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Clinical evaluation of microbiological profile of abnormal vaginal discharge and It's antimicrobial susceptibility pattern

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Abstract
Vaginal discharge, the major sign of vaginitis is the common reason for which women consult a gynaecologist. Vaginal discharge that, suddenly differs in colour, consistency and odour, and a significant increase or decrease in amount, may indicate an underlying abnormality of which infections are most common. Hence based on the above findings the present study was planned to detect the clinical profile of the patients with vaginal discharge, as well as spectate and perform antimicrobial susceptibility tests.
The present study was planned in the Department of Microbiology in Government Medical College, Bettiah. In the present study high vaginal swabs from 100 clinically suspected vaginitis cases were processed for the identification of causative organism(s) over a period of one year, attending the Gynaecology.
The prevalence of bacterial vaginosis was relatively high and was affected by individual hygiene. Therefore, comprehensive healthcare education aimed at reducing bacterial vaginosis is needed. One of the most common complaints by women attending Gynecology OPD is vaginal infection and it still remains one of the least understood. Though bacterial vaginosis is a global problem, we should detect the principle pathogen and treat them properly. Extensive and indiscriminate use of antibiotics has created a major problem- drug resistance. Several antimicrobial agents have been used to treat symptomatic bacterial vaginosis.

Keywords: vaginal discharge, vaginosis, antimicrobial, susceptibility

Introduction
Vaginal discharge is a mixture of liquid, cells, and bacteria that lubricates and protects the vagina. This mixture is constantly produced by the cells of the vagina and cervix and it exits the body through the vaginal opening. The composition, amount, and quality of discharge varies between individuals as well as through the various stages of sexual and reproductive development. Normal vaginal discharge may have a thinner, watery consistency or a thick, sticky consistency, and may be clear or white in color. Normal vaginal discharge may be large in volume but typically does not have a strong odor, nor is it typically associated with itching or pain. While most discharge represents normal functioning of the body, some changes in discharge can reflect infection or other pathological processes. Infections that may cause changes in vaginal discharge include vaginal yeast infections, bacterial vaginosis, and sexually transmitted infections. The characteristics of abnormal vaginal discharge vary depending on the cause, but common features include a change in color, a foul odor, and associated symptoms such as itching, burning, pelvic pain, or pain during sexual intercourse [1].
Abnormal discharge can occur in a number of conditions, including infections and imbalances in vaginal flora or pH. Abnormal vaginal discharge may also not have a known cause. In one study looking at women presenting to clinic with concerns about vaginal discharge or a foul smell in their vagina, it was found that 34% had bacterial vaginosis and 23% had vaginal candidiasis (yeast infection). 32% of patients were found to have sexually transmitted infections including Chlamydia, Gonorrhea, Trichomonas, or Genital Herpes. Diagnosing the cause of abnormal vaginal discharge can be difficult, though a potassium hydroxide test or vaginal pH analysis may be used. When abnormal discharge occurs with burning, irritation, or itching on the vulva, it is called vaginitis [2] The most common causes of pathological vaginal discharge in adolescents and adults are described below.
Bacterial vaginosis (BV) is an infection caused by a change in the vaginal flora, which refers to the community of organisms that live in the vagina. It is the most common cause of pathological vaginal discharge in women of childbearing age and accounts for 40–50% of cases. In BV, the vagina experiences a decrease in a bacterium called lactobacilli, and a relative increase in a multitude of anaerobic bacteria with the most predominant being Gardnerella vaginalis. This imbalance results in the characteristic vaginal discharge experienced by patients with BV. The discharge in BV has a characteristic strong fishy odor, which is caused by the relative increase in anaerobic bacteria. The discharge is typically thin and grey, or occasionally green. It sometimes is accompanied by burning with urination. Itching is rare. The exact reasons for the disruption of vaginal flora leading to BV are not fully known. However, factors associated with BV include

antibiotic use, unprotected sex, douching, and using an intrauterine device (IUD). The role of sex in BV is unknown, and BV is not considered an STI. The diagnosis of BV is made by a health care provider based on the appearance of the discharge, discharge pH > 4.5, presence of clue cells under the microscope, and a characteristic fishy odor when the discharge is placed on a slide and combined with potassium hydroxide ("whiff test"). The gold standard for diagnosis is a gram stain showing a relative lack of lactobacilli and a polymicrobial array of gram negative rods, gram variable rods, and cocci. BV may be treated with oral or intravaginal antibiotics, or oral or intravaginal lactobacillus [3].

Most pregnant women have vaginal discharges that are either physiologic or pathologic. The challenge to the clinician is to separate the vaginal infections with potentially serious input for pregnancy from annoying but not serous secretions, irritation and pruritus. Infectious vaginitis is usually caused by yeast, such as Trichomonas vaginalis, bacterial vaginosis, gonorrhoea, Chlamydia trachomatis, Mycoplasma, Group B streptococcus or herpes. Normal vaginal secretions consist of water, electrolytes, epithelial cells, microbial organisms, fatty acid and carbohydrate compounds. The concentration of anaerobic bacteria is usually five times than that of aerobic organisms. The most prevalent organisms in the vagina are lactobacilli, Streptococci, Staphylococcus epidermidis, Gardnerella vaginalis and Escherichia coli. Anaerobic species that are frequently isolated include Peptostreptococcus, anaerobic lactobacilli and bacteroides [5].

Vaginal pH, glycogen content and amount of secretion influence the quantity and type of organisms present in the vagina. Lactobacilli restrict the growth of other organisms by producing lactic acid, thus maintaining a low pH. These organisms also produce hydrogen peroxide, which is toxic to anaerobes. The normal vaginal bacterial population assists in inhibiting the growth of pathologic vaginal organisms. If the normal vaginal ecosystem is altered, there is a greater chance of proliferation of pathogenic organisms. The challenge of treating vaginitis in pregnancy is the necessity of making accurate diagnosis and treating correctly [4]. True infections (some of which can have dangerous effect on gestation) must be separated and distinguished from the exaggeration of physiologic discharge by pregnancy. Infection with bacterial vaginosis, Chlamydia trichomonas or Group B Streptococcus has been associated with septic abortion, premature rupture of membranes and premature delivery [4, 6].

Vaginal discharge, the major sign of vaginitis is the common reason for which women consult a gynaecologist. Vaginal discharge that, suddenly differs in colour, consistency and odour, and a significant increase or decrease in amount, may indicate an underlying abnormality of which infections are most common. Hence based on the above findings the present study was planned to detect the clinical profile of thelt patients with vaginal discharge, as well as spectate and perform antimicrobial susceptibility tests.

Methodology

The present study was planned in the Department of Microbiology in Government Medical College, Bettiah. In the present study high vaginal swabs from 100 clinically suspected vaginitis cases were processed for the identification of causative organism(s) over a period of one year, attending the Gynaecology.

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study.

Following was the inclusion and exclusion criteria for the present study.

Inclusion criteria: Women of reproductive age within 15-65 years, both pregnant and non-pregnant, with abnormal vaginal discharge, with or without mild vulver itching or burning are considered as patients.

Exclusion criteria: 1. Below 15 yr & over 65 yrs. 2. Known case of malignancy or AIDS patient. 3. History of taking antimicrobial agents or vaginal medication for vaginitis within the last one month. 4. Menstruating women. 5. Patient having history of vaginal douche on the day of examination Two high vaginal swabs were collected with sterile swabs from posterior vaginal, fornix using a sterile sim's speculum. Swabs were transported to the microbiology laboratory in Amie's transport medium. Following sample collection speculum is withdrawn and sniff test was performed by the addition of 10% KOH onto the discharge collected on the speculum and the release of an intense fishy odour noted. Smear is prepared from the swab and gram staining was done and observed for presence of pus cells, clue cells, Gram negative coccobacilli, Gram negative cured rods (Mobiluncus). Smears were graded according to Nugent's scoring system. Another swab was incoculated on to human blood agar with columbia blood agar base, Mac conkey agar, chocolate agar and blood agar plate was kept in the candle jar with a blotting paper to provide 5 to 10% CO2 and incubated for 48 hours. Culture plates were observed for diffuse β hemolytic tiny colonies on blood agar and no growth on Macconkey agar. Catalase and Oxidase tests are done from Chocolate agar and they were negative. Sugar fermentation test was done using 1% starch, 1% maltose and 1% glucose using phenol red as indicator. Antibiotic susceptibility testing was done on human blood agar by Kirby – Bauer disc diffusion method using Metronidazole 10 µg, 25µg and 50µg, Ampicillin 25µg, gentamicin 10µg, ciprofloxacin 10µg, tetracycline 10µg, streptomicin 10µg, kanamicin 30µg and cotrimoxazole 25µg.

Results & Discussion

The data from the 100 patients were collected and presented as below. Gynaecological infections, if not diagnosed and treated in time may lead to severe or irreversible complications. Due to changing microbiological profile of infection and sensitivity of microorganisms and emergence of β-lactamase and methicillin resistant pathogen and resistance to Azole group of drugs in nonalbicans is a major problem throughout the world in various clinical infections including Gynecological infections. Early microbiological diagnosis will help to plan accurate, appropriate and effective therapy. In present study an attempt is made to know the Microbiological profile of abnormal vaginal discharge and its antimicrobial susceptibility pattern. The results are compared with other studies and discussed.

Vaginal infections have various implications for women's health, being the most common gynaecological problem. Prevalence studies indicate that there is a potentially large reservoir of bacterial vaginosis infection in the population.

Microbes play a critical role in determining the biochemical and inflammatory profile of the vaginal environment. An understanding of the composition of the vaginal microbial ecosystem is essential for comprehensive understanding of the etiology of vaginal infection and for the prevention and treatment of the disease.

Table 1: Basic Characteristics of the Patients

Characteristics	Number	Bacterial vaginosis (%)	
Age in years		**Yes**	**No**
15–24 years	26	11	15
25–44 years	56	25	31
45–64 years	18	12	6
Total	100	48	52
Marital status			
Unmarried	31	16	15
Married	51	24	27
Divorced	18	9	9
Total	100	49	51
Education			
Illiterate	22	10	12
Primary school	28	15	13
Secondary school	31	21	10
College	19	7	12
Total	100	53	47
History of abortion			
Yes	25	14	11
No	75	35	40
Total	100	49	51
Previous BV/GTI			
Yes	45	21	24
No	55	29	26
Total	100	50	50

Table 2: Species and No. of Isolates

Species	Number of isolates
S. aureus	24
S. agalactiae	5
S. pyogenes	2
E. coli	28
Klebsiella pneumoniae	18
Klebsiella ozaenae	2
Enterobacter aerogenes	11
Citrobacter freundii	4
Citrobacter diversus	2
Proteus mirabilis	1
Providencia rettgeri	3
Total	100

Table 3: Antimicrobial susceptibility of G.Vaginalis from NSV cases

Drug	Conc in µg/disc	No. sensitive
Metronidazole	10	0
Metronidazole	25	0
Metronidazole	50	37
Ampicillin	25	51
Gentamicin	10	37
Ciprofloxacin	10	33
Co- Trimoxozole	25	7
Streptomycin	10	22
Kanamycin	30	22
Tetracycline	10	25

Bacterial vaginosis is the most common infection in female worldwide leading to vaginal disorders. It may be a polymicrobial syndrome but recent studies have shown Gardnerella vaginalis (G. vaginalis) also can be a primary pathogen in half of the cases of bacterial vaginosis.

During pregnancy, the vagina shows an increase in susceptibility to infection by candida. It is thought that during pregnancy high level of hormone by providing higher glycogen content in the vaginal tissue, provide an excellent carbon source for candida and oestrogen enhances adherence of candida cells to vaginal mucosa. The distribution of organisms vary from place to place. The organisms localize where their survival needs are met and have exemption from the infection preventing destructive capacity of host. It is thought that during pregnancy high level of hormone by providing higher glycogen content in the vaginal tissue, provide an excellent carbon source for candida and oestrogen enhances adherence of candida cells to vaginal mucosa [7].

The prevalence of various disease varies considerably from place to place which could be attributable to the changes in environmental condition and socioeconomic habits of people in developing countries [8].

The presence of co-morbidities like hospitalization, immunosuppression and co-existent reproductive tract infections have to be evaluated accordingly. It is known that vaginal infections, due to a disruption of normal vaginal flora, increase the risk of sexually transmitted infection, especially human immunodeficiency virus (HIV). However, our study did not identify any association with HIV, and diabetic status records were not available. No patients had taken hormone supplements or any other medications that could interfere with the results.

Several microorganisms were isolated in our study, and those with the highest frequencies were Escherichia coli. We also found Escherichia coli to be the most common pathogenic bacteria isolated from culture. In fact, Escherichia coli was found to be the most prevalent pathogen isolated not only from high vaginal swabs but also urine, pus, blood and wounds, as seen in a study conducted by Dutta S *et al*., in Dhaka [9].

Gopal Kumar, *et al* found Candida species in 2% of patients [10]. It is known that vaginal infections due to a disruption of normal vaginal flora increase the risk of sexually transmitted infections especially HIV [11]. A recent study in Chicago collecting cervicovaginal lavage specimens found gram stains indicative of Bacterial vaginosis to be significantly associated with a newly identified HIV-Inducing factor that induces HIV-1 Gene expression [12]. Newer antibiotics like Imipenam are effective but very expensive [13]. Amikacin and Gentamycin still continue to be drugs to which most of the organisms are sensitive. Lakshmi, *et al* found most of their gram negative isolates to be highly sensitive to Amikacin, gentamycin and Ceftazidine. They also found that MRSA strains were all found to be susceptible to Vancomycin.

Bacterial vaginosis is a situation that occurs when lactobacilli are replaced by the overgrowth of anaerobic bacteria, primarily G. vaginalis and Mobiluncus spp. Other bacteria (Escherichia coli, Klebsiella spp., Acinetobacter spp., Staphylococcus spp., enterococci, and Streptococcus agalactiae (group B streptococci), however, have been termed “intermediate flora” in some studies or have been included with bacterial vaginosis in others [14]. Still others consider them as distinct bacterial floras that cause aerobic vaginitis which has been thought to be a better candidate than bacterial vaginosis as a cause of pregnancy

complications such as preterm rupture of the membranes and preterm delivery [15]

The vagina is a highly nutrient-rich chamber for microbes. As a result, the composition of the vaginal microbiota is affected by numerous host factors, including age, changes in hormone levels (during the menstrual cycle, during menopause, pregnancy, or as a result of hormone contraceptive use), other genital infections, as well as sexual and hygiene practices. Most of these commensal microbiotas do exist in mutualistic relationships with their human hosts, although a few are opportunistic pathogens that can cause chronic infections, preterm delivery, or life-threatening maternal and fetal diseases.

Diagnosis of these infections based on culture sensitivity is a definite step in treatment of these infections. In regular practice, fixed protocols are followed. Inadequate treatment with antimicrobials due to non-compliance or under the prescription of drugs results in high incidence of recurrence. Extensive resistance rates have emerged among commonly used antibiotics due to indiscriminate use. Newer antibiotics like imipenem and meropenem are highly effective but expensive [13]. Short term effects of antimicrobial regimens have been tested and proved through clinical trials to be effective in achieving clinical and microbiological cure. Newer therapeutic approaches include the development of new drugs, phage therapy (bacterial viruses can be robust anti-bacterial agents in vitro), photodynamic inactivation of micro-organisms and immunomodulators [16]. A significant proportion of pathogens causing vaginal infections are resistant to the conventionally used antibiotics. This study is a step in familiarizing sensitivity and resistance patterns to used antibiotics, preventing resistance and thus preventing the chronic sequelae.

Conclusion

The prevalence of bacterial vaginosis was relatively high and was affected by individual hygiene. Therefore, comprehensive healthcare education aimed at reducing bacterial vaginosis is needed. One of the most common complaints by women attending Gynecology OPD is vaginal infection and it still remains one of the least understood. Though bacterial vaginosis is a global problem, we should detect the principle pathogen and treat them properly. Extensive and indiscriminate use of antibiotics has created a major problem- drug resistance. Several antimicrobial agents have been used to treat symptomatic bacterial vaginosis.

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