned that a post-antibiotic era will result in frequent infections and small injuries may result in death if we fail to act against antibiotic resistance; Multi-drug resistant bacteria causing more deaths worldwide. More than 63,000 patients from the United States of America (USA) die every year from hospital-acquired bacterial infections (Aminov and MackieI, 2007). Every year, an estimated 25,000 patients die due to multiple drug resistance (MDR) bacterial infections in Europe (Freire-Moran ,*et al.,* 2011). Many countries are facing the burden of nosocomial Staphylococcus aureus (S. *Aureus*) infections as waves of clonal dissemination. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are rapidly spreading globally (Lowey, 2003). Estimated costs due to multidrug-resistant bacterial infection might result in extra healthcare costs and productivity losses (Freire-moran, *et al.,* 2011). It has been a standard practice for most of the pharmaceutical companies to distribute antibiotics that may no longer be effective or lacking regulatory approval (Levy and Marshall, 2004). Evidence shows that increased antibiotic use may result in a positive association with a higher prevalence of resistant microorganisms, while reduced antibiotic use showed lower resistance rates. There is clear evidence that patients historically treated with antibiotics are more likely to have antibiotic resistance (Laximinarayan R and Brown, 2001). Further, re-administration of antibiotics from the initial cycle accelerates resistance mechanisms (Anderson, 1991). Antibiotics encourage selective pressure for bacteria to evolve when administered frequently or irrationally. Individuals and states play a role in the evolution of antibiotic resistance (Laxminarayan and Brown, 2001). For example, Clarithromycin consumption and its resistance similarly increased fourfold in Japan between 1993 and 2000 in comparison to other countries (Perez *et al*., 2002).

**2.6 Regulatory issues related to antibiotic resistance**

Congruent international management guidelines for daily antibiotic practices are yet unavailable. Hence, regulatory guidelines vary in different countries. Some countries have acted swiftly offering guidance e.g. United Kingdom, while other nations have yet to move toward interventions. The WHO has offered recommendations such as children in developing countries that antibiotics should only be used for the treatment of severe bloody diarrhea and cholera (WHO. CAH. Geneva, 1995). Since the beginning of the industrial revolution, we have dumped increase amounts of organic and inorganic toxins into streams, rivers, oceans, land, and air. In the personal care industry, there are insufficient guidelines for monitoring the home hygiene products which are likely to cause more risk for resistance because these products contain a high concentration of antibacterial ingredients (Laxminarayan and Brown, 2001).

With an abundance of evidence, there is no scope to ignore global antibiotic resistance. Antibiotic resistance can be more prevalent where antibiotic consumption is found to be higher. Lack of regulation and control in using antibiotics is prominent and needs to be targeted at a global capacity. Developing nations are at the greatest risk. Low prices of antibiotics, ease of availability and unnecessary use of antibiotics are causing more burdens in developing countries (Levy and Marshall, 2004). Antibiotic use is relatively uncontrolled among the countries where there is no universal health coverage for its citizens (Zaman and Hossain, 2017). Hence, irrational use of drugs has become a major concern. According to a study done in the United Kingdom, among the participants, 11.3% reported that they did not finish their last antibiotic course as prescribed. When asked about the reason why not comply with the course, 65% of the respondents stated that they felt better or forgot to take an antibiotic in time (Woodhead and Finch, 2007).

We are all affected by this multi-face ted public health issue. An all-encompassing problem that doesn’t just pertain to clinical personnel and microbiologists, but service personnel, industry stakeholders, specialists and the general public. We have to take necessary steps to tackle this complicated challenge. Social awareness, motivation, commitment in responsible sectors, stringent rules and regulation have to be prioritized. Further, we need the coupled action for the proper Urinary Tract Infection(UTi)lization of antibiotics, best management practices, and behavioral shifts across all industries that we can then combat against this public health burden. Application of modern technology can help the patient to take the antibiotic timely (Zaman , 2017). At present, the most notorious superbug is the Gram-positive organism *Staphylococcus aureus* (Lowey , 2003). This pathogen is frightening as its resistance to antibiotics is dramatically increasing. With an intimate history so closely tied to humans, *Staphylococcus aureus* is feared and at times, misunderstood (Lowey , 2003). These tendencies are causing higher resistance rates resulting in imminent hazards in human health. Notably, irrationality is observed in using antibiotics in livestock. Animals are given antibiotics for faster growth and disease prophylaxis. Strict and enforced regulations in the agricultural industry are needed to curb the harmful ripple effects.

Treatments for bacterial infections are becoming intensified every day. Infections remain as antibiotics gain resistance; treatment failure is common due to antibiotic resistance and multi-drug resistance, for e.g. tuberculosis. Newer and effective antibiotics that have no known resistance to bacteria are in high demand. Alternative treatment procedures are under consideration to fight bacterial infection. Passive immunization or administration of antibodies to non-immunized to prevent bacterial infections have been found effective (Keller and Stiehn, 2000). Another effective intervention is phage therapy, whereas bacteriophages are used to treat pathogenic bacterial infections (Monk et *al.,* 2010). Many newer classes of antimicrobials to fight antibiotic resistance are in the pipeline for clinical trials (Devasahayam et *al.,* 2010). Intervention strategies are aimed not only at targets but rather at the biological networks that may help to create new antibacterial therapies (Kohanski et *al.,* 2010). Combination therapies coupling antibiotics with antibiotic-enhancing phage have demonstrated the potential to be a promising antimicrobial intervention (Aminov, 2010).

**2.7 Mechanism of antimicrobial resistance**

There are number of ways by which microorganisms are resistant to antimicrobial agents. These include:

1. Bacteria produce enzymes which destroy the antimicrobial agents before it reaches its targets e.g. Beta lactamase enzyme hydrolyses beta lactam drugs which develop resistance.

2. Impermeable cell for antimicrobial drugs e.g. Gram negative bacteria may become resistant to Beta lactam antibiotics by developing permeability barrier.

3. Mutation e.g. Ribosome methylation of ribosomal RNA develop macrolide resistant.

4. Bacterial efflux pump that expels antimicrobial drugs from cell before it can reach its targets.

5. Specific Metabolic pathways in the bacteria are genetically altered so that antibacterial agents cannot exert an effect. (Marie et *al.,* 2005; Rice et *al.,* 2007).

**CHAPTTER THREE**

**3.1 MATERIALS AND METHODS**

This is a study conducted at Department of Microbiology of Godfrey Okoye University, Enugu state. A total of 6 high vaginal swabs were collected both from indoor and outdoor patients from Apollos laboratory which were presented with symptoms of virginal discharge such as: malodor, dysuria, dyspareunia, itching and fever. Patients who have vaginal instrumentation recently and history of usage of past two weeks antibiotics were excluded from the study. Sterile swabs were labeled with a unique identifier. The collection of sample was done using cottonswab which was inserted deep into the vagina. The samples were brought to Godfrey Okoye University for further analysis. Collected sample was inoculated in blood agar and chocolate agar by streaking method. Plates were incubated aerobically for 18-24 hours. Preliminary identification was done on the basis of morphological characteristics (shape, color, and spore formation), Gram stain and biochemical reactions (catalase test, oxidase test, IMViC test). Antibiotic sensitivity testing was done using (modified Kirby-Bauer’s) disc diffusion method. Antimicrobials tested for sensitivity were Amikacin, Ampicillin, Amoxicillin Clavulanic acid, Imipenem, Ciprofloxacin and Sulzone. After overnight incubation plates were examined to read the susceptibility zone. Data obtained were presented as biochemical tests result, antibiotic sensitivity of bacteria isolated from vaginal swab and number and percentage of patients from which the microorganisms were isolated. The frequency and antimicrobial sensitivity patterns of microbes were presented in percentages.

**3.2 PREPARATION OF MACCONKEY AGAR**

MacConkey Agar media was prepared in flask by dissolving 1.68gm of powdered agar media in 1litre of distilled water or the equivalent of this in smaller volumes. The flask was shaken for some time, heat on a Burnsen flame to dissolve the medium completely and then sterilized inautoclave (at 121°C temperature for 15 minutes).

**3.3 PREPARATION OF BLOOD AGAR**

Nutrient ager was prepared by dissolving 3.46 grams of powdered ager into a liter of distilled water, in a flask shaken for some time, heated to dissolve the medium and then sterilized by autoclaving at 121°C for 15 minutes. It was transferred to a 50°C water bath. When the agar base was cooled to 50°C, sterile blood agar was added aseptically and mixed well gently. Formation of air bubbles was avoided.  The blood was warmed to room temperature at the time of dispensing to molten ager base. A volume of 15 ml was dispensed into sterile-Petri plates aseptically. The medium was labeled with the date of preparation and name of agar. The plates are stored at 2-8°C, preferably in sealed plastic bags to prevent loss of moisture.

**3.4 SUBCULTURE**

Isolates were separately sub cultured onto blood and chocolate agar plates using a sterile wire loops and incubated at 37oc for 24 hours.

**3.5 AGAR SLANT SUB CULTURE IN BIJOU BOTTLES**

Nutrient agar was prepared by dissolving the nutrient agar into distilled water. It was boiled for sixty seconds to dissolve the agar completely, dispensed into the Bijou bottles up to halve of each bottle and sterilized by autoclaving at 121ocfor 15 minutes. It was then allowed to cool in a slant manner. Isolates were individually sub cultured onto agar slants, incubated for 18h and subsequently stored in the refrigerator at 4oC as stock culture

**3.6 GRAM STAINING**

Using a sterile wire loop, a loopful of colony of microorganisms was collected and fixed on a sterile glass slide and smeared then allowed to air dry. It was then gently flooded with crystal violet, tilting the slide and allowed to stand for 60seconds then rinsed with water then blot dry. Gently flood the smear with Grams iodine and again allowed for 60seconds. Then rinsed with water. Decolorize using Acetone, tilting the side slightly and immediately flushed with water. Finally it was flooded with safranin and allowed to stand for 45seconds then rinsed. Then it was blot dry and viewed under the light microscope under oil immersion.

**3.7 BIOCHEMICAL TESTS**

**3.7.1 CATALASE TEST**

An aliquot, 2ml of hydrogen peroxide solution was put into a test tube. A sterile wire loop was used to collect a loop full of the test organism and immersed in hydrogen peroxide solution and was observed for production of bubbles.

**3.7.2 OXIDASE TEST**

**Procedure for oxidase test using dry filter paper method**

Filter paper was soaked with 1% solution of reagent. Little culture is smeared on it with a sterile wire loop and is checked for result.

**3.7.3 UREASE TEST**

**Procedure**

A broth medium was inoculated with a loop full of pure culture of the test organism and was streaked on the surface of the agar slant. The cap was loosely covered and incubated at 35oc for 24hours.

**3.7.4 METHYL RED TEST**

**Procedure**

Prior to inoculation, allow medium to equilibrate to room temperature. Using organisms taken from an 18-24 hour pure culture, lightly inoculate the medium.

Incubate aerobically at 37 degrees C. for 24 hours. Following 24 hours of incubation, aliquot 1ml of the peptone glucose broth to a clean test tube. Reincubate the remaining broth for an additional 24 hours. Add 2 to 3 drops of methyl red indicator to aliquot. Observe for red color immediately.

**3.7.5 INDOLE TEST**

**Procedure using conventional tube method**

A culture of the microorganism was sterilized in a test tube containing tryptophan broth and incubated at 37oc for 24 hours. 0.5 ml of kovac’streagent was added to the broth culture and observed for the presence or absence of red ring.

**3.7.6 VOGES PROSKAUER TEST**

**Procedure**

Voges proskauer broth was inoculated with a pure culture of the test organism and incubated for 24 hours at 37oC . 1.0ml of the incubated broth was removed to a separate tube for vp testing. The reagent was allowed to warm to room temperature prior to use and 9 drops of Naphthol followed by 0.2ml of KOH the tube was shaken gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10 to 15minutes..

**3.7.7 CITRATE TEST**

**Procedure**

Simmons citrate agar lightly inoculated on the slant by streaking it to the microbial colony. It was then incubated at 37oC for 24 hours and observed for colour development

**3.8 ANTIBIOTIC CULTURE SENSITIVITY**

In third stepa 100 ml of muller hinton was prepared under sterile condition and poured into sterile test plates. A sterile cotton swab was dipped into a sample from well-mixed colonies in distilled water and applied onto nutrient agar plate. Sensitivity was checked by using 6 different commercially available anti-microbial discs (wafers) that were, Amoxicillin, Oflaxacin, Ciproflaxin and Gentamycin, and, were placed on the plate by means of multi-disc dispenser and pressed firmly onto agar plate with sterile forceps by using the agar-disc diffusion. The inoculated plates containing the antibiotics were incubated at 37oC for 24 hours after which the diameter of zone of inhibition around each antibiotic disc were then measured to the nearest millimeter (mm). Zones of inhibition of growth were examined around the disc; susceptible: zone of inhibition of ≥15mm. Resistant: zone of inhibition of ≤15mm. The discs were dispensed onto agar surface aseptically by forceps and firm contact was ensured. A distance of 24mm was maintained between the discs. After overnight incubation at 37OC the plates were examined for the zone of inhibition. Zone diameters were measured by calipers and strains were reported sensitive or resistant according to the chart supplied.

**CHAPTER FOUR**

**4.1 RESULTS**

Results of the biochemical tests indicating the presumptive identity of the isolates are shown in tables 1 and 2 below. Table 2 shows that most of the isolates are Gram negative rods while table 1 shows that that they are members of the genera *Escherichia, Pseudomonas, Proteus*, and *Citrobacter*.

TABLE: 1. Biochemical tests resuls

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Isolate | Catalase | Oxidase | Indole | Methyl red | Voges proskuer | Citrate | Probable organisms |
| A | + | + | + | + | + | + | E. coli |
| B | + | \_ | \_ | \_ | + | + | Klebsiella |
| C | + | + | \_ | \_ | \_ | + | pseudomonas |
| D | + | \_ | \_ | + | \_ | \_ | citrobacter |
| E | + | \_ | \_ | + | \_ | + | proteus |
| F | + | \_ | \_ | \_ | \_ | + | Acitenobacter |

TABLE 2: Gram reaction

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| isolate | GRAM REACTION | SHAPE | COLOUR | FLAGELLA | CAPSULE | SPORE | PROBABLE ORGANISM |
| A | Gram negative | Rod | purple | flagellated | variable | Non sporolating | *e. coli* |
| B | Gram negative | Rod | purple | non  flagellated | capsulated | non | *klebsiella* |
| C | Gram negative | Rod | purple | flagellated | Non capsulated | Non sporolating | *pseudomonas* |
| D | Gram negative | Rod | purple | flagellated | Non capsulated | non | *citrobacter* |
| E | Gram negative | Rod | purple | flagellated | Non capsulated | non | *proteus* |
| F | Gram negative | cocobacillus | purple | Non flagellated | capsulated | Non sporolating | *acitenobacter* |

TABLE 3:Antibiotic sensitivity pattern of bacteria isolated from high vaginal swab

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| bacteria | Amikasin | Ampicillin | Amoxicillin | Ciprofloxacin | Imipenem | Cefixime |
| E. coli | 22mm | 6mm | 3mm | 28mm | 27mm | 21mm |
| *Klebsiella* | 13mm | 18mm | 18mm | 19mm | 24mm | 21mm |
| *Pseudomonas* | 9mm | 8mm | 2 | 15mm | 21mm | 16mm |
| *Citrobacter* | 4mm | 7mm | 16mm | 18mm | 25mm | 14mm |
| *Proteus* | 19mm | 9mm | 1mm | 24mm | 27mm | 23mm |
| *Acinetobacter* | 23mm | 19mm | 22mm | 25mm | 21mm | 15mm |

**CHAPTER 5**

**5.1 DISCUSSION**

Vaginal flora contains a range of microorganisms normally.*Lactobacillus* spp. play fundamental role in maintaining acidic vaginal pH and prevent the overgrowth of potentially harmful and opportunistic bacteria. Vaginal infections are a great threat for women’s health related to common gynecological problem. This study demonstrates the prevalence of vaginal pathogens among the women study group. Vaginal infections are increasing due to vagina colonization by pathogenic bacteria other than the protective bacteria. The results of this study are similar to the study conducted by Lakshmi et al.,(2001). They reported the prevalence of vaginal infections in India.(Larsen, 2001)In our study *E.coli* was the most frequent bacteria followed by *Klebsiella* , *PseudomonasCitrobacter* , *Proteus* and *Acinetobacter* respectively, similar to a study conducted by Dutta *et a.,.(1995)*. McDonald *et al.,*(1994) also found *E. coli* to be the important bacteria associated with bacterial vaginosis. The most useful antibiotics against gram negative rods in our study were Imipenem (27mm) and Ciprofloxacin (28). Antibiotics like Imipenem are extremely effective but expensive. Tariq *et al.,*(2017)reported similar findings. Whereas the antimicrobials with least affectivity against gram negative rods were Penicillins (Ampicillin, amoxicillin-clavulanic acid), Amikacin due, probably, to indiscriminate use of antibiotics.

**CONCLUSION**

High prevalence of gynecological infections demands that the patients who suffer from the symptoms of gynecology must be investigated carefully. The elevated prevalence of vaginal bacteria in the recent study stress that high vaginal swab culture provides a good laboratory means for the identification of causative bacteria.It is therefore recommended for roUrinary Tract Infection(UTi)ne use. There is a need for stakeholders to be aware of antibiotic resistance in order to avoid improper use and frequent abuse of available antibiotics. Treatment schedule must be designed subsequent to proper laboratory investigations

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