CHAPTER-I

1 INTRODUCTION

Vaginitis is the inflammation and infection of the vagina, most often caused by bacteria, fungi or parasites (Olner-Hanssen et al., 1989). It may also be the result of an allergic reaction to an irritating chemical such as spermicides, douche or bath soap that disturb the microbial balance of vaginal area.

Bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomoniasis are the common types of vaginal infection (Sobel, 1997) and are common disorder in women (CDC, 1998). BV is a condition characterized by raised vaginal pH and milky discharge in which the normal vaginal flora (Lactobacilli) is replaced by a mixed flora of aerobic, anaerobic and microaerophilic spp (Aggrawal et al., 2003). *Gardenella vaginalis* is found in almost all women with BV and also in 50% of healthy vaginal flora (Livengood, 2000). BV is not considered to be a Sexually Transmitted Infections (STIs) (CDC, 2006). Pregnant women and women with STIs are especially at risk for getting BV (Sumati et al., 2009). It is associated with racial origin, smoking, sexual activity, vaginal douching and psychological stress (Culhane et al., 2001) and may either be symptomatic or asymptomatic.

Prevalence of BV varies from 10-65% and approximately 10-30% of pregnant women experience BV during pregnancy (Sumati et al., 2009; Hiller et al., 1995). In Nairobi vaginitis was found among 20% of pregnant women and the prevalence of VVC, BV and trichomoniasis was 26.2%, 20.6% and 19.9% respectively (Thomas, 1996). In Nepal, the prevalence of BV was found to be 69% in symptomatic and 62% in asymptomatic women and trichomoniasis to be 14% (Aryal, 1997).

VVC is a fungal infection of lower genital tract mainly vulva and vagina especially among sexually active women. *Candida albicans* is both the most frequent colonizer and causative agent of most cases of VVC (Singh, 2003). Three quarters of the women experience at least one episode of VVC in their reproductive life and about one half of these women experience a recurrence (Sobel, 1997). One third to three fourth of the affected women are asymptomatic (Sobel, 1990). VVC is not considered as a STD (Singh, 2003) but frequent sexual intercourse is the strongest risk factor for VVC (Foxman, 1990). Pregnancy, STIs, antibiotics use, oral contraceptives, contraceptive devices, high oestrogen levels, diabetes and HIV increase the risk of VVC (Hedayati, 2010). During pregnancy VVC becomes more chronic or recurring due to hormonal changes (Foxman, 1990).

Trichomoniasis "trich" is a STI resulting in inflammation of the vagina, cervix and urethra in women caused by *Trichomonas vaginalis*. About 7.4 million new cases of trichomonasis are reported each year (CDC, 2006). Trichomoniasis during pregnancy can increase the risk of premature rupture of membranes and preterm delivery.

The condition in which disturbed microflora with the absence of lactobacilli in the vagina is replaced by a significant number of aerobic facultative pathogenic flora from the bowel such as *E. coli*, Enterococci, *Staphylococcus* species and Group B Streptococci is called aerobic vaginitis. This colonization of different pathogenic microorganism is favored by different host behavioral factors and moreover due to increase in the vaginal pH as a result of BV (Donders et al., 2002).

Urinary tract infection (UTI) is defined as the presence of bacteria undergoing multiplication in urine within urinary drainage system and presence of more than 10^5 organisms/ml in midstream urine (Jha and Bapat, 2005; Leigh, 1996). One woman in five develops a UTI during her life time. Each year UTI accounts about 7 million outpatient visits (Braunwald et al., 2001), two third of the patients are women (Mandell et al., 2000). UTI during pregnancy may lead to fetal death if untreated.

Association between BV and UTI and vice versa probably begins with an increase in pH of vagina because of reduction of vaginal lactobacilli producing lactate and H_2O_2 (Hillerband et al., 2002). Pregnant women are more prone to asymptomatic bacteriuria because the higher urinary pH allows the more rapid colonization of vagina by uropathogens. Frequent sexual intercourse, which is also linked to both BV and UTI, may also contribute to this phenomenon (Harmanli, 2000). Women suffering from BV are at great risk of UTI than others (Hillerbrand et al., 2002; Harmanli et al., 2000).

In Nepal one in three women wants consultation for vaginal discharge (Pradhan, 2001). It is very difficult for women to get treatment for vaginal discharge/ infection due to the lack of proper diagnosis in the Outpatient Department (OPD) in much hospital of Nepal (Padhye, 2003). Little work has been done previously in this subject in Nepal. Vaginitis may lead to an immense physical and psychological problem that may require instant medical attention. Vaginitis and UTI during pregnancy have risk to both the mother and fetus and a single step of early diagnosis and treatment can save both the lives. Evaluation of UTI in women with BV is effective as it reduces the complications associated like low birth weight, acute pyelonephritis, hypertension, preclamsia, habitual abortion, congenital disease in baby and fetal mortality. Since this study focuses on infection rate and cause of vaginitis, it may also provide a data base for planning, effective case management and control of the problem and also fulfills the gap in research carried out in the field of vaginitis. Selection of antimicrobial agents to treat the bacteriuria giving special consideration to maternal and fetal toxicity may also be assessed by this study. Thus, with the objective to find the prevalence and distribution factors of different types of vaginitis and the association between BV and UTI among suspected pregnant women, the present study has been designed.

CHAPTER-II

2 OBJECTIVES

2.1 General Objectives

To describe the prevalence and distribution of different types vaginitis and the association between BV and UTI among suspected pregnant women visiting Thapathali Maternity and Women's Hospital, Thapathali, Kathmandu

2.2 Specific Objectives

1. To describe the prevalence of vaginitis among suspected pregnant women
2. To determine distribution factors associated with vaginitis among infected pregnant women
3. To associate different risk factors with vaginitis
4. To find out the relationship between BV and UTI
5. To determine antibiotics resistance among bacterial isolates of vagina and urinary tract

CHAPTER-III

3 LITERATURE REVIEW

3.1 Definition of vaginitis

Vagina is the muscular passageway between the uterus and the external genital area. Vaginitis is the infection and inflammation of the vagina mostly often caused by bacteria, fungi or parasites (Olner-Hanssen, 1989). It is a common clinical syndrome being found in 28% of women attending STD clinics, obstetrics and gynecology units and outpatients departments (CDC, 1979). Three or more episode of vaginitis per annum is described as recurrent vaginitis (Maruotti et al., 1989). Factors responsible for recurrent vaginitis are unhygienic practices, improper care during menstruation, multiple sexual partners and contraceptive trends (Thulkar, 2010). In acute vaginitis, the squamous epithelial lining of the vaginal wall is invaded and inflammed causing discomfort, pruritus or pain in addition to discharge. In premenopausal women, infection is the most common cause of vaginitis. After menopause, a low level of estrogen often leads to atropic vaginitis.

Organisms associated with vaginitis are part of the host's own microflora or exogenous microorganisms that must interact with species present as part of the host's indigenous flora. These microrganisms are frequently isolated from asymptomatic women (Krieger et al., 1988). Bacterial, yeast, viruses, chemicals in creams, sprays, spermicides and feminine hygiene products can cause vaginitis. Sometimes vaginitis occurs from organisms that are passed between the sexual partners. Antibiotics, douching, contraceptives, sexual intercourse, stress hormones, feminine hygienic sprays, certain soaps or bubble baths, diabetes, pregnancy and infections, can change the vaginal environment and allow pathogens to grow (Kent, 1991; Sobel, 2000).

3.2 Endogenous vaginal flora and microenvironment

The normal vaginal environment is characterized by a dynamic interrelationship between *Lactobacillus species* and other endogenous flora, estrogen, glycogen, vaginal pH and metabolic by-products of flora and pathogens. The healthy vaginal flora is dominated by *Lactobacillus* species which use flavoproteins for terminal oxidation and produce H_2O_2 and eliminate other bacteria unable to synthesize catalase. In the healthy adult female, each ml of vaginal fluid contains more than 10^5 lactobacilli, principally *Lactobacillus crispatus*, *Lactobacillus jensenii* (Smayevsky et al., 2001). Strictly anaerobic bacteria such as Bacteroides and *Prevotella* spp are recovered in similar numbers from only approximately one sixth of women (Ferris, 1998). Other H_2O_2 producers include *Lactobacillus acidophilus, Lactobacillus rhamnosus,* and so on. H_2O_2 can be auto inhibitory or toxic to adjacent bacteria, viruses or mammalian cells, particularly in the presence of peroxidase-mediated antimicrobial system in vitro. Vaginal lactobacilli are also powerful organic acid producers providing the normal vaginal pH between 3.8 and 4.2 using glycogen in the vaginal epithelium as the substrate. They also synthesize bacteriocins, biosurfactants and lactic acid that inhibit other bacterial species. They also coaggregate with pathogens and stimulate the superficial vaginal immune system to enhance local defense mechanism against non-resident bacteria (Furr et al., 1991).

3.3 Vaginal colonization by different bacteria during pregnancy

The human vagina is frequently colonized by various microorganisms which may be normal flora or potential pathogens. Lactobacilli are the predominant organisms (about 70%) found in the cervix and vagina of healthy pregnant and non pregnant women (Davies, 1984). Lactobacilli are regarded as a normal flora because of their production and tolerance of high acidity which limit the growth of other bacteria. Though *Staphylococcus epidermidis* and Diptheroides have been found among 30-60% of pregnant women, due to their relative inert nature, they are regarded as autochthonous species.

Aerobic vaginitis is a condition in which disturbed microflora with the absence of lactobacilli is not overwhelmed by anaerobic bacteria as in typical BV but rather contains a significant number of aerobic facultative pathogenic flora from the bowel. In aerobic vaginitis lactobacilli is replaced by aerobic facultative intestinal pathogens, such as *E. coli*, enterococci, *Staphyloccous* species and GBS, vaginal leucocytosis and parabasal cells (Donders et al., 2003). Other potential pathogens include Group D *Streptococcus* (10-40%), α-haemolytic and β-haemolytic *Streptococcus* (10-25%), *Candida* species (20-30%), *E. coli* (5-20%), *Neisseria* species (15-20%), *Klebsiella* species, *Proteus* species (<10%) and *Staphylococcus aureus* (<5%). Among them GBS and *E. coli* is most common causative organism in serious maternally transmitted infections in neonates (Davis, 1984). BV is a condition in which there is relative immune suppression, in response to bacterial overgrowth whereas in aerobic vaginitis there is rather a sepsis like local overreaction of the immune response (Donders, 2007). Symptoms of severe aerobic vaginitis often include a red, inflamed vaginal mucosa, a yellowish stick discharge, a high pH above 6 and an odor that is unpleasant but not like fishy odor (Donders et al., 2002). In aerobic vaginitis, an elevated host immune reaction can be demonstrated by an increased number of leucocytes, which sometimes have a toxic appearance. This condition produces large amount of pro-inflammatory cytokines that can cause preterm labor and mid trimester pregnancy losses by initiating prostaglandin cascade (Donders, 2003).

3.4 Normal and abnormal vaginal discharge

Vaginal discharge may be normal or abnormal (Omole-Ohons et al., 2006). The flow of discharge is part of the natural cleansing and lubricating process. Normal discharge is usually clear to cloudy white, relatively odorless, non irritating and sometimes may have a yellowish appearance after it dries. Normal vaginal discharge is physiologic, such as occurs during pregnancy, sexual arousal or at specific period in the menstrual cycle. It comprises of vaginal transudates containing desquamated epithelial cells, cervical and vaginal mucus

and secretions from endometrial glands. The frequency of vaginal discharge varies with age, emotional stress, nutritional status, menstrual cycle, pregnancy and usage of medication including birth control pills and sexual arousal (Trabert et al. 2007). During pregnancy vaginal discharge increases due to increased estrogen and blood flow that the body needs. During pregnancy, vagina is more susceptible to infection, resulting in a higher incidence of colonization and symptomatic vaginitis (Osoba et al., 1993). The clinical attack rate is increased maximally during the third trimester and symptomatic reoccurrence is also more common (Trabert et al. 2007).

Abnormal vaginal discharge in female of reproductive age is the commonest and one of the most frequent gynecological complaints in the clinics (Nwankwo et al., 2010). The abnormal vaginal discharge may be green, yellow, brown or red in color with foul smelling odor, pruritis, irritation, dysuria and dysparenia depending on type of infection (Osoba et al., 1993). The causes of vaginal discharge include foreign body such as ring pessary inserted for uterogenital prolapse or for induction of abortion by untrained Dais in Nepal (Padhye, 2003), a change in normal balance of vaginal bacteria, bacteria, viruses, yeasts and organisms that are passed between sexual partners. Other causes include women's health, personal hygiene, medications, hormones particularly oestrogens, health of her sexual partners and chemicals in creams or sprays and clothing.

3.5 Types of vaginitis

Infectious vaginitis, which includes the three most common types of vaginal infections i.e. bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomoniasis (Trich), is a common disorder in women (CDC, 1998; Sobel, 1997). Mixed infections commonly occur dictating that all 3 be sought in symptomatic patients.

3.5.1 Bacterial vaginosis (BV)

BV was first reported by Gardner and Dukes in 1995 and has been referred as *Haemophilus vaginalis*, nonspecific vaginitis, anaerobic vaginitis, *Corynebacterium vaginalis*, and *Gardnerella vaginalis* over the period of 100 years. It is a condition characterized by raised vaginal pH and milky discharge in which the normal vaginal flora is replaced by a mixed flora of aerobic, anaerobic and microaerophilic species (Hill, 1985). BV is actually not an infection but a condition resulting from an imbalance in the vaginal flora (Pradhan, 2001).

BV is characterized by decreased or absent *Lactobacillus* species and increased concentrations of potentially pathogenic bacteria. Other characteristic changes include elevated pH > 4.5, formation of clue cells, odor due to increased concentrations of diamines, polyamines and organic acids, in vaginal fluid, an upregulation of inflammatory cytokines such as Interleukin-1beta, a noticeable absence or rare presence of white blood cells in the vaginal discharge, and a decrease in naturally protective molecules like secretory leukocyte protease inhibitor (Cauci et al., 2004).. The normal vaginal epithelium is covered by a thin layer of mucin. In BV, this protective layer is replaced by a *Gardenella vaginalis* specific biofilm (Swidsinski et al., 2005).

BV is not considered to be a STI (CDC, 2006). This is common in women of reproductive age (Sobel, 2000) and one in 3 women will develop the condition at some point in their lives (Wang, 2000). Fifty percent of all patients with BV present with no symptoms in the lower genital tract (Ferris, 1998).

3.5.1.1 Causative organisms

The aetiology and pathogenesis of BV is still unclear. Microbiology of BV is complex involves various organisms such as *Gardnerella vaginalis*, mixed anaerobes such as *Mobiluncus* species, *Prevotella* species, *Peptostreptococcus* species, *Bacteroides* species,

Eubacterium species, *Mycoplasma hominis,and* facultative bacteria such as *Mycoplasma* species, *Staphylococcus epidermidis*, *Streptococcus* species etc. (Hill, 1985). Both anaerobes and *Gardnerella vaginalis* are normal inhabitants of the vagina, but overgrowth of normal lactobacilli dominated flora by these bacteria results in BV (Smayevsky et al., 2001). This overgrowth results in the degradation of the mucus membrane and shedding of the vaginal epithelium resulting in a discharge. The destruction of these mucins exposes the epithelium to other organisms, with subsequent appearance of clue cells.

3.5.1.2 Risk factors

The main factors responsible for the downward shift of lactobacilli, the consequential development of BV include early sexual activity, multiple sexual partners and new sexual partners (Barbone, 1990; Paavonen, 1983), intercourse without a condom which, history of STI and BV (Ness et al., 2006), women having sex with women (Marrazo et al., 2002). Other factors are vaginal douching (Ness et al., 2002), use of broad spectrum antibiotics and intrauterine device, smoking (Mijac et al., 2006), frequent use of scented soaps and indigent population (Georgijevic et al., 2000).

The vulnerability of pregnant women to BV is as a result of increased levels of oestrogen during pregnancy which creates a climate for growth of these agents (Hopsu-Havu, 1980). BV is also twice as common in black women as in white women (Ness et al., 2003).

3.5.1.3 Symptoms of BV

The clinical symptoms of BV are thin, homogenous, gray, maladrous vaginal discharge without significant pruritus or pain (Eschenbach, 1999). Increased vaginal discharge is a more frequent but less specific symptom of BV. The classical symptom is a fishy order due to the production of amines by the anaerobic bacteria which volatilize increasingly with rising pH. An elevated vaginal pH of 5 to 6.5 is always associated with BV (Hill, 1993) and

amine test odor is related to the increase in pH (Greenhood, 1983). However *Gardenella vaginalis* was most often observed adhering to the surface of exfoliated vaginal epithelial cells (clue cells) in higher number than anaerobic bacteria suggesting the *Gardenella vaginalis* is responsible for clue cell formation which may be due to presence of glycocalyx layer as detected by wet mount or Gram stained smears (David, 1997).

3.5.1.4 Diagnosis

BV has been diagnosed using Amsel's criteria, with three of the four findings required to establish the diagnosis (Amsel et al., 1983). The criteria includes (i) thin, homogenous discharge (ii) positive whiff test (iii) presence of clue cells on microscopy (iv) vaginal pH >4.5. Based on these criteria, 90 percent of women with BV can be diagnosed correctly (Amsel et al., 1983; Thomason et al., 1990). Vaginal culture of *Gardenella vaginalis* has no place in the evaluation of asymptomatic women because this organism is carried by 30-40% of women without BV (Mirza et al., 1983). Culture is also time consuming, expensive and misleading, as it can lead to considerable over and under treatment.

3.5.1.5 BV during pregnancy and adverse outcomes

During pregnancy the vagina is more susceptible to the infection resulting in a higher incidence of colonization and symptomatic vaginitis. Approximately 10% of women with BV experience adverse pregnancy outcomes. The clinically attack rate is increased maximally during 3rd trimester and symptomatic recurrence is also more common (Silver et al., 1989). BV during pregnancy may lead to preterm labor, spontaneous preterm delivery, low birth weight, premature rupture of membranes, postpartum metritis, intra-amniotic fluid infection (Kurki et al., 1992; Gravelt, et al., 1986). BV also increases risk of PID, postabortion uterine infection and breast abscess (Policar, 1996). BV has been associated with increased HIV shedding, HIV acquisition and co-infection with STIs such as *Trichomonas vaginalis, Chlamydia trachomatis* (Koumans et al, 2007; Martin et al., 1999).

3.5.2 Vulvovaginal Candidiasis (VVC)

VVC is also known as candidosis, moniliasis, candidal vaginitis and vaginal yeast infection. It is the most common form of mucosal candidiasis of the female lower genital tract the vulva and the vagina caused by *Candida* species especially among the sexually active group (Sobel, 2000). It is usually secondary to overgrowth of normal flora *Candida* species in the vagina (Hedayati, 2010). VVC is not considered as STD (Singh, 2003) because it does affect celibate women, children and also *Candida* species is seen in normal vaginal flora in healthy women. However, *Candida* can be sexually transmitted (CDC 2002). It can be recurrent or relapsing (Ferris et al., 2002). Recurrent or relapsing VVC is four or more episodes of VVC per year and it affects less than 5% of healthy women (Reeds et al., 2003).

3.5.2.1 Causative agent

Candida albicans is both the most frequent colonizer and responsible for most cases of VVC (Singh, 2003). *Candida* species are the part of the lower genital tract flora in 20-50% of healthy asymptomatic women (McClelland et al., 2009). Establishing *Candida* species as the cause of vaginitis can be difficult because as many as 50% of asymptomatic women have candidal organisms as part of their endogenous vaginal flora (Sobel, 1993). Over the last decades there has been an increment in the frequency of VVC caused by non-albicans species with *Candida glabrata* consistently being the leading one (Ray et al., 2007). *Candida albicans* is responsible in approximately 85% cases while others such as *Candida glabrata, Candida krusei, Candida tropicalis* and *Candida stellatoidea* cause vaginitis to the less extent (Van Dyck et al, 1999). VVC has increased in past three decades due to antifungal resistance to *Candida* species, a change in women's health quality and the failure to eradicate *Candida* species from the female genital tract (Saporiti et al., 2001).

3.5.2.2 Predisposing factors

Pregnancy is the major risk factor for VVC. The use of antibiotics can inadvertently kill normal bacteria in the vagina and cause the overgrowth of *Candida* species (Patricia, 2006). Frequent sexual intercourse is the strongest risk factor for VVC (Besty, 1990). Others include use of broad spectrum antibiotics (Singh. 2003), poorly controlled diabetes (Leon et al, 2002), diaphragm, spermicide, topical antimicrobial agents, tight clothing and underwear, douches and perfumed feminine hygiene sprays, thyroid disorders and corticosteroid (Johnson, 1990), intrauterine contraceptive devices (Chassot et al., 2008), young age at first intercourse and receptive oral sex (Foxman, 1990; Geiger et al., 1995), HIV infections/ AIDS (Hedayati et al., 2010; Duerr et al., 2003).

3.5.2.3 Sign and Symptoms

Women with VVC frequently complain of white, thick curd like, clumpy (cottage cheese-like) discharge with no odor and a normal pH. Pruritus, vaginal irritation, dysuria, vulvar and vaginal edema and erythema and occasionally scaling and fissures of vulvar tissue and pain during intercourse are also observed. The commonest cause of vulva pruritus is VVC which may be associated with the lowered renal threshold for sugar which occurs in pregnant women (Leon et al., 2002). Other symptoms during pregnancy include painful urination, pain during sex, enlarged or swollen vulva and white discharge from vagina, itching or discomfort in vaginal area.

3.5.2.4 Pathogenesis of VVC during pregnancy

VVC is more serious and dangerous during pregnancy because hormonal changes in the body and the infection become chronic or recurring. It occurs in 10% of women during the first trimester of pregnancy and in one third to one half of women in third trimester. Symptomatic occurrence is maximally increased during third trimester and pregnancy brings symptomatic yeast infections often multiple ones per pregnancy (Sobel, 1993).

Candida and pregnancy often go hand in hand and during pregnancy the immune system of mother weakens making it easier for them to come down with other illness or diseases.

The changes in the vaginal environment, such as an increase in glycogen production in pregnancy or altered estrogen and progesterone levels from the use of oral contraceptives, enhance the adherence of *Candida albicans* to vaginal epithelial cells and facilitate the germination of yeast (Sobel et al., 1998). A cytosol receptor or binding system for female reproductive hormones has been documented in *Candida albicans*. These changes may transform asymptomatic colonization into symptomatic infection during pregnancy. Molecules present in the most external layers of *Candida* cells are essential for the adherence to host surfaces. Receptors for fibrinogen, fibronectin, surface proteins and other components of extracellular matrix have been found in Candidal surfaces. Adherence is essential in the early stages of colonization and tissue invasion. It is achieved by the combination of specific (ligand receptor interactions) and non-specific (electrostatic charges, vanderwaals forces) mechanisms, which allow the yeast to attach to a wide range of tissue types and inanimate surfaces (Cotter and Kavanagh, 2000).

3.5.2.5 Adverse outcome

Although VVC is both treatable and mild, when left untreated complications like PID, infertility, ectopic pregnancy, pelvic abscess, menstrual disorders, spontaneous abortion and premature birth may occur. Epidemiologically, vaginal *Candida* infections are important as they may increase the risk of acquisition of HIV/AIDS and also viral shedding in HIV infected women (Hedayati et al., 2010). Neonates may develop invasive candidiasis due to premature rupture of amniotic membrane (Tiraboschi et al., 2009).

3.5.2.6 Diagnosis of VVC

For the specific diagnosis of VVC a number of steps are recommended i.e. determination of vaginal pH (normal 4-4.5) which means that a higher pH more than 5 is suggestive of BV

or trichomoniasis (CDC, 2002), wet mount preparation of the vaginal discharge for the identification of yeast cells and mycelia (Marrazzo, 2002); a 10% KOH prepared of vaginal discharge (Geiger et al., 1995). Gram stain preparation may also be used since yeast is Gram positive. If microscopic studies are negative and suspicion of VVC continues to be high, HVS for fungal culture on SDA is done (Sherrard 2001; Sobel et al., 1998).

3.5.3 Trichomoniasis

Trichomonas vaginalis, a motile protozoon with four flagella (Carr et al., 1998), is the third most common cause of vaginitis (Egan and Lipsky, 2000). It affects 180 million women worldwide and currently accounts for 10 to 25% of vaginal infections (Sobel, 1993). This genitourinary tract infection is more common in females i.e. 25 to 50% of sexually active women are infected while only about 5% of men are infected (Madigan et al., 2003). The incidence of trichomoniasis is decreasing in most industrialized countries (Kent, 1991).

Trichomonads are transmitted sexually and may be identified in 30 to 80% of the male sexual partners of infected women (Sobel, 1993). It principally infects the squamous epithelium of the genital tract and may act as a vector for other venereal diseases (Laga et al., 1993, Cotch et al., 1991). Incubation period is between 4 to 28 days. Infection may persist for longer periods in females, but for less than 10 days in males. It increases the transmission rate of the HIV (Laga et al., 1993). Risk factors include use of an IUD, cigarette smoking and multiple sexual partners and inadequate standards of personal hygienic practices (Barbone et al., 1990, Haukkamma et al., 1986).

3.5.3.1 Signs and symptoms

From 20 to 50% of women with trichomoniasis are asymptomatic (Lossick et al., 1991). Since most men do not present symptoms with trichomoniasis, the infection is often not diagnosed until the women develop the symptoms of vaginitis. Symptoms of trichomoniasis can appear as early as 4 days after sex with infected partner. Classic

manifestations of vaginal trichomoniasis in women include a purulent, frothy, yellow/green discharge with a watery to milky consistency and an abnormal/foul odor, itching and tenderness in and around vagina, swollen labia and pain during sex and intercourse. The physical examination may reveal superficial vulvovaginal erythema, and the discharge usually has an elevated pH (Haefner, 1999).

3.5.3.2 Trichomoniasis during pregnancy and its adverse outcomes

The worldwide prevalence of trichomoniasis is 174 million and it accounts for 10% to 25% of vaginal infection (WHO, 2001). In patients with trichomoniasis, changes in estrogen and progesterone levels, as well as elevations of vaginal pH and glycogen levels, may enhance the growth and virulence of *Trichomonas vaginalis.* Sexual partners should be treated and instructed to avoid sexual intercourse until both partners are cured (CDC, 1998).

Complications include premature rupture of membranes and preterm delivery (Cotch et al., 1991), increased risk of HIV transmission, low-birth weight infant and increased risk of inflammation of fallopian tubes (Berg, 1984).

Trichomoniasis can be treated with metronidazole or tinidazole (Greenhood et al., 1997). The CDC advices treatment of symptomatic pregnant women with a single dose of 2 gm of metronidazole but not asymptomatic pregnant women (Owen and Clenney, 2004).

3.5.4 Other types of vaginitis

(i) Chlamydia vaginitis

(ii) Gonococcal vaginitis

(iii) Viral vaginitis

(iv) Noninfectious vaginitis (Atrophic vaginitis)

3.6 **Prevalence of BV, VVC and Trichomoniasis**

Vaginitis has been observed in 61-90% pregnant vaginal carriers (Carrel, 1983). Prevalence of vaginal discharge in India is 30% and in Delhi is 29.9% (NFHS-2, 1999). BV is the most prevalent form of vaginal infection and accounts for 10 to 30% of cases of infectious vaginitis in women of childbearing age (Hay, 2000; Owen and Clenney, 2004). The prevalence of BV varies from 10% to 65% (Sumati et al., 2009) and 15 to 23% in pregnant women with up to 50% of women being asymptomatic (Amsel, 1983; Gravelt, 1986, Hill, 1988). BV has been found in 15 to 19% of ambulatory gynecology patients, 10 to 30% of pregnant patients and 24 to 40 % of patients in STI clinics (Bump and Buesching, 1988). BV is particularly common in Sub-Saharan Africa with prevalence ranging from 20%-49% among women presenting to STDS clinics with vaginal discharge in east Africa (Fonck et al., 2000) from 29%-52% among pregnant women attending antenatal clinics in Central and West Africa (Blankhart et al, 1999; Govender et al., 1996).

About 30% of vaginitis is caused by *Candida* species (Pappas et al., 2009). Three quarters of women experience at least one episode of VVC in their life time and about one half of these women experience a recurrence (Hedayati et al., 2010). Also half of college women by the age of 25 will have one episode of VVC diagnosed by a physician (Sobel, 1997). VVC is the second most common cause of vaginitis in the United States and the most common cause in Europe (Kent, 1991).

In the study of Medical College in India, the most common cause of vaginal infection was BV by 29% followed by VVC by 18.8% and *T. vaginalis* by 11.3% (Acharya, 1988). Also in a community study conducted by Bang et al. (1989) from India found that 14% of women had trichomoniasis, 62% of women had BV and 34% had VVC. The study from Egypt showed that 18% of women had trichomoniasis, 22% had BV and 11% had VVC. Similarly in a study by Harms (1995) in Madagascar among 231 women, 90% women had vaginal discharge; the most prevalent was BV (37%) followed by trichomoniasis by (31%)

and candidiasis by (30%). In Quettte among 500 women vaginitis was found in 33.48%, BV in 30.7%, VVC in 10% and trichomoniasis in 7.2% (Sami and Baloch, 2005). Similarly, among 2,927 Danish pregnant women, 13.7% had BV (Thorsen et al., 2006).

In a study conducted among 206 pregnant women in New Guinea, VVC was observed in 23% of women, *Trichomonas vaginalis* in 19% and BV in 23% (Klufio et al., 1995). Evan (2000) found the prevalence of BV in 8.4%, VVC in 32.9% and trichomoniasis in 2.9% in London. In South Africa the vaginal infection was found in 20-49% of antenatal and family planning clinics attenders. Among them 60.9% had vaginal discharge, 46.8% had BV and in 50% cases mixed infection of *Candida* and *Trichomonas* was found (Kanter, 1996). Thomas (1996) found vaginitis among 205 pregnant women and the prevalence of VVC, trichomoniasis and BV was 26.2%,19.9%, 20.6% respectively the study conducted in Jakarta among 4519 pregnant found that 18% had BV and 3.8% had trichomonioasis (Joesoef et al., 1995). In the study conducted in among 1223 pregnant women from Amir-Almmemenin general Hospital in Iran, the prevalence of BV and *Trichomonas vaginalis* were detected in 16% and 5.5% respectively (Azargoon and Darvishzadesh, 2006).

In a study conducted in Nepal, VVC was found in 6.4%, Trichomoniasis in 0.9%, trichomoniasis with BV in 1.3% and BV in 19.5% (Padhye, 2003). The prevalence of BV was found to be 69% in symptomatic women and 62% in asymptomatic women. It also showed the prevalence of trichomoniasis to be 14.1% (Aryal, 1997). Rizvi and Luby (2004) in Nepal found BV in 25% and trichomoniasis in 17%. A study conducted in 500 women of reproductive age at gynecological OPD of TU teaching Hospital, BV was found in 2.5% and among BV positive patients, 67.3% were asymptomatic and 32.7% were asymptomatic (Manandhar et al., 2005).

3.7 Diagnosis of vaginitis

3.7.1 Microscopy

Microscopic examination of a wet-mount preparation is done for observing motile Trichomonads, clue cells, fungal hyphae, increased numbers of polymorphonuclear cells (seen in trichomoniasis) or round parabasal cells (seen in atrophic vaginitis). Clue cells are vaginal epithelial cells that are coated with the coccobacilli. The examination of clue cells is best made by examining the edges of the cells. A normal cell has sharp, clear, linear edges whereas a clue cell has a granular, cloudy, rough edge. The presence of clue cells is the most reliable of the criteria indicating a diagnosis of BV (Thomason et al., 1990).

Gram stain of vaginal discharge may be gold standard for diagnosis of BV. In BV, the normal *Lactobacillus*, which manifests as gram-positive rods, is supplanted by a large numbers of gram variable coccobacilli which may be seen adhering to the epithelial cells (clue cells) or in clumps in the vaginal materials.

3.7.2 KOH preparation and Whiff Test

The vaginal discharge is placed on a slide with 10% KOH solution. A coverslip is placed on the slide before examination under a microscope using low power. This is useful for detecting candidal hyphae, mycelial tangles and spores. The whiff test is positive if a "fishy" or amine odor is detected when KOH is added to the vaginal discharge. The odor results from the liberation of amines and organic acids produced from the alkalization of anaerobic bacteria. A positive whiff test is suggestive of BV (Hill, 1993).

3.7.3 Litmus testing for pH determination

A normal vaginal pH is between 3.8 and 4.2. The pH level can be determined by placing litmus paper in vaginal secretions or against the lateral vaginal wall. A pH greater than 4.5 is found in 80 to 90% of patients with BV and frequently in patients with trichomoniasis

(Carr et al., 1998). The lactobacilli flora and normal pH is maintained in patients with VVC but the flora is altered and the pH elevated in BV and trichomoniasis. Thus a normal vaginal pH in symptomatic women suggests a diagnosis of VVC whereas an elevated pH supports a diagnosis of BV or trichomoniasis. The lactobacillary vaginal flora and hence the vaginal pH is unaffected by pregnancy (Hay, 2000).

3.8 URINARY TRACT INFECTION (UTI)

3.8.1 Introduction of UTI

UTI can be defined as the detection of both bacteriuria (the multiplication of the organisms in urinary tract) and the presence of more than a hundred thousand organisms per ml of midstream sample of urine (Chakraborty, 1995). It is also defined as the detection of both bacteriuria, 10^5 cfu/ml and pyuria i.e. 10 leucocytes/HPF (Goya et al., 1997). UTI is very common which result in more than 7 million outpatient visits each year (Braunwald, 2001) and two thirds of the patients are women (Mandell, 2000). UTI has become the most common hospital acquired infection accounting for as many as 35% of nosocomial infections and it is the second most common cause of bacterimia in hospitalized patients (Stamm, 2002; Kolawole et al., 2009). Approximately 10% of people will have a UTI at some time during their lives. The infection rate is highest in women and 20-50% of will suffer a clinical episode during their lifetime (Leigh, 1996). UTI accounts for approximately 10% of office visit by women and 15% of women will have UTI at some time of their life. In pregnant women the incidence can be as high as 8% (Mikhail, 1995).

Usually UTI is caused by bacteria that can also live in digestive tract, in the vagina, or around the urethra, which is at the entrance to the urinary tract. Most often these bacteria enter the urethra and travel to the bladder and kidneys. *E. coli* accounts for the majority of isolates leading to uncomplicated UTI. Others are *Staphyloccus saprophyticus, Klebsiella* species, *Proteus* species, *Staphylococcus aureus, Enterococcus* species and *Enterobacter*

species (Gupta et al., 2001). *E. coli* is present in between 80-90% of UTI and up to 95% of acute pyelonephritis (Conolly et al., 1999; Delzell, et al 2000) and Novobiocin resistant *Staphyloccus saprophyticus* is the true gram positive pathogen of UTI (Johnson, 1990) and found in 5-10% (Nicolle, 2008). *E. coli* with multidrug resistant strains has been found to be the commonest cause of UTI among pregnant women (Dalzell, 2000).

The signs and symptoms include burning feeling during urination, frequent or intense urges to urinate, pains in the back or lower abdomen and cloudy, dark, bloody or unusual smelling urine, fever or chills (Nkudic, 2005)

3.8.2 UTI during pregnancy

The organisms that cause UTIs during pregnancy are the same as those found in non pregnant patients. *E. coli* accounts for 80 to 90% of infections and others such as *Proteus mirabilis, Klebsiella pneumonia,* Group B Streptococci and *Staphylococcus saprophyticus* are also common. Enterococci, *Gardnerella vaginalis* and *Ureaplasma ureolyticum* are less common (Kariuki, 2007).

UTI has been reported among 20% of the pregnant women and it is the most common cause of admission in obstetrics wards (Mikhail, 1995). In pregnancy women are at higher risk and about 6% have been found to have asymptomatic bacteriuria due to anatomical and physiological changes such as changes in hormonal level (Kass, 1970). UTI may manifest as asymptomatic bacteriuria (ASB) or symptomatic bacteriuria (SB). The prevalence of ASB has been previously reported to be 2% to 13% in pregnant women (Delzell et al, 2000; Christensen 2000) compared with that of SB which occurs in 1-18% during pregnancy (Masinde et al, 2009). Approximately 25-33% of women who experience bacteriuria during pregnancy will have reinfection. Although pregnancy does not increase the rates of ASB, it does increase the risk that it will progress to a full-blown infection. In early pregnancy, frequent urination, a common symptom of UTI, is most likely due to

pressure on the bladder. However in early pregnancy frequent urination, a common symptom of UTI is most likely due to pressure on the bladder.

3.8.3 Predisposing factors during pregnancy and pathogenesis

The main factors predisposing to bacteriuria are pregnancy and sexual intercourse (Lucas, 1993). Sexual activities increase the chances of bacterial contamination of female urethra. Having intercourse may also cause UTI in women because bacteria can be pushed into the urethra. Bladder infection in women often occurs from the massaging effect of sexual intercourse on the urethra which introduces bacteria from urethra to the urinary bladder. This anatomical relationship of the female urethra to the vagina makes it liable to trauma during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during pregnancy/childbirth (Kalawole et al., 2009). Nearly 80% of all urinary tract infections occur within 24 hours of intercourse.

During pregnancy 2-11% of pregnant women have ASB and of those 13-17% will develop a kidney infection late in their term. Beginning in week 6 and peaking during weeks 22 to 24, approximately 90% of pregnant women develop ureteral dilatation, which will remain until delivery. The combination of mechanical, hormonal and physiologic changes during pregnancy contributes to significant changes in the urinary tract, which has a profound impact on the acquisition and the natural history of bacteriuria during pregnancy (Patterson et al., 1997). Pregnancy predispose to upper UTI due to

(i) Dilation of ureter and renal pelvis
(ii) Increased bladder volume and decreased bladder tone, decreased ureteral tone that leads to increased urinary stasis and ureterovesical reflux (Patterson, 1997)
(iii) Atony-reduce tone in ureteric musculature during pregnancy results from inhibitory effect of progesterone

(iv) The physiologic increase in plasma volume during pregnancy decreases urine concentration and increases urinary progestins and estrogens, which may lead to a decreased ability of lower urinary tract to resist including bacteria. This decreased ability may be caused by decreased ureteral tone or possibly by allowing some strains of bacteria to selectively grow (Patterson, 1997; Lucas, 1993)

(v) Up to 70% of pregnant women develop glycosuria, which encourages bacterial growth in the urine (AI-Issa, 2009).

(vi) Temporal incompetence of vesico-urethral valves (Chakraborty, 1995)

The upshot of these changes is that it takes longer for urine to pass through your urinary tract, giving bacteria more time to multiply and take hold before being flushed out, and it also becomes easier for the bacteria to travel up to the kidneys.

3.8.4 Clinical presentation of UTI among pregnant women

In pregnant women UTIs have 3 principle presentations ie asymptomatic bacteriuria, acute cystitis and pyelonephritis.

3.8.4.1 Asymptomatic bacteriuria (ASB)

SB has been historically defined as finding more than 10^5 CFU per ml of urine (Stamm, 1993). SB may exist in asymptomatic patients which subsequently increase the risk of developing pyelonephritis. ASB is common, with a prevalence of 10% during pregnancy. Untreated ASB leads to the development of symptomatic cystitis in approximately. It is also associated with an increased risk of intrauterine growth retardation and low birth weight (Harris, 1976). 30% of patients with untreated ASB develop symptomatic cystitis and upto 50% develop pyelonephritis (Kass, 1970). The relatively high prevalence of ASB during pregnancy, the significant consequences for women and for the pregnancy, plus the

ability to avoid sequelae with treatment justify screening pregnant women for bacteriuria. is recommended. It recommended that a urine culture should be obtained at the first prenatal visit or between 12 to 16 weeks gestation (ACOG, 1996).

3.8.4.2 Acute cystitis

Acute cystitis is distinguished from ASB by the presence of symptoms such as dysuria, urgency and frequency in a febrile patients with no evidence of systematic illness. It is also known as bladder infection and is the most common type of UTI. The most common symptoms of a bladder infection are burning with urination (dysuria), frequency of urination, an urge to urinate, no vaginal discharge and no significant pain (Nicolle, 2008).

3.8.4.3 Pyelonephritis

The infection of the upper urinary tract or kidney is known as pylonephritis, and is potentially more serious. Acute pyelonephritis during pregnancy is a serious systemic illness that can progress to maternal sepsis, preterm labor and premature delivery. The diagnosis is made when the presence of bacteriuria is accompanied by systemic symptoms or signs such as fever, chills nausea vomiting and flank pain. Symptoms of lower tract infection (i.e. frequency and dysuria) may or may not be present. Pyelonephritis occurs in 2% of pregnant women and upto 23% of these women have a recurrence during the same pregnancy (Gilstrap, 1981). The most common reason for treatment failure in pyelonephritis is antibiotics resistance. UTI recur in approx 4 to 5% per pregnancies and the risk of developing pyelonephritis is the same as the risk with primary UTIs.

3.9 Association between BV and UTI

Association of BV with UTI (vice versa) probably begins with an increase in the pH of the vagina because of reduction of vaginal lactobacilli-producing lactate and hydrogen peroxide. The normal vaginal flora may be replaced by predominantly anaerobic flora

(Hillerbrand et al., 2002). Frequent sexual intercourse, which was also linked to both these infections, may also contribute to this phenomenon (Harmanli, 2000). Factors causing colonization of gram-negative bacilli around urethra are unknown, but it seems that urethral massage during sexual activity has a facilitating role; furthermore, it seems that proximity of urethra to anus, shortness of female urethra, its location under labia, warm and moist environment of perineum have important roles to play. It might be necessary to carry out test for urinary tract infections in women with BV (vice versa).

Harmanli (2000) diagnosed BV in 67 women of whom 15 (22.4%) also had UTI. Among those who didn't have BV 6(10%) had UTI. In another study of 67 women with BV, 15(22.4%) had UTI, compared with 6(9.7%) of 62 women who didn't have BV. They concluded that women with BV were 2.79 times more likely to develop a UTI (Carson, 2000). Hilliarbrand et al. (2002) observed that among 503 pregnant women suffering from BV 13.6% also had UTI where as only 6.6% of women without BV had UTI. Sharami (2007) found that among 322 pregnant women 23.6% had BV and 23.6% with BV had UTI compared to 9.8% of those without BV. Pregnant women with BV are at increased risk for UTI. Sumati (2009) found that out of 55 BV positive pregnant women, 42.27% had UTI.

Treatment of women with UTI also needs treatment of genital tract infections. In 1989, the relationship between BV and UTI in women using diaphragms was reported (Hootan, 1989). In 2000 there was a report that women suffering from BV are at a greater risk of urinary tract infection than others (Hillerbrand, 2002). The same investigator in 2002 reported the association of UTI in pregnant women with BV.

3.10 Antibiotic susceptibility test (AST)

The major concern of AST is to determine whether the bacterial etiology of concern is capable of expressing resistance to the antibiotics that are potential choices as therapeutic agents for managing the infection (Jane et al., 2006). So this test measures the ability of an

antibiotic to inhibit bacterial growth in vitro. This ability may be estimated by either the dilution method or the diffusion method.

Multidrug resistance organisms (MDROs) are defined as microorganisms predominately bacteria that are resistance to one or more classes of antimicrobial classes of antimicrobial agents (Jane et al., 2006). Although the names of certain MDROs describe the resistance to only one agent eg methicillin resistance *Staphylococcus aureus* (MRSA) and vancomycin resistance enterococccci (VRE) these pathogens are frequently resistant to most available antimicrobial agents. In addition to MRSA and VRE, certain Gram negative bacteria (GNB) including those producing extended spectrum beta lactamases (ESBLs) and others that are resistant to multiple classes of antimicrobial agents are of particular concern. In addition to *E. coli* and *Klebsiella pneumonia, Acinetobacter baumannii* is resistant to all antimicrobial agents or all except impenem (Mahgoubs et al., 2002; Gales et al., 2001) and organisms such as *Stenotrophomonas maltophilia* (Hanes et al., 2002) are intrinsically resistant to the broadest spectrum of antimicrobial agents. Multidrug resistant *Streptococcus pneumonia* is resistant to penicillin and other broad spectrum agents such as macrolides and floroquinolones (Ryan et al., 2006). Some strains of *Staphylococcus aureus* have intermediate resistance to vancomycin ie vancomycin intermediate *Staphylococcus aureus* (VISA), vancomycin resistant *Staphylococcus aureus* (VRSA) (Whitener et al., 2004) have affected specific population such as hemodialysis patients.

Studies have documented increased mortality, hospital lengths of stay and hospital charge associated with multidrug resistance gram negative bacilli (MDR-GNBs) including an outbreak of ESBL producing *Klebsiella pneumoniae* (Stonza et al., 2003) and the emergence of 3rd generation cephalosporin resistance in *Enterobacter* species (Cosgrove et al., 2002). Prevalence of MDROs varies temporally Prevalence of MDROs varies temporally, geographically and by healthcare settings (Zinn et al., 2004). Antibiotics resistant rates are strongly correlated with hospitalized tertiary level care and facility type

(Diekema et al., 2004). The prevalence of MDROs has increased steadily during last several decades in US hospitals and medical centers (Klevens et al., 2006). By the early 1990s, MDR accounted for 20-25% of *Staphyloccus aureus* isolates from hospitalized patients (Boyce et al., 1994) and by 2003, 59.9% of Staphylococcus aureus isolates in National Nosocomial Infection Surveillance (NNIS) ICUs were MRSA (NNIS, 2003).

Gram negative Bacteria resistant to ESBLs, floroquinolones, carbapenems and aminoglycosides also have increased in prevalence. A study in 1997 in US found that among *Klebsiella pneumoniae* strains isolated in the US, resistance rate to ceftazidime and other third generation cephalosporins were 6.6%, 9.7%, 5.4% and 3.6% for blood stream, pneumonia, wound and UTI respectively (Jones, 2001). However, in 2003, 20.6% of all the isolates of *Klebsiella pneumonia* were resistance to these drugs. Similarly between 1999 and 2003, *Pseudomonas aeruginosa* resistance to floroquinolones antibiotics increased from 23% to 29.5% in ICUs (Fridkin, 2001).

WHO recommended modified Kirby-Bauer disc diffusion technique, is used by the most laboratories to test routinely for antibiotic susceptibility. Using this test, antibiotic resistance is detected by allowing the antibiotics to diffuse from a point source, commonly in the form of an impregnated filter paper disc, into an agar medium that has been seeded with the test organisms. Visible growth of bacteria occurs on the surface of the agar where the concentration of antibiotic has fallen below its inhibitory level for the test strain (Collee *et al.,* 1999).

CHAPTER- IV

4 MATERIALS AND METHODS

4.1 Materials

A list of the materials used in this study is given in Appendix-II

4.2 Study Design

Cross sectional descriptive study was done to determine the prevalence and distribution factors of different types vaginitis and the association of BV with UTI among the pregnant women visiting "Paropakar Maternity and Women's Hospital" Thapathali, Kathmandu. The hospital is located centrally at the capital city, Kathmandu.

4.3 Study Population

Pregnant women (N=230) presenting to the hospital for antenatal care over a period of six months starting from June 2010 to December 2010 were enrolled in this study.

4.4 Data collection

Primary data was collected from the study population. Structured questionnaire was administered to collect relevant information from each target population.

4.5 Consent

Verbal consent was taken from each participant before filling up the questionnaire and specimen collection. Detailed clinical history and demographic features were recorded.

4.6 Laboratory diagnostic procedures

4.6.1 Sample collection

4.6.1.1 Collection of HVS and urine specimen

Duplicate HVS were collected using sterile cotton swab with the help of gynecologist, trained nurse and metron. Exposing the posterior fornix with a sterile vaginal speculum (coscos), a sterile swab stick was inserted to pick a HVS. The sterile swab was provided from the laboratory. The pregnant women whose vaginal swab was collected were given a sterile dry, clean wide neck and leak proof collection bottle for urine collection (5-10ml). They were instructed properly for the collection of midstream urine (Appendix V).

4.6.1.3 Transportation of specimen

One HVS was inserted in the labeled sterile test-tube and capped tightly for pH determination and whiff test. And another swab was inserted in the labeled sterile test tube containing 0.5 ml of normal saline for culture, wet mount and gram staining. The specimen containing containers were labeled with date, name, time of collection and admission no. of the patient along with the request form. The collected specimen i.e. HVS and urine were immediately taken to the laboratory and processed according to the standard methods.

4.7 Processing of the specimen

4.7.1 High vaginal swab

For one of the HVS kept into sterile capped test tube, pH determination and whiff test was done. Another HVS was used for culture, wet mount and Gram stain. The pH determination was done by using suitable indicator paper.

4.7.1.1 Whiff test

i. The High HVS was rolled on a clean and grease free new slide.

ii. A drop of 10% KOH was added to the slide.

iii. The slide was hold close to nose to detect the amine order. The sample was considered as positive if fishy smell was noticed. The odor should be noticed as soon as possible as the specimen would quickly become odorless upon standing.

4.7.1.2 Wet mount microscopy

It was done for viewing yeast cells, clue cells and motile parasites i.e. *Trichomonas vaginalis.*

Procedure for wet mount microscopy

(i) A drop of normal saline was added to the center of clean and grease free slide.
(ii) Thin and homogenous suspension of HVS was made.
(iii) The suspension was covered with a cover slip.
(iv) Then it was examined under microscope at X10 and X40.

The examination should be done before drying up the preparation.

4.7.1.3 Gram stain microscopy

Gram staining of vaginal swab was done to observe Gram positive yeast cells, clue cells and Gram variable coccobacilli. The procedure for Gram staining is given in Appendix V.

4.7.1.4 Culture for *Candida* species

Primary inoculum was made at one corner of labeled petriplate having Sabouraud's Dextrose Agar (SDA) media. With the sterile loop, the primary inoculum was streaked on SDA media. The plate was incubated in incubator at 37°C for 24-48 hours. The cream colored pasty colonies if appeared after 24-48 hours of incubation were considered as positive for fungal pathogens. The colonies were Gram stained and if Gram positive yeast cells were observed, then it was preceded for germ tube test.

Procedure for Germ Tube Test (Cheesebrough, 2000)

(i) 500 micro liter of human serum was pipette into a small test tube.
(ii) A yeast colony from the SDA plate was inoculated on the serum using a sterile loop. The tube was incubated in the incubator at 35-37°C for 2-3 hours.
(iii) Using a Pasteur pipette, a drop of serum yeast culture was transferred to a glass slide, and covered with a cover slip.
(iv) The preparation was examined using the 10X and 40x objectives with the condenser iris diaphragm closed sufficiently to give good contrast.
(v) Sprouting yeast cells that are tube-like outgrowths from the cells, known as germ tubes were examined. If sprouting yeast cells were seen, the culture was reported as *Candida albicans* isolated.

4.7.1.5 Bacteriological culture of High Vaginal Swab

Petri plates with sterile Blood Agar (BA) and MacConkey Agar (MA) were taken. A loopful of normal saline suspension was taken streaked on the entire surface of each media with the help of loop. After the incubation of 24 hours at 37°C aerobically, the culture plates were observed for the growth.

4.7.2 Culture of urine specimen

The urine sample was cultured onto MA and BA by the semi-quantitative culture technique using a standard loop. After mixing the urine sample in the container thoroughly, a loopful of sample touched to the center of the plate, from which the inoculums was spread in a line across the diameter of the plate.

(i) Without flaming the loop was drawn across the entire plate, crossing the first inoculums streak numerous times to produce isolated colonies.
(ii) The plats were incubated aerobically at 35-37°C overnight.

(iii) The approximate number of colonies was counted and number of bacteria i.e. colony forming unit (CFU) per ml urine estimated in accordance to the volume of urine inoculated previously. For e.g., 100 colonies on inoculating 0.001 ml of urine would correspond to 10^5 CFU/ml.

Reporting of culture positivity

(i) Less than 10^4/ml organisms : Not significant
(ii) 10^4-10^5/ml organism: Doubtful significance (suggest repeat specimen)
(iii) More than 10^5 organisms: Significant bacteriuria

However if the culture indicated the appearance of 3 or more organism types with no predominating organism, this was interpreted as due to possible contamination of the specimen and asked for another specimen (Forbes et al., 2002).

4.7.3 Isolation and identification of organisms from HVS and urine

Identification of isolates was done by using microbiological techniques like morphological appearance of the colonies, Gram's staining reactions and biochemical properties (Bailey & Scott's, 1990; Cheesebrough, 2000; Mackie and McCartey, 1998).

Each of the organisms was isolated in pure form before performing biochemical and other tests. Gram staining of the isolates was done from the primary culture. For gram negative organism an speck of single isolated colony from MA and for Gram positive organism from BA was transferred into the nutrient broth and incubated at 37°C for 4 hours. It was then sub-cultured on dried NA plate and incubated at 37°C for 24 hours. Thus obtained overnight incubated culture of organism on NA was used to perform catalase, oxidase, other biochemical tests and antibiotics susceptibility test. Pure colonies on the media plates were inoculated onto appropriate biochemical tests for the identification of the bacterial isolates.

Gram positive organisms were identified primarily on the basis of their response to Gram's staining, catalase, oxidase and cogulase tests. The biochemical tests used for the identification of Gram negative bacterial isolates include catalase test, oxidase test, indole test, MR test, Vogus Proskeuer test, Citrate Utilization test, Oxidation Fermentation test, TSI test, Motility test and Gas production test (Cheesebrough 2000). The composition and preparation of the media and reagent used in the biochemical tests are mentioned in the Appendix III.

4.7.4 Antibiotics Susceptibility Testing

The AST was performed according to Kirby-Bauer sensitivity testing method (NCCLS, 1999).

(i) Mueller Hinton Agar (MHA) was prepared and sterilized as instructed by the manufacturer.

(ii) The pH of the medium 7.2-7.4 and the depth of the medium at 4 mm (about 25 ml per plate) were maintained in petridish.

(iii) Using a sterile wire loop, a single isolated colony of which the sensitivity pattern is to be determined was touched and inoculated into a nutrient broth tube and was incubated for 2-4 hrs.

(iv) After incubation in a good light source, the turbidity of the suspension was matched with the turbidity standard of McFarland 0.5.

(v) Using a sterile swab, a plate of MHA was inoculated with the bacterial suspension using carpet culture technique.

(vi) Using sterile forceps, appropriate antimicrobial discs (6 mm diameter) was placed, evenly distributed on the inoculated plates, not more than 6 discs were placed on a 90 mm diameter Petri plate.

(vii) Within 30 minutes of applying the discs, the plates were taken for incubation at 35^0C for 16-18 hrs.

After overnight incubation, the plates were examined to ensure confluent growth and the diameter of each zone of inhibition in mm was measured and results interpreted.

4.7.5 Purity plate

The purity plate was used to ensure that the inoculation used for the biochemical tests is pure and also to see whether the biochemical tests are performed in an aseptic condition or not. Thus, while performing biochemical tests, the same inoculum was subcultured in respectively medium and incubated. The media was then checked for the appearance of pure growth of organisms.

4.7.6 Quality control for test

Quality of each test was maintained by using standard procedures. The quality of each agar plates prepared was tested by incubating one plate of each lot on the incubator. During identification of organism, for each test ATCC control positive and control negatives was taken simultaneously. Quality of sensitivity tests was maintained by maintaining the thickness of Muller Hinton agar at 4mm and pH at 7.2-7.4. Similarly antibiotics disc containing the correct amount as indicated were used. Strict aseptic conditions were maintained while carrying out all the procedures.

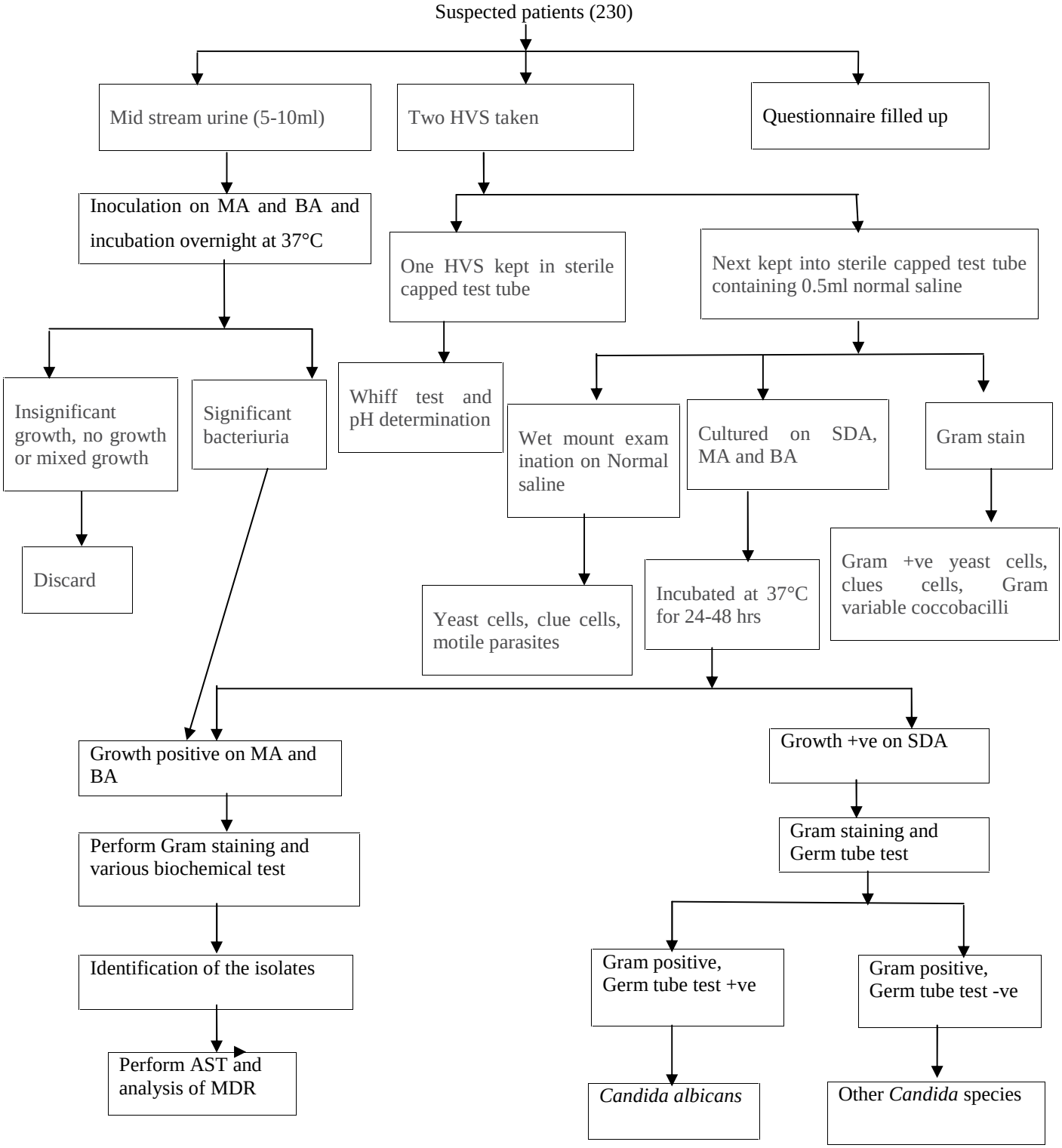
4.7.7 Safe disposal of sample and contaminated tools

After handling the specimen, it was discarded in the specific container and all the equipment used should be placed in the beaker containing Lysol which then should be sterilized. The swab should be incinerated.

4.8 Data Analysis

The data were analyzed using statistical software SPSS version 13.0. Chi-square was applied to find the association at 95% confidence level.

Figure 1 Flow diagram for the processing of HVS and urine



CHAPTER-V

5 RESULTS

Among 230 HVS sample, 40.0% samples were positive for infectious vaginitis while 60.0% were negative. The overall prevalence rate of UTI was found to be 13.9%.

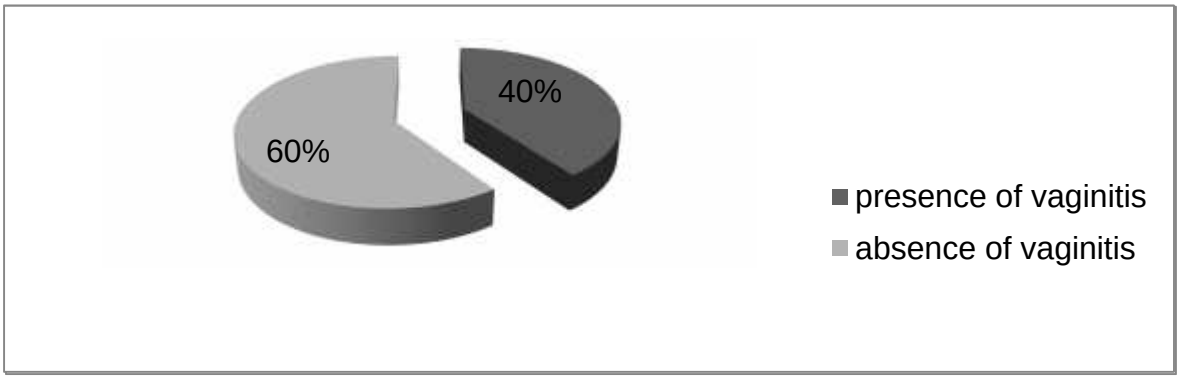


Figure 1 Prevalence of vaginitis among suspected pregnant women

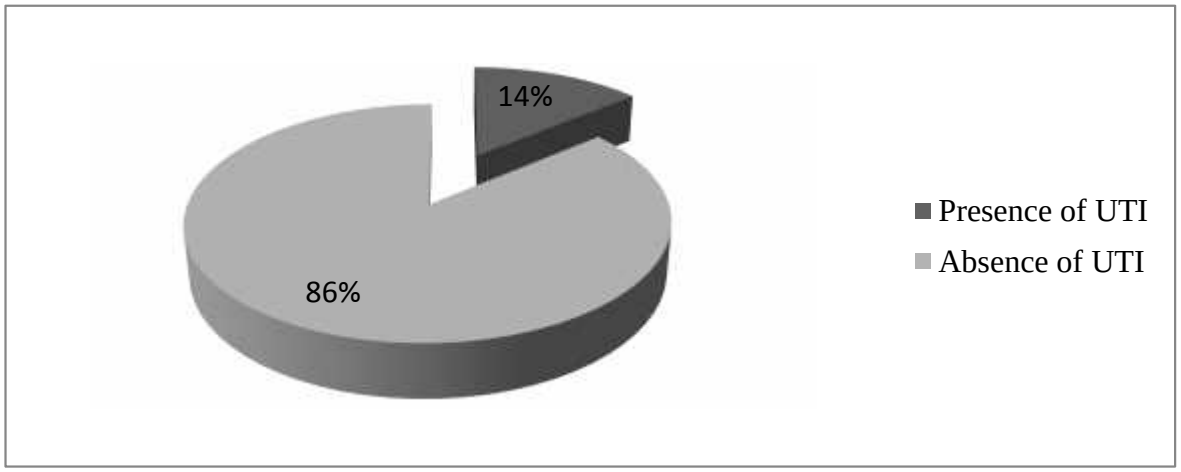


Figure 2 Prevalence of UTI among pregnant women

5.1 Demographic distribution of vaginitis cases

Out of 230 suspected HVS sample, 64.3% were collected from outpatient department (OPD) of the hospital and 35.7% were from patients admitted to the hospital (inpatients). Majority of the infected patients were from inpatient department 26.9%. The numbers of patients from urban areas were higher 72.2% than from rural areas 27.8% and the infection was found higher among women from rural area 30.8%.

Table No. 1 Distribution of various demographic factors

S.N	Demographic characteristics	Number (%)	Presence of vaginitis (%)	Statistics (chi-square test)
1	Origin : Inpatient	148 (64.3%)	62(26.9)	P-value >0.05
	Outpatient	82 (35.7%)	30(13.0)	
3	Address :Rural	166(72.2)	71(30.8)	P-value >0.05
	: Urban	64(27.8)	21(9.1)	
4	Occupation: Housewife	167 (72.6%)	66(28.6)	P-value >0.05
	Employed	63 (27.4%)	26(11.3)	
5	Education: Literate	152 (66.1)	40(17.4)	P-value <0.05
	Illiterate	78 (33.9)	52(22.6)	

(Figure in parenthesis indicate percentage)

Most of them were housewife 72.6%, and literate 66.1%. The higher number of the infected women was housewife 28.6% and illiterate 22.6%. The education status was significantly associated with vaginitis ($P < 0.05$) (Table 1).

5.2 Frequency of infection types among pregnant women

BV was most the prevalent type of vaginitis 27.8% followed by VVC 24.3% and then trichomoniasis 0.4%. Out of 92 positive samples, 69.6% had monomicrobial infection and 30.4% had mixed infection (Table 2).

Table No. 2 Distribution of different types of vaginitis among pregnant women

Type of infection	Frequency	Percentage (%)
BV	36	15.7
VVC	28	12.2
BV+VVC	27	11.7
BV+ VVC +Trichomoniasis	1	0.4
Total (N=230)	92	40.0

Out of 56 *Candida* isolates (single infection VVC - 28 and mixed infection-28), 37 (66.0%) were *Candida albicans* and 19 (34.0%) were other *Candida* species (Table 3).

Table No. 3 Frequency of *Candida* isolates

Isolates	Number	Percentage (%)
Candida albicans	37	66.0
Candida species	19	34.0
Total	56	100.0

5.3 Distribution of vaginitis types among different age group

The highest number of patients 61.7% visiting to the hospital was of 20-29 years age group and also had highest infection rate. Infection rate was high among the age group 20 to 29 (44.4%) and least infected group was 40 and above (20.0%). BV was found high in age group 20-29, candidiasis in <20 and mixed infection in 30-39 age group. There was no statistical significance between age and different types of vaginitis (P >0.05) (Table 4).

Table No. 4 Age wise distribution of vaginitis among infected women

Age category	Total	Infection				Positive	Statistic Chi-square test
		BV	VVC	BV+VVC	BV+VVC+ Trichomoniasis		
<20	24	1(4.2)	6(25.0)	0(0.0)	0(0.0)	7(29.0)	P-value >0.05
20-29	142	29(20.4)	16(11.3)	19(13.4)	0(0.0)	63(44.4)	
30-39	59	5(8.5)	6(10.2)	8(13.6)	1(1.7)	21(35.6)	
40and	5	1(20.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	
Total	230	36(15.7)	28(12.2)	27(11.7)	1(0.4)	92(40.0)	

(Figure in parenthesis indicate percentage)

5.4 Distribution of infection types in trimesters of pregnancy

The higher rate of infection was found in third trimester 57(42.5%) of pregnant women in their third trimester were infected followed by second trimester and then first trimester. However the difference was not statistically insignificant (p >0.05)(Table 5).

Table No 5 Distribution of infection by trimesters in pregnancy

Trimester of pregnancy	Total	Infection			Total infected (%)	Statistics Chi-square Test
		BV	VVC	Trichomoniasis		
First	25(10.9)	6(24.0)	2(8.0)	0(0.0)	7(28.0)	P-value= >0.05
Second	71(30.7)	24(33.8)	16(22.5)	0(0.0)	28(39.4)	
Third	134(58.2)	34(25.3)	38(28.3)	1(0.7)	57(42.5)	
Total	230	64(27.8)	56(24.3)	1(0.4)	92(40.0)	

(Figure in parenthesis indicate percentage)

5.5 Association of risk factors with infection status

The infection was higher among women who had complications in previous pregnancy 20(57.1%) compared to those who didn't have complications in previous pregnancy 72(36.9%) and this was found significantly associated (P <0.05). The infection was higher among women who had history of vaginal infection 35(47.9%) than those without such infections 57(36.3%). However, the statistical association between vaginitis and history of vaginal infection was found to be insignificant (P >0.05) (Table 6).

Table No 6 Association of risk factors with vaginitis

History of pregnant women		Infection status		Total	Statistics (Chi-square test)
		Yes (%)	No (%)		
Complications in previous pregnancy	Yes	20(57.1)	15(42.9)	35	P-value <0.05
	No	72(36.9)	123(63.1)	195	
History of vaginal infection	Yes	35(47.9)	38(52.1)	73	P-value >0.05
	No	57(36.3)	100(63.7)	157	

(Figure in parenthesis indicate percentage)

BV was more than twice common among smokers than non-smokers. A greater 28(56.07%) of smokers were found among having BV while only 36(20.0%) of non-smokers had BV. The association between BV and smoking was significant (P<0.05) (Table 7).

Table No 7 Association between bacterial vaginosis with smoking

Smoking Habit	Bacterial Vaginosis		Total	Statistics
	Present	Absent		
Yes	28(56.0)	22(44.0)	50(21.7)	Chi-square test (P<0.05)
No	36(20.0)	144(80.0)	180(78.2)	
Total	64(27.8)	166(72.2)	230(100)	

(Figure in parenthesis indicate percentage)

5.6 Relation of infection with laboratory and clinical findings

BV was diagnosed on the basis of presence of 3 or more than 3 of the following criteria. Out of 64 BV positive sample, homogenous vaginal discharge, presence of clue cells, positive whiff test and vaginal pH higher than 4.5 were found among 87.5%, 68.7%, 90.6% and 84.3% respectively (Table 9).

Table No 8 Laboratory findings of BV (Amsel's criteria)

Amsel's criteria	Total positive	Positive (%)	Statistics (Chi-square test)
Vaginal discharge	95	56 (87.5)	P value <0.05
Presence of clue cell	46	44 (68.7)	P value <0.05
Positive whiff test	65	58(90.6)	P value <0.05
Vaginal pH (.4.5)	66	54(84.3)	P value <0.05
Total	-	64 (100)	

Among 230 pregnant women, 113 were asymptomatic while 117(50.9%) were symptomatic with symptoms like discharge, itching and burning. The infection rate was higher among symptomatic women 64(54.7%) while only 28(24.8%) of asymptomatic women were infection. The difference was statistically significant (P<0.05) (Table 10).

Table No. 9 Association of vaginitis in relation to signs and symptoms

Signs and symptoms	Infection status		Total	Statistics Chi-square test
	Negative	Positive		
Asymptomatic	85(75.2)	28(24.8)	113(100)	P<0.05
Symptomatic	53(45.3)	64(54.7)	117(100)	
Total	138(60.0)	92(40.0)	230(100)	

(Figure in parenthesis indicate percentage)

Among the symptomatic, BV was the most prevalent 19.6% followed by VVC and then mixed infection of BV and VVC but no trichomoniasis. Similarly in asymptomatic cases, BV (19.6%) was followed by mixed infection of BV and VVC (18.8%), VVC (15.3%) and finally by BV+VVC+trichomoniasis (Table 11).

Table No 10 Distribution of organism on the basis of signs and symptoms

Signs and symptoms	Total	Infection				positive	Statis-tics
		BV	VVC	BV+ VVC	BV+VVC+ Trichomoniasis		
Asympto-matic	113	13(11.5)	10(8.8)	5(4.4)	0(0.0)	28(24.8)	P-value <0.05
Symptomatic	117	23(19.6)	18(15.3)	22(18.8)	1(0.9)	64(54.7)	
Total	230	36(15.7)	28(12.2)	27(11.7)	1(0.4)	92(40.0)	

(Figure in parenthesis indicate percentage)

Among 92 infected pregnant women, 35.9% had white curdy discharge mostly in women with candidiasis and 34.8% had mucopurulent discharge mostly found among women with BV and mixed discharge was found in only 2.1% of the infected patients (Table 11).

Table No 11 Distribution of different causative agents of vaginitis based on vaginal discharge

Types of discharge	Organisms					Statistics (Chi-square test)
	BV	VVC	BV+VVC	BV+VVC+ Trichomoniasis	Total	
No discharge	6(60.0)	10(66.7)	2(13.)3	0(0.0)	15(16.3)	P-value <0.0%
White curdy	4(12.1)	16(48.8)	11(33.3)	0(0.0)	33(35.9)	
Mucopurulent	2(25.0)	0(0.0)	6(75.0)	0(0.0)	8(8.7)	
Serous watery	24(75.0)	2(6.2)	6(18.8)	0(0.0)	32(34.8)	
Blood mixed	0(0.0)	0(0.0)	2(100.0)	0(0.0)	2(2.1)	
Mixed	0(0.0)	0 (0.0)	0(0.0)	1(50.0)	2(2.1)	
Total	36(15.7)	28(12.2)	27(11.7)	1(0.4)	92(100.0)	

(Figure in parenthesis indicate percentage)

5.7 Microorganisms isolated from vaginal swab of infected patients

Of the total 64 bacterial isolates obtained from high vaginal swab, the most predominant organism isolated was *E. coli* (53.1%) followed by *Klebsiella pneumoniae (*18.7%), *Staphyloccous aureus (*15.6%), *Proteus mirabilis* (7.8%), *Streptococcus* spp (3.1%) whereas CoNS accounted for *(*1.6%) of the total isolates (Table 14).

Table 12 Frequency of bacterial isolates isolated from vaginal swab

Microorganism	Frequency	Percentage infected (%)
Escherichia coli	34	53.1
Klebsiella pneumoniae	12	18.7
Staphylococcus aureus	10	15.6
Proteus mirabilis	5	7.8
Streptococcus species	2	3.1
CoNS	1	1.6
Total	64	100

5.8 Demographic distribution of Urinary Tract Infection

The overall prevalence of urinary tract infection was 32/230 (13.9%). The majority of infected were from inpatient department 24 (10.4%), rural area 21(9.1%), housewife 23(10.0%) and literate 20(8.6%). Higher number of infected women were between the age of 20 to 29 (8.2%), during third trimester (8.2%) of their and women having first pregnancy (6.5%). However the association between origin, address, age, trimester, no of pregnancies, occupation, education with urinary tract infection was found statistically insignificant (P >0.05) (Table 15).

Table No 13 Distribution of various demographic factors

Demographic characteristics	Presence of UTI (%)	Total	Statistics
Origin: Outpatient	8(3.5)	82	P >0.05
Inpatient	24(10.4)	148	
Address: Rural	21(9.1)	166	P >0.05
Urban	11(4.8)	64	
Age: <19	4(1.7)	24	P >0.05
20-29	19(8.2)	142	
30-39	9(4.0)	59	
40- above	0(0.0)	5	
Trimester: First	3(1.3)	25	P >0.05
Second	10(6.0)	71	
Third	19(8.2)	134	
Occupation :Housewife	23(10.0)	167	P >0.05
Employed	9(4.0)	63	
Education: Illiterate	12(5.2)	78	P >0.05
Literate	20(8.6)	152	
Total	32(13.9)	230	-

5.9 Microorganisms isolated from urine of the infected patients

Out of 32 isolated bacterial pathogens *E. coil* (43.8%) was predominant, followed by *Klebsiella pnemoniae* (21.9%), *Proteus mirabilis* (15.7%), *Staphyloccous aureus* (9.3%), CoNS (6.2%), and *P. aeruginosa* (3.1%). The gram positive and negative bacteria accounted for 15.6% and 84.3% respectively (Table 14).

Table 14: Frequency of isolated organisms among UTI patients

Microorganism	Frequency	Percentage infected (%)
E. coli	14	43.8
Klebsiella pneumoniae	7	21.9
Proteus mirabilis	5	15.7
Staphylococcus aureus	3	9.3
CoNS	2	6.2
Pseudomonas aeruginosa	1	3.1
Total	32	100

5.9 Association between BV and UTI

Urinary tract infection was more than twice common among women with bacterial vaginosis than without it. 15(23.4%) of BV positive patients had UTI while only 17 (10.2%) of women without BV had UTI. The association between BV and UTI was found to be statistically significant (P <0.05) (Table 17).

Table No 15 Association between BV and UTI

UTI	BV		Total	Statistics
	Present	Absent		
Present	15(23.4)	17(10.2)	32(13.9)	Chi-square test (P-value <0.05)
Absent	49(76.6)	149(89.8)	198(86.1)	
Total	64(27.8)	166(72.2)	230(100)	

(Figure in parenthesis indicate percentage)

5.10 Antibiotic susceptibility pattern of isolated microorganisms

S. aureus was mostly resistant to Cephalexin and sensitive to Gentamicin, Amikacin and Cefotaxime. Only 2 *Streptococcus* spp were isolated and all were resistant to Nalidixic acid and 50% isolates were sensitive Gentamicin, Ciprofloxacin, Cefotaxime and Ampicillin. A single isolate of CoNS isolated was resistant to Ciprofloxacin, Nalidixic acid and Ceftazidime and sensitive to remaining drugs (Table 16).

Table No 16 Antibiotic resistance patterns of gram positive isolates from HVS

Microorganisms	Antibiotics used							
	G	AK	CF	NA	Ce	A	COT	CP
Staphylococcus aureus (n=10)	1(10)	1(10.0)	5(50)	4(40.0)	1(10)	3(30)	5(50.0)	6(60.0)
*Streptococcus species (*n=2)	0(0.0)	1(50.0)	0(0.0)	2(100)	0(0.0)	0(0.0)	1(50.0)	1(50.0)
CoNS (n=1)	0(0.0)	0(0.0)	1(100)	1(100)	1(100)	0(0.0)	0(0.0)	0(0.0)

(Figure in parenthesis indicate percentage)

Key: G=Gentamicin, AK=Amikacin, CP=Cephalexin, CF=Ciprofloxacin, NA= Nalidixic acid, COT=Cotrimoxazole, A=Ampicilin, Ce=Cefotaxime

Table No17 Antibiotics resistance pattern of gram negative isolates from HVS

Microorganism	Antiobiotics used							
	G	AK	CF	NA	Ce	A	COT	CP
E.coli (n=34)	8 (23.5)	6 (17.6)	14 (41.2)	12 (35.3)	14 (41.2)	12 (35.3)	10 (29.4)	13 (38.2)
Klebsiella pnemoniae(n=12)	1 (8.3)	3 (25.0)	3 (25.0)	1 (8.3)	4 (33.3)	3 (25.0)	4 (33.3)	3 (25.0)
Proteus mirabilis (n=5)	1 (20.0)	0 (0.0)	0 (0.0)	1 (20.0)	1 (20.0)	2 (40.0)	2 (40.0)	2 (40.0)

Majority of *E. coli* (38.2%) showed resistance to Cephalexin followed by Nalidixic acid (35.3%) and sensitive to Amikacin (17.6%). *Klebsiella pneumoniae* was resistant to Cephalexin and Cotrimoxazole and sensitive to Nalidixic acid and Gentamycin. *Proteus mirabilis* also showed higher resistance to Ampicilin, Cotrimoxazole and Cephalexin and sensitive to Amikacin and Ciprofloxacin. (Table No 17)

Table No 18: Detection of MDR and Non –MDR pathogens from HVS isolates

Isolated microorganism	Drug resistant		Statistics (chi-square test)
	MDR	Non-MDR	
E. coli (n=34)	18(52.9)	16(47.1)	P-value >0.05
Klebsiella pneumoniae(n=12)	4(33.3)	8(66.7)	
Proteus mirabilis (n=5)	2(40.0)	3(60.0)	
Staphylococcus aureus (n=10)	6(60.0)	4(40.0)	
CoNS (n=1)	1(100.0)	0(0.0)	
Streptococcus species (n=2)	1(50.0)	1(50.0)	
Total (N=64)	32(50.0)	32(50.0)	

(Figure in parenthesis indicate percentage)

Among the total isolated microorganisms, CoNS (100.0%) was found to be the most predominant organism having MDR properties followed by *E. coli* 18(52.9%) then *S. aureus* 6(60.0%) and *Klebsiella pneumoniae* 4(33.3%) (Table No. 20).

Staphylococcus aureus was mostly resistant to Cephalexin, Nalidixic acid, Gentamycin and Cotrimoxazole (66.7%) while sensitive to Amikacin. CoNS was resistant to Nalidixic acid, Cephalexin, Ciprofloxacin and sensitive to Gentamicin, Amikacin, Ampicillin and Ceftazidime. (Table 19)

Table No 19Antibiotic resistant pattern of gram positive isolates from urine

Microorganism	Antibiotics used							
	G	AK	CP	CF	NA	A	COT	Ce
Staphylococcus aureus (n=3)	2 (66.7)	0 (0.0)	2 (66.7)	1 (33.3)	2 (66.7)	2 (66.7)	2 (66.7)	1 (33.3)
CONS *(n*=2)	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)	2 (100.0)	0 (0.0)	1 (50.0)	0 (0.0)

(Figure in parenthesis indicate percentage)

Key: G=Gentamicin, AK=Amikacin, CP=Cephalexin, CF=Ciprofloxacin, NA= Nalidixic acid, A=Ampicilin, COT= Cotrimoxazole, Ce= Cefotaxime

Majority of *E. coli* (57.1%) showed resistance to Nalidixic acid and were mostly sensitive to Amikacin and Cotrimoxazole. *Klebsiella pneumoniae* showed resistance to Ciprofloxacin and was sensitive to *Proteus mirabilis* also showed higher resistance to Ampicilin, Nalidixic acid, Gentamicin and Amikacin and Cephalexin (40.0%). A single isolate of *Pseudomonas aeroginosa* isolated was resistant to Ciprofloxacin, Cephalexin, Ampicillin and Cotrimoxazole and sensitive to others. (Table 20)

Table 20 Antibiotic resistant pattern of gram negative isolates from urine

Microorganism	Antibiotics used							
	G	AK	CP	CF	Ce	NA	A	COT
E. coli (n=14)	5 (35.7)	3 (21.4)	7 (50.0)	5 (35.7)	5 (35.7)	8 (57.1)	6 (42.9)	3 (21.4)
Klebsiella pneumoniae (n=7)	2 (28.6)	0 (0.0)	3 (42.9)	4 (57.1)	2 (28.6)	3 (42.9)	2 (28.6)	1 (14.3)
Proteus mirabilis (n=5)	2 (40.0)	2 (40.0)	2 (40.0)	1 (20.0)	1 (20.0)	2 (40.0)	2 (40.0)	1 (20.0)
Pseudomonas aeruginosa (n=1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)

(Figure in parenthesis indicate percentage)

Key: G=Gentamicin, AK=Amikacin, CP=Cephalexin, CF=Ciprofloxacin, NA= Nalidixic acid, A=Ampicilin, COT= Cotrimoxazole, Ce= Cefotaxime

All the isolates of CoNS and *Pseudomonas aeruginosa* (100.0%) were MDR followed by *Staphylococcus aureus* (66.7%) and then *E. coli* (57.1%) (Table 21).

Table 21: Detection of MDR and Non –MDR pathogens from urine isolates

Isolated microorganism	Drug resistant		Statistics (Chi-square test)
	MDR	Non-MDR	
E.coli (n=14)	8 (57.1)	6 (42.9)	P-value>0.05
Klebsiella pneumoniae (n=7)	3 (42.9)	4 (57.1)	
Proteus mirabilis (n=5)	2 (40.0)	3 (60.0)	
Staphylococcus aureus (n=3)	2 (66.7)	1 (33.3)	
CoNS (n=2)	2 (100.0)	0 (0.0)	
Pseudomonas aeruginosa (n=1)	1 (100.0)	0 (0.0)	
Total (N=32)	18 (56.3)	14 (43.8)	

(Figure in parenthesis indicate percentage)

CHAPTER-VI

DISCUSSION AND CONCLUSION

6.1 Discussion

Vaginitis can cause different types of morbidities in women especially of reproductive age group (Aryal, 1997). In Nepal, one in three women wants consultation for vaginal discharge (Padhye, 2003). Women are more prone to infection during pregnancy. In pregnancy women are at higher risk of having asymptomatic bacteriuria due to anatomical and physiological changes such as changes in hormonal level. In Nepal very few studies has been done to determine the prevalence of vaginitis among pregnant women and its association with urinary tract infections. Therefore, this cross-sectional descriptive study was carried out to find out the distribution pattern of different types of vaginitis among pregnant women and association between BV and UTI among pregnant women. The association of vaginitis with different demographic characteristics and risk factors is also assessed by the study.

In this study the frequency of vaginitis among pregnant women from inpatient department (26.9%) was twice more than those from outpatient department (13.0%) and distribution was statistically significant (P=0.43). Treatment is not sought until the symptoms severely affect the women's health as vaginal discharge is considered as a shameful STI and accepted as social stigma in Nepalese society. Other factors determining the infection include nosocomial infections and immunosuppressed condition among inpatients.

A higher vaginal infection rate was found among pregnant women from rural area (30.8%) and mostly among housewives (28.6%) compared to urban area (9.13%) and employed women (11.3%). Manandhar et al. (2005) also reported the higher infection rate among rural housewives compared to urban pregnant women. The differences may be due to

variation in sexual norms and practices which may affect exposure to the infection as well as by differences in willingness to report symptoms or be examined. Women in rural area may be more secluded and live in a more conservative sexual milieu. The higher infection rate among rural population can be correlated with that of housewives due to higher proportion of infection women from rural area who were housewives. The poor sanitary practices, poor living standard, stress, ignorance, lack of proper health education, hesitation to disclose the reproductive problems and difficulty in accessibility towards immediate health care facilities may attribute to the higher rate of vaginitis among rural housewives.

The level of education has effect on the incidence of vaginitis in the study as the difference was statistically significant (P-value <0.05). In accordance to this, a study done by Adeyeba (2003) found that vaginitis decreased with the level of education i.e. higher among illiterate and lower among educated. This may be due to lack of health education, unhygienic health practices, low economic status, sexual behaviors, and random use of different antibiotics without consultation with physicians, lack of female doctors at health center and so on.

The overall prevalence rate of infectious vaginitis was 40.0% indicating high rate of infection among Nepalese pregnant women. All the three major types of vaginitis, BV, VVC and trichomoniasis were found in the study. The prevalence rate of infectious vaginitis was found in the range of 20.0% in Nairobi to 71.0% in South Africa (Thomas, 1996; Sami and Baloch, 2005; Adeyeba, 2003; Carrel, 1983).

In the present study the prevalence of BV was highest followed by VVC and then trichomoniasis (0.4%). Organisms associated with vaginitis are part of the host's own microflora or exogenous microorganisms that must interact with species present as part of the host's indigenous flora (Krieger, 1988). Similar trend of infection has been reported by various authors (Kamara et al., 2000 in Jamaica; Bang et al., 1989 in India; Acharya, 1988 in India; Bro, 1989 in Denmark and Patricia et al., 2006 in Peru). While study done by Klufio et al. (1995) in New Guinea; Evans (1995) in London and Thomas (1996) in Nairobi

showed the highest prevalence of VVC, then BV and trichomonasis showed lowest prevalence. In a study conducted among Nepalese women by Padhye (2003), VVC was found in 36.8%, BV in 19.5%, Trichomoniasis among 0.9%, VVC with BV in 6.4% and trichomoniasis with BV in 1.3%. Similarly in a study conducted by Rizvi et al. (2004) among both pregnant and non pregnant Nepalese women attending OPD in a large public hospital in Kathmandu, the prevalence of VVC (78%), BV (25%) and trichomoniasis (17%).

Pickering et al. (2005) in Southeast Uganda, Helen et al. (2005) in Tamilnadu, Adeyeba, (2003) in Nigeria found the highest prevalence of BV, followed by trichomoniasis and then VVC. Namkinga et al. (2005) found the highest prevalence of trichomoniasis followed by BV and then VVC. The change in the trend might be due to the differences in the living standard, sexual practices, socioeconomic factors, awareness towards health education and geographical, ethnic and environmental factors such as hygiene and nutrition (Hansen, 2004).

The overall prevalence of BV was found to be 27.7%. BV is actually not an infection but as a condition resulting from imbalance in the vaginal flora (Pradhan, 2001). It accounts for 10 to 30% of cases of infectious vaginitis in women of childbearing age (Owen and Clenney, 2004). Similar prevalence rate of was found in Bangui Larsson, 2007 (29.1%), in US by Hansel et al., 2010 (29%); in Nairobi by Thomas 1996 (26.2%) and in Vientiane by Sihavong et al., 2006 (24.8%). Different studies by Sobel (1997); Hill (1993); Hay (1998); Sobel (1990), Hillier (1995), Kurki (1992), Platz –Christensen (1993) have documented the prevalence rate of BV ranging from 6% to 32% among pregnant women. However, lower prevalence of 6.2% in South Africa (Amsel et al.,1983), 6.4% in Burkino Faso (Kirakoya-Samadoulougou et al., 2008), Thorsen (2006) in Danish pregnant women (13.7%) and Sharami, 2007 (13.6%), Pelapermpoonsiri et al. (1996) in Japanese and Thai pregnant women (13.6% and 15.9% respectively); Joesef et al. (1995) in Indonesian pregnant

women (18%) and higher prevalence in Bangladesh from 28%-68% (Saleem et al., 2006), in sub Saharan Africa from 29%-52% (Blankhart et al., 1999) have been reported.

The study on BV in pregnancy is difficult to compare, mostly because of the heterogeneity among study population, differences in gestational age at examination, differences in the diagnostic criteria, clinical settings and socio-demographic factors (Trabert, 2007). The fluctuations on different studies may also be due to vaginal hygienic practices such as douching (Bruce et al., 2000) and sexual behaviors (Koumans et al., 2007) which are the main determinants of BV. And also because determining the BV is difficult as one third to three quarters of affected women are asymptomatic (Hay, 1998; Sobel, 1990). Some factors for higher infection rate include poor health habits (eating poorly, no exercise), emotional stress, douching, new sexual partners or multiple sexual partners (Krohn, 1989). The lack of symptoms among women with BV is a major constraint in its diagnosis and treatment.

The prevalence of VVC was found to be 24.3%. Similar results were found by Gracia et al. (2004) in Peru (28%); Donbraye-Emmanuel (2010) in Nigeria (26%); Klufio et al. (1995) in Papua (23%); Smoes et al. (1998) in Brazil (19.2%) and Lisiak et al. (2000) in Poland (20.8%). Higher prevalence of VVC has been documented by Guzel et al. (2011) in Turkey (37.4%), Livia et al. (2004) (42.3%). This higher prevalence rate in their study was due to intake of oral contraceptives IUCD, previous history of VVC and others. This change in the status is due to the differences in the living standards, socio-economic status, hygienic conditions, awareness towards their health education, and so on. Establishing *Candida* species as the cause of vaginitis can be difficult because as many as 50% of asymptomatic women have *Candida* species as part of their endogenous vaginal flora (Sobel, 1993).

During pregnancy, the vagina shows increased susceptibility to infection by *Candida* species, resulting in both higher prevalence of vaginal colonization and a higher rate of symptomatic vaginitis (White and Larsen, 1997). High levels of reproductive hormones

provide an excellent carbon source for *Candida* species by providing higher glycogen content in the vaginal tissues. Also the estrogen enhances adherence of yeast cells to the vaginal mucosa. A cytosol receptor or binding system for female reproductive hormones has been documented in *C. albicans.* Several investigators demonstrated in vitro binding of female sex hormones to *Candida* spp as well as the capacity of certain hormones to enhance yeast mycelia formation and hence virulence (Zhao et al., 1995).

The prevalence of Trichomoniasis was found to be 0.4% in this study. Similar prevalence rate was found among pregnant women of Bangladesh by Afroza, 2003 (1.4%). Higher prevalence rate of 9.84% by Livia et al., (2004), Amsel et al., 1983 (15.2%), Adeyeba, (2003) (11.1%) was also observed. There has been a consistent decline in prevalence of trichomoniasis globally (Sobel, 1997). However results vary widely from 0% to 34% (Mirza et al., 1983; Hart, 1993). The high prevalence rate in some countries may be due to improper use of antibiotics, tropical climate and sexual habits (Livia et al., 2004).

BV was most freqently asociated with yeasts in this study. Similar observation was found in the study done by Jihan M Al-Muk, 2001. However this finding was in contrast with the reports by Hillier (1993) where *Trichomonas vaginalis* was the most frequently associated organisms with BV. However these studies were carried out in non pregnant women and the vaginal yeast carriage and concomitant infection is more frequent in pregnancy (Hillier, 1993; Schgal, 1990). Mixed infections have also been shown by Patricia et al., (2006) and Garcia et al., (2006).

Candida albicans was responsible for 66.6% of VVC whereas other *Candida* species infection rate was 33.3% in this study. Donbraye- Emmanuel et al. (2010) showed similar results whereas a study done by Hedayati et al. (2010) of in US showed 70-90% done by *Candida albicans* and while the remainder of infections was caused by other *Candida* species. The frequency of non-albicans spp (e.g.*Candida glabrata*) has increased possibly secondary to greater use of over-the–counter antifungal products (Horowitz, 1990).

Among 230 pregnant women between age 15 to 45, the infection rate was highest among pregnant women of age group 20 to 29 (44.4%) and least for 40 and above age group (20.0%).The highest prevalence in the age group 20 to 29 might be due to the age being the most reproductively active and high sexual exposure at this group. Younger women are particularly susceptible to infection because they have fewer antibodies to fight the pathogens (Prasad, 2005). The least infected group in this study was 40 and above which might be due to dryness of the vagina and less sexual activities at this age. Similarly the higher rate of vaginitis in age group 16-25 was also found by Adeyeba (2003), Manadhar et al. (2005) and Helen et al. (2005).

In this study BV was most prevalent among pregnant women with age group 20-29. Similarly, Larsson et al. (2007) found higher rate of BV among women less than 26 years. BV is most common among sexually highly active group (Sihavong, 2006). The frequency was did not increase with increasing age as suggested by Moi (1990). BV prevalence increase with age has been in an argument that BV is not a sexually STI as other STIs are more common among younger women (Larsson, 2007). With respect to VVC prevalence by age group, it was slightly higher in pregnant women younger than 20 years. This result corroborates findings of another report (Kent, 1991; Bohbot, 1995) that age is not a factor that seriously affects candidiasis prevalence.

In present study, the pregnant women in overall in their 3rd were mostly infected followed by 2nd trimester and then 1st trimester. BV was higher in 2nd trimester followed by 3rd trimester. The incidence of VVC was found highest during 3rd (28.3%). Sobel (1995) reported VVC to occur in 10% of women during 1st of pregnancy and in one third to half of women in third trimester. The incidence of VVC is almost doubled in pregnant women particularly in their 3rd compared with non pregnant women. It recurs during pregnancy as a result of the increased levels of estrogens and corticoids reducing vaginal defense mechanisms against such opportunistic infections as C*andida* spp (Sobel, 1997). Pregnancy

disrupts the balance between levels of *Candida* in the vagina and vaginal acid levels which increases the risk of VVC, especially during the 2nd and 3rd trimester (Geiger et al., 1995).

In this study vaginitis was found significantly more among those with a history of complication during previous pregnancy such as at least one late miscarriage and low birth weight infant. Also women with history of vaginal infection were more likely to get vaginitis. BV is an independent risk factor for premature delivery of low birth weight infants (Hillier et al. 1995). Hootan et al. (1996) showed that women with a history of recurrent vaginitis were more likely to be vaginally colonized with uropathogens and thus develop UTI. Gonger (2005) also found a significant positive association between BV and a history of vaginal infection, preterm delivery, and premature rupture of membranes. Thus, it can be concluded that BV is clearly linked to adverse gynecological and reproductive outcomes such as fetal loss, low birth weight and neonatal infectious mortality. BV causes PID, upper genital tract infection and also stimulates the production of cytokines which in turn causes premature birth or may even lead to miscarriages. So, the pregnant women with a history of early or late pregnancy loss should be screened for BV and treated.

Smoking is risk factor for having BV in pregnancy (Kalinka et al., 2002; Hay et al., 1994). In this study smoking was found strongly associated with BV as indicated by more than double prevalence of BV among smokers (56.0%) as compared to nonsmokers (20.0%). BV was twice common among smokers than nonsmokers (Liahl- Camp, 1996; Hay, 1994; Larsson et al., 2007, Boris et al., 1998; Hellberg et al., 2001). Women who smoke may have risk behaviors that would predispose them to BV and also may not notice the malodor caused by BV. Cigarette smoking exposes various chemicals like nicotine, cotine and benzo[a]pyrenediol epoxide (BPDE) which have been demonstrated in cervical epithelium mucus of smokers and may directly alter the vaginal microflora or may act by depleting Langerhans cells in cervical epithelium leading to local immunosupression (Schwebke, 1999). Also the effect of smoking on acquisition of BV could be due to a reduction in

placenta's ability to produce estrogens that results in decreased growth of Lactobacilli spp. Maternal smoking influences placental enzyme production (Barnea, 1994) and placental hormones secretions (Shurtz- Swirski et al., 1992) which greatly enhanced proliferation of bacterial in the murine vagina by oestrogen administration (Furr et al. 1991).

The diagnosis of BV is based on clinical findings and laboratory testing. Clinically Amsel's criteria are the most widely accepted for the diagnosis of BV (Iftikhar, 2003) and the sensitivity and specificity of Amsel's criteria was found to be 92% and 96% resp. (Begum et al., 2003). In this study, BV was diagnosed on the basis of Amsel's criteria in 64(27.8%). Out of total 64 BV positive sample, vaginal discharges, presence of clue cells, positive whiff test and vaginal pH higher than 4.5 was found in 87.5%, 68.7%, 90.6% and 84.3% respectively. Among the 95 pregnant women with vaginal discharge, 87.5% had BV while only 12.5% of BV positive sample had no discharge. Begum et al. (2003) observed that out of 80 BV positive samples 91.2% had homogenous milk like discharge, 95.0% had positive whiff test, 90.0% had clue cells and 93.7% had pH greater than 4.5. This showed that vaginal discharge is also one of the diagnostic criteria for BV as the difference was statistically significant.

Nearly half (50.8%) of the pregnant women were symptomatic and the infection rate was more among symptomatic women (54.7%) in comparision to (24.8%) asymptomatic women. Similar result was found by Dadhwal et al. (2010) who showed that 43.3% of symptomatic pregnant women had vaginitis. Similarly, Adeyeba et al. (2003) showed higher rate of vaginitis among women with symptoms such as discharge and itching than without symptoms and also showed the significant association between them. However, study done by Amsel et al. (1983) and Hill et al. (1988) showed that up to 50% of infected pregnant women being asymptomatic.

The prevalence of BV was found higher among the symptomatic women (87.5%) compared to that of asymptomatic (12.5%) and the association was statistically significant.

Similarly Dadhwal et al. (2010) showed the higher prevalence of BV among symptomatic pregnant women and their significant association. However, different studies done by Owen and Clenney, (2004); Liahi-camp et al. (1996) showed BV common among asymptomatic women. The higher infection among symptomatic women in our study might be due to the reason that most of the pregnant women in our study were the suspected cases and in 3rd trimester of their pregnancy and the colonization and symptomatic recurrence is maximally increased during third trimester.

The white curdy discharge was mostly found among women with VVC and BV was characterized by mucopurulent discharge. Donbraye-Emmanel et al. (2010) and Akha et al. (2010) also showed that VVC among pregnant women showed the symptoms like thick white curd like discharge and vaginal itching. Similarly Eschenbach (1999) showed thin, homogenous mucopurulent discharge as the typical clinical symptoms of BV.

This study showed that out of 230 HVS from pregnant women, 27.8% were growth positive among which *E. coli* (53% of the total isolate) was the predominant bacteria colonizing vagina, followed by *Klebsiella pnemoniae, S. aureus* and others. Similarly, Masinda (2009) showed 24.6% of sample with bacterial growth and the organisms isolated from HVS were *E. coli* (43.7%), *S. aureus*(37.5%) and *Proteus* species (18.7%).Vaginal colonization by *E coli*, *Klebsiella* spp, *Proteus* spp Staphylococci, Streptococci and *P. aeruginosa* during pregnancy has also been shown by Carel (2005); McDonald (1992), Bayo et al. (2002).

Higher number of enteric bacteria in the vagina may indicate that bacteria from bowel that may get access to the vagina in these women. During pregnancy vagina is less acidic, has increased vascularity and increased estrogen content and these factors influence growth and colonization of different pathogenic microorganisms in vagina. Other factors include bowel movement, cleansing habits, presence of haemorrihs and use of sanitary pads. In accordance with the result found by Donders et al. (2007), this study also found that mixed

aerobic vaginitis (AV) and bacterial vaginosis (BV) among pregnant women. And if BV represents a mixed flora of AV and BV, it may be speculated that the aerobic component of the microflora may be the main factor during pregnancy. Other factors influencing vaginal colonization by pathogens may be poor health, aggressive intercourse, declining hormone levels, poor diet or abnormal microflora populations.

Vaginitis may lead to UTI because the distribution factors were of similar patterns for both vaginitis and UTI. Majority of the pregnant women having UTI were from inpatient department compared to outpatient. High prevalence of UTI was found in pregnant women of rural area who were housewives compared to urban and employed women. A study carried out by Bookallil et al. (2005) showed the higher prevalence of UTI among women living in rural and remote area. The high prevalence of UTI in pregnant women from rural housewives might be due to their unhygienic practices including low frequency of bathing, lack of health education and health facilities, inadequate cleaning of genitals, incorrect way of washing anal areas such as from back to front and exposure to various types of microorganisms at their workplace.

Pregnant women in 3rd trimester (8.2%) were more than infected followed by 2nd (6.0%) and then 1st trimester (1.3%). Similar trend was observed by Leigh 1996; Tugrul et al. (2005). Al-Haddad (2004) showed an increase in frequency of bacteriurea with progress of pregnancy, with higher infection during 3rd trimester. This may be due to decrease in urinary progestines and oestrogens in various trimester of pregnancy. As ASB is a strong predictor of symptomatic UTI later in pregnancy (MacLean, 2001) the higher prevalence of UTI in 3rd and 2nd trimester in this study might be due to unidentified ASB in 1st trimester which developed in symptomatic UTI in later stages of pregnancy and another reason might be urethral dilatation during late pregnancy (Delzell et al., 2000).

Majority of the infected women were illiterate and between the age group of 20-29. Haider et al. (2010); Fatima and Ishrat (2006) also reported higher infection rate among illiterate

pregnant women.. Education status was found to be high risk factor for the occurrence of UTI in pregnancy and education improves the attitude and beliefs of women and has a protective role against most of the morbidities. Onyemelukwe et al. (2003) and Onuh et al. (2006) also reported no relationship of either age or parity with bacteriuria. Women who are sexually active and especially if they use diaphragm and spermicide for contraception are at increased risk of UTI. Colonization of the vaginal and urethral mucosa with intercourse facilitates migration of the organism into the bladder (Komaroff, 1986).

Out of 230 urine samples, 32(13.9%) were culture positive of which (84.4%) were Gram negative bacteria and of them *Escherichia coli* (n=14) was the most common pathogens and *Staphyococcus aureus* (n=3) was found to be predominant in Gram positive bacteria. Similar prevalence rate of UTI among pregnant women was observed by Hamdan et al., 2011 (14.0%), Masinda et al., 2009 in Tanzania (14.6%); Assefa et al., 2008 in Ethiopia (11.6%). The minor differences in the prevalence rate in our country could be due to inclusion of both symptomatic and asymptomatic pregnant women in this study, differences in the environment, social habits of the community, the standard of personal hygiene and differences in education (Valiquette, 2001; Pummer, 1993), sexual activity and washing genital precoitus (Dimetry et al, 2007). Higher rate of UTI among pregnant women was found by Zaria (2010) and Okonko et al. (2009).

E. coli was the most common both as vaginal colonizer and causative agent of UTI. However, Mumtaz S (2008) reported *E. coli* to be the most prevalent vaginal pathogens followed by enteric gram negative bacteria. *E. coli* was found the most predominant causative agent of UTI among pregnant women (Okonko et al., 2009, Al- Haddad, 2004; Masinde et al., 2009 and Zaria, 2010). The predominance of *E. coli* as a causative agent of UTI may be due to *E. coli* preference for urinary stasis, a condition which is common during pregnancy (Zaria, 2010). Other isolated from were from urine were *S. aureus*,

Klebsiella pneumoniae, Proteus species and *Pseudomonas aeruginosa* was the least prevelant one (Okonko et al. 2009).

Out of the 64 BV positive pregnant women, 15(23.4%) had UTI and of 166 BV negative pregnant women 17 (10.2%) had UTI. Sharami et al. (2007) also found that 23.6% of pregnant women with BV had UTI compared to 9.8% of those without BV. Harmanli, 2000 reported that 22.4% of women with BV had UTI while 10% of those without BV had UTI. However, Sumati et al. (2009) documented a higher prevalent rate of 42.27% and Hillerbrand et al. *(*2002) reported a lower prevalence of 13.6% of the pregnant women suffering from BV also had urinary tract infection (UTI) among pregnant women. Thus, it can be concluded that BV in pregnancy increases the risk of UTI.

UTI in women develop when uropathogens, almost always from the fecal flora colonize the vagina; ascend into the bladder and in some cases the kidney. Loss of the vaginal Lactobacilli may predispose women to acquisition of genitourinary infections (Boris, 1998; Hootan et al., 1997). The colonization of different pathogenic microorganism is favored by host behavioral factors such as spermicides use, sexual intercourse (Hooton et al., 1996) and moreover due to increase in vaginal pH as a result of decrease or alteration of normal flora of vagina i.e. Lactobacilli to the vagina as a result of BV. BV diagnosed from the lower genital tract has been related to (i) an increased potential for other vaginal pathogens to gain access to the upper genital tract, (ii) the presence of enzymes that reduce the ability of leukocytes to reduce infection, and (iii) an increased level of endotoxins stimulating cytokine and prostaglandin production (Platz-Christensen, 1993; Briselden et al., 1992) and lead to UTI.

The most effective chemotherapeutic agents against *E. coli* isolated from vagina were Amikacin, Gentamicin and Cotrimoxazole whereas Cephalexin, Nalidixic acid and Ampicillin were least effective. The member of enteric Gram negative bacilli colonizing

vagina were mostly resistant to Cephalexin and Ampicillin and sensitive to Amikacin. Among Gram positive bacteria, *Staphylococcus aureus* was susceptible to Gentamycin, Amikacin and Cefotaxime while resistant to Cephalexin, Cotrimoxazole and Ciprofloxacin. Shamim et al. (2008) repoted *S. aureus* resistant to Sulphonamides and Cephalexin while sensitive to cefotaxime. Of the total *S. aureus* isolated from HVS, 60.0% were MDR followed by 52.9% of *E. coli*. However, a single isolate of CoNS was also MDR.

The drug of choice for treatment of UTI over the years includes Septrin (Cotrimoxazole), Ciprofloxacin, Ofloxacin, Nitrofurantoin, Gentamycin, Ampicillin (Orenstein et al, 2004) and Tetracyclin, Amoxicillin and Nalididxic acid (Romac et al., 1992) but in recent time the organisms have developed resistance to some of these antimicrobial agents. Our study concludes that it *E. coli* is one of the important causative agents of UTI during pregnancy. Most of *E. coli* isolates (57.1%) showed multiple antibiotics resistance, maximum resistance was found against Nalidixic acid and Cephalexin whereas least resistance was detected against Amikacin and Cotrimoxazole. Zaria et al. (2010) also reported the higher resistant of *E. coli* to Nalidixic acid and higher sensitivity to Cotrimoxazole. Similarly higher sensitivity of *E. coli* to Cotrimoxazole and Amikacin was shown by Raco et al. (2000) and Farshad et al. (2010) in different studies. However, *E. coli* showed moderate sensitivity towards Ampicillin (42.9%) and Cephalexin (50.0%) which are among the drugs of choice for the treatment of UTI during pregnancy. In contrast Okonko et al. (2009) reported *E. coli* to be highly sensitive to Nalidixic acid. Moreover, the differences in sensitivity pattern of the isolates could be attributed to the time differences between different studies, environmental factors such as practice of self medication, the drug abuse and indiscriminate misuse of antibiotics among general population which has favored the emergence of resistant strains (Okonfua et al., 1989; Ominigho et al., 2001)

All the isolates of *S. saprophyticus* and *P. aeruginosa* isolated from urine were MDR (100%). It was followed by *S. aureus* (66.7%) and then *E. coli* (57.1%). Different studies

showed that the multidrug resistant (MDR) percentage of *E. coli* from urine varies with countries 7.1% in US to 65.0% in Iran (Sahin et al., 2000; White et al., 2001).

Inappropriate practices like misuse, abuse and underuse of antibiotics and unskilled practioners can lead to emergence of resistant bacteria. Expired antibiotics, self medication, counterfeit drugs, improper antibiotics prescription, inadequate hospital control measures can as well promote the development of resistance in clinical isolates (Chikere, 2008). In developing countries like Nepal, self medication is a common practice and this might be a major cause of antibiotics resistance in clinical isolates since patients only think of going to hospitals when they are unable to treat themselves. Also lack of sufficient data about bacterial resistance to the antimicrobial agents may be responsible for the increasing emergence of MDR.

E. coli was found to the most common bacteria in both aerobic vaginitis and UTI. Also *S. aureus* showed the higher rate of MDR pattern in both of the cases. So, it can be concluded that the BV creates the environment for vaginal colonization by the bacterial isolates which ultimately leads to UTI.

6.2 CONCLUSION

Vaginitis is prevalent among suspected pregnant women of Nepal. BV was the most prevalent followed by VVC and trichomoniasis. The infection does not seem to depend on demographic distribution. Vaginitis was found associated with complications during previous pregnancy and history of vaginal infections. Smoking was found to be the risk factor for BV. Urinary tract infection was significantly associated with BV. Multidrug resistance among bacterial isolates isolated from vagina and urinary tract was high.

CHAPTER-VII

7 SUMMARY AND RECOMMENDATION

7.1 Summary

1. The 230 HVS were collected from suspected pregnant women visiting Thapathali Maternity and Women's Hospital and among them 40.0% had vaginitis and 13.9% had UTI.
2. Out of 230 cases, the infection rate was higher in inpatients (26.9%) compared to that of outpatients (13.0%). Among total cases, rural pregnant women (30.8%) and housewife (28.6%) were mostly infected compared to urban (9.1%) and employed (11.3%) pregnant women respectively. The infection rate was higher in illiterate women (22.6%) compared to literate women (17.4%) (P value <0.05)).
3. BV was the most prevalent type of vaginitis (27.8%) followed by VVC (24.3%) and then trichomoniasis (0.4%).
4. Out of 56 total *Candida* species isolated from HVS, 66.0% were *Candida albicans* and 34.0% were other species of *Candida*.
5. Vaginitis was most prevalent in age group 20 to 29 (44.4%) while least infected age group was 40 and above (20.0%). BV was found high in age group 20 to 29, VVC in <20 and mixed infection in 30-39 age group.
6. Pregnant women in their 3rd trimester were mostly infected (42.5%) followed by 2nd trimester (39.4%) and then 3rd trimester (28.0%). BV was most common during 2nd trimester whereas VVC and trichomoniasis during 3rd trimester.
7. Vaginitis was higher among women who had complications in previous pregnancy (57.1%) than those without any complications (36.9%).
8. BV was found more common among pregnant women who smoke (56.0%) than those who don't smoke (20.0%).

9. Among 64 BV positive cases diagnosed by Amsel's criteria , vaginal discharge, presence of clue cells, positive whiff test and vaginal pH greater than 4.5 were found among 87.5%, 68.7%, 90.6% and 84.3% respectively.
10. Vaginitis was mostly prevalent in symptomatic cases (54.7%) compared to asymptomatic cases (24.8%). All the types of vaginitis i.e. BV, VVC and mixed infection were common among symptomatic pregnant women.
11. Serous watery discharge was mostly found in BV positive (75.0%) cases while VVC was characterized by white curdy discharge (48.8%).
12. Of the total 64 bacterial isolates isolated from HVS, *E. coli* was the most predominant organism (53.1%) followed by *Klebsiella pneumoniae* (18.7%) and CoNS (1.6%) were least predominant.
13. The majority of the women with UTI were from inpatient department (10.4%), rural area (9.1%), housewife (10.0%) and literate (8.6%) compared to outpatient (3.5%), urban area (4.8%), employed (4.0%) and illiterate (5.2%) respectively. Pregnant women between the age group 20 to 29 were mostly infected (8.2%).
14. Among 32 uropathogens, *E. coli* was the most predominant (43.8%) followed by *Klebsiella pneumoniae* (21.9%), *Pseudomonas aeruginosa* (3.1%) was least predominant.
15. Out of 64 BV positive cases, 23.4% had UTI while only 10.2% of pregnant women without BV had UTI.
16. *E. coli* isolated from HVS was mostly resistant to Ciprofloxacin (41.2%), Cephalexin (38.2%) and Nalidixic acid (35.3%) and sensitive to Amikacin. Most of isolated *S. aureus* was resistant to Cephalexin, Cotrimoxazole and Ciprofloxacin and sensitive to Gentamicin, Amikacin and Cefotaxime.
17. Of 34 *E. coli* isolated from HVS, 52.9% were MDR and of 10 *S. aureus* isolated, (60.0%) were MDR. *Klebsiella pneumoniae* (33.3%), *Proteus mirabilis* (40.0%), CoNS (100.0%), *Streptococcus* species (50.0%) were MDR.

18. *E. coli* isolated from urine were mostly resistant to Nalidixic acid (57.1%) and Cephalexin (50.0%) and sensitive to Amikacin and Cotrimoxazole. *S. aureus* was resistant to Gentamicin, Cephalexin, Nalidixic acid, Ampicillin and Cotrimoxazole (66.7) and sensitive to Amikacin.
19. Out of 14 *E. coli* isolates isolated from urine, 57.1% were MDR and out of 3 *Staphylococcus aureus* isolates, 66.7% were MDR. 42.9% of *Klebsiella pneumoniae*, 40.0% of *Proteus mirabilis*, 100.0% of each CoNS and *Pseudomonas aeruginosa* were MDR.

7.2 Recommendation

1. Antenatal health care facilities should incorporate screening of vaginitis among pregnant women and its timely management.
2. As the occurence of vaginitis is higher among pregnant women from rural areas, housewives and illiterate women, such groups should be targeted for basic health knowledge and awareness regarding reproductive health, hygienic practices and encouraged to seek medical help if any problem arises regarding reproductive health.
3. Smoking being a risk factor for BV should be strictly prohibited.
4. The pregnant women with BV should be screened for UTI and treated.
5. Antibiotics therapy should be used only after a thorough culture and antibiotics sensitivity testing have to be carried out to avoid emergence of drug resistance among bacteria. Also the proper use of antibiotics should be encouraged.
6. To alleviate the problem of MDR in developing countries like Nepal, clinicians should prescribe antibiotics wisely and sufficiently and there should be periodic supervisions on the drug consumptions by respective organization.
7. The etiology of BV should be established and more advanced techniques like PCR and DNA probe methods must be recommended for more sensitivity and specificity.

8. CHAPTER-VIII

9. REFERNCES

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APPENDIX-I

QUESTIONNAIRE

Patient ID……. Date: ……..

1. Name of the patient:…….
2. Age:……..
3. Address.
   a. Rural b. Urban
4. Origin of the patient:
   a. Inpatient b. Outpatient
5. Trimester of pregnancy:
   a. First b. Second c. Third
6. Education:
   a. Illiterate b. Literate
7. Occupation:
   a. Housewife b. Employed
8. Complications in previous pregnancy:
   a. No b. Yes
9. History of vaginal infections:
   a. No b. Yes
10. Smoking habit
    a. No b. Yes
11. Clinical signs and symptoms:
    a. Symptomatic b. Asymptomatic
12. Presence of vaginal discharge:
    a. Yes b. No

    If yes type of vaginal discharge:

    a. Curdy white b. Mucopurulent c. Serous watery d. Blood mixed e. Mixed
13. Smell of the discharge:
    a. Normal b. Offensive c. Fishy odor

APPENDIX-II

DIFFERENT MATERIALS USED DURING THE RESEARCH PERIOD

Glassware

i. Petri plates
ii. Measuring cylinder
iii. Conical flask
iv. Test-tubes
v. Beakers
vi. Slides
vii. Coverslip
viii. Glassrod

	Media	Equipments	
i.	Sabouraud's Dextrose Agar	i. Incubator	Heraeus D-6450, Hanau
i.	MacConkey Agar	ii. Autoclave	ELCON
ii.	Nutrient Agar	iii. Waterbath	Napco, 220 model
ii.	Blood Agar	iv. Refrigerator	Godrej, Cold-gold
iii.	Mueller Hinton Agar	v. Centrifuge	Kabota model KC 25, Japan
iv.	TSI Agar	vi. Hot air oven	Ambassadors, Lab Electronic Oven
v.	Simmon's Citrate Agar	vii. Gas burner	
vi.	Urease Agar		
vii.	Nutrient Broth		
viii.	MR-VP Broth		

Chemicals

i. Crystal violet
ii. Iodine
iii. Methanol
iv. Safranine
v. 10% Potassium Hydroxide
vi. Normal saline (0.9% NaCl)
vii. Lysol
viii. 3% hydrogen peroxide
ix. Acetone alcohol
x. Blood plasma
xi. Methyl red
xii. Mineral oil
xiii. Barium chloride
xiv. Sulphuric acid
xv. Barrit's reagent
xvi. Kovac's reagent
xvii. Glycerol
xviii. Glucose oxidase kit
xix. NNNN-tetramethyl paraphenyldiamine dihydrochloride

Miscellaneous

i. Cotton
ii. Cotton-tipped swab
iii. Candle
iv. Heater
v. Magnetic stirrer
vi. Gloves
vii. Weighing machine
viii. Dropper
ix. Blotting paper
x. Tissue paper
xi. Inoculating loop
xii. Forcep
xiii. Immersion oil
xiv. Sticker
xv. Distilled water

ZONE SIZE INTERPRETATIVE CHART

Antimicrobial Agent	Symbol	Disc Content	Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
Amikacin	Ak	30 µg	14	15-16	17
Amoxycillin When testing Gram-negative enteric organisms When testing Staphylococci When testing *Haemophilus* sp.	AC	20 µg	13 28 18	14-16 - 19-21	17 29 22
Ampicillin When testing Enterobacteriaceae When testing Staphylococci	A	10 µg	13 28	14-16 -	17 29
Ceftazidime	Ca	30 µg	14	15-17	18
Cephalexin	Cp	30 µg	14	15-17	18
Chloramphenicol	C	30 µg	12	13-17	18
Ciprofloxacin	Cf	5 µg	15	16-20	21
Cloxacillin	OB	5 µg	11	12-13	14
Cotrimoxazole	CO	25 µg	10	11-15	16
Erythromycin When testing Staphylococci When testing Streptococci	E	15 µg	13 15	14-22 16-20	23 21
Gentamicin	G	10 µg	12	13-14	15
Imipenem	I	10 µg	13	14-15	16
Nitrofurantoin	Nf	300 µg	14	15-16	17

Meropenem	Mr	10µg	13	14-15	16
Norfloxacin	Of	10 µg	12	13-16	17
Ofloxacin			12	13-15	16
When testing Streptococci	Of	5µg	12	13-15	16
When testing Staphylococci			14	15-17	17

(Source: Product Information Guide, Hi-Media Laboratories Pvt. Limited, Bombay, India).

APPENDIX-III

COMPOSITION AND METHODS OF PREPARATION OF DIFFERENT CULTURE MEDIA AND REAGENTS USED

Different types of culture media such as selective media and transport media were used. Composition and preparation of different types of culture media are given below. The culture media used were from two companies

A. Hi-Media Laboratories Pvt. Limited, Bombay, India.
B. Oxoid Unipath Ltd. Basingstoke, Hampshire, England

(All compositions are given in grams per liter and at 25°C temperature)

I. Composition and Preparation of Different Culture Media

1. Saboraud's Dextrose Agar (SDA)

Ingredients	gm/litre
Mycological peptone	10.0
Dextrose	40.0
Agar	15.0

Final pH (at 25°C) 5.6+/-0.2

65.0 grams of powder media was suspended in 100ml distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121° C (15 lbs pressure) for 15 minutes**.**

2. Blood agar (BA)

Blood agar base (infusion agar) + 5-10% sheep blood

Ingredients	gm/liter
Beef heart infusion	500.0
Tryptose	10.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25^0C) 7.3±0.2

42.5 grams of the Blood Agar base medium was suspended in 1000 ml distilled water and sterilized by autoclaving at 121^0C (15lbs pressure) for 15 minutes. After cooling to 40-50^0C, 50 ml sterile defibrinated sheep blood was added aseptically and mixed well before pouring.

3. MacConkey Agar (MA)

Ingredients	gm/liter
Peptone	20.0
Lactose	10.0
Sodium taurocholate	5.0
Sodium chloride	5.0
Neutral Red	0.04
Agar	20.0

Final pH (at 25^0C) 7.4±0.2

(Without sodium taurocholate, without salt and crystal violet)

55 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes.

4. **Mueller Hinton Agar (MHA)**

Ingredients	gm/liter
Beef, Infusion form	300.0
Casein Acid Hyrolysate	17.5
Starch	1.5
Agar	17.0

Final pH (at 25^0C) 7.4±0.2

38 grams of the medium was suspended in 1000 ml distilled water and the medium was warmed to dissolve. 10 ml was distributed in test tubes and sterilized by boiling in water bath for 10 minutes.

5. **Nutrient Agar (NA)**

Ingredients	gm/litre
Peptone	10.0
Sodium Chloride	5
Beef Extract	10.0

Yeast Extract	1.5
Agar	12.0

Final pH (at 25^0C) 7.4±0.2

37 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes.

6. **Nutrient Broth (NB)**

Ingredients	gm/litre
Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5

Final pH (at 25^0C) 7.4±0.2

13 grams of the medium was dissolved in 1000 ml distilled water and autoclaved at 121^0C for 15 minutes.

II. Biochemical Test Media

1. MR-VP Medium

Ingredients	gm/litre
Buffered Peptone	7.0
Dextrose	5.0

Dipotassium Phosphate	5.0

Final pH (at 25^0C) 6.9±0.2

17 grams was dissolved in 1000 ml distilled water. 3 ml of medium was distributed in each test tube and autoclaved at 121^0C for 15 minutes.

2. Hugh and Leifson's Medium

Ingredients	gm/litre
Tryptone	2.0
Sodium Chloride	5.0
Dipotassium Phosphate	0.3
Bromothymol Blue	0.08
Agar	2.0

Final pH (at 25^0C) 6.8±0.2

9.4 grams of the medium was rehydrated in 1000 ml cold distilled water and then heated to boiling to dissolve completely. The medium was distributed in 100 ml amounts and sterilized in the autoclave for 15 minutes at 15 lbs pressure (121^0C). To 100 ml sterile medium aseptically added 10ml of sterile Dextrose and mixed thoroughly and dispensed in 5 ml quantities into sterile culture tubes.

3. Sulphide Indole Motility (SIM) medium

Ingredients	gm/litre
Beef Extract	3.0

Peptone	30.0
Peptonized Iron	0.2
Sodium Thiosulphate	0.025
Agar	3.0

Final pH (at 25^0C) 7.3±0.2

36 grams of the medium was suspended in 1000 ml distilled water and dissolved completely. Then it was distributed in tubes to a depth of about 3 inches and sterilized.

4. Simmon Citrate Agar

Ingredients	gm/litre
Magnesium Sulfate	0.2
Mono-ammonium Phosphate	1.0
Dipotassium Phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Agar	15.0
Bromothymol Blue	0.08

Final pH (at 25^0C) 6.8±0.2

24.2 grams of the medium was dissolved in 1000ml distilled water. 3ml medium was distributed in test tubes and sterilized by autoclaving at 121^0C for 15 minutes. After autoclaving tubes containing medium were tilted to form slant.

5. Triple Sugar Iron (TSI) Agar

Ingredients	gm/litre
Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Beef Extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosulphate	0.3
Phenol Red	0.024
Agar	12.0

Final pH (at 25^0C) 7.4±0.2

65 grams of the medium was dissolved in 1000ml of distilled water and sterilized by autoclaving at 15 lbs (121^0C) pressure for 15 minutes. The medium was allowed to set in sloped form with a butt about 1 inch of thickness.

6. Christensen Urea Agar

Ingredients	gm/litre
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Dipotassium Phosphate	1.2
Mono-potassium Phosphate	0.8
Phenol Red	0.012
Agar	15.0

Final pH (at 25^0C) 7.4±0.2

24 grams of the medium was suspended in 950 ml distilled water and sterilized by autoclaving at 121^0C for 15 minutes. After cooling to about 45^0C, 50 ml of 40% urea was added and mixed well. Then 5 ml was dispensed in test tube and set at slant position.

III. Staining and Test Reagents

1. For Gram's Stain

a) Crystal Violet solution:	Crystal Violet	20.0 g
	Ammonium Oxalate	9.0 g

Ethanol or Methanol 95 ml

Distilled Water (D/W) to make 1 litre

Preparation: In a clean piece of paper, 20 gm of crystal violet was weighed and transferred to a clean brown bottle. Then, 95 ml of ethanol was added and mixed until the dye was completely dissolved. To the mixture, 9 gm of ammonium oxalate dissolved in 200 ml of D/W was added. Finally the volume was made 1 litre by adding D/W.

(b) Lugol's Iodine: Potassium Iodide 20.0 g

Iodine 10.0 g

Distilled Water 1000 ml

Preparation: To 250 ml of D/W, 20 gm of potassium iodide was dissolved. Then 10 gm of iodine was mixed to it until it was dissolved completely. Finally the volume was made 1 litre by adding D/W.

(c) Acetone-Alcohol Decoloriser: Acetone 500 ml

Ethanol (Absolute) 475 ml

Distilled Water 25 ml

Preparation: To 25 ml D/W, 475 ml of absolute alcohol was added, mixed and transferred to a clean bottle. Then immediately 500 ml acetone was added to the bottle and mixed well.

(d) Safranin (Counter Stain) Safranin 10.0 g

Distilled Water 1000 ml

Preparation: In a clean piece of paper, 10 gm of safranin was weighed and transferred to a clean bottle. Then 1 litre D/W was added to the bottle and mixed well until safranin dissolved completely.

3. Normal saline: Sodium Cholride 0.85 g

Distilled Water 100 ml

Preparation: The sodium chloride was weighed and transferred to a leak-proof bottle premarked to hold 100 ml. Distilled water was added to the 100 ml mark, and mixed until the salt was fully dissolved. The bottle was labeled and stored at room temperature.

4. Test Reagents

a. For Catalase test: Catalase Reagent (3% H_2O_2)

Hydrogen peroxide 3 ml

Distilled Water 97 ml

Preparation: To 97 ml of D/W, 3 ml of hydrogen peroxide was added and mixed well.

b. For Oxidase Test: Oxidase Reagent (impregnated in Whatman's No. 1 filter paper)

Tetramethyl *p*-phenylene diamine dihydrochloride (TPD) 1 gm

Distilled Water 100 ml

Preparation: This reagent solution was made by dissolving 1 gm of TPD in 100 ml D/W. To that solution strips of Whatman's No. 1 filter paper were soaked and drained for about 30 seconds. Then these strips were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

c. For Indole Test: Kovac's Indole Reagent

Isoamyl alcohol 30 ml

p- dimethyl amino-benzaldehyde 2.0 g

Hydrochloric acid	10 ml

Preparation: In 30 ml of isoamylalcohol, 2 g of *p*-dimethyl aminobenzaldehyde was dissolved and transferred to a clean brown bottle. Then to that, 10 ml of conc. HCl was added and mixed well.

d. For Methyl Red Test: Methyl Red Solution

Methyl red	0.05 g
Ethyl alcohol (absolute)	28 ml
Distilled Water	22 ml

Preparation: To 28 ml ethanol, 0.05 gm of methyl red was dissolved and transferred to a clean brown bottle. Then 22 ml D/W was added to that bottle and mixed well.

e. For Voges-Proskauer Test (Barritt's Reagent)

Solution A	α-Napthol	5.0 g
	Ethyl alcohol (absolute)	100 ml

Preparation: To 25 ml D/W, 5 g of α-Napthol was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

Solution B	Potassium hydroxide	40.0 g
	Distilled Water	1000 ml

Preparation: To 25 ml D/W, 40 gm of KOH was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

f. Bile Salt Solution

Commercially available sodium deoxycholate	10 g
Distilled Water	100 ml

Preparation: A 10% solution of sodium deoxycholate was prepared by adding 10 gm sodium deoxycholate powder in 100 ml distilled water and transferred in a clean brown bottle and was autoclaved.

g. Potassium hydroxide

10% potassium hydroxide was prepared by dissolving 5gms of KOH crystals in 50 ml of of sterile distilled water.

g. Mac Farland standard 0.5

It is prepared by adding 0.6 ml of 1% w/v barium chloride solution to 99.4 ml of 1% v/v solution of sulphuric acid.

APPENDIX-IV

BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIA

A. Catalase test

During aerobic respiration, in the presence of oxygen, microorganisms produce hydrogen peroxide, which is lethal to the cell itself. Catalase enzyme breaks down hydrogen peroxide into water and oxygen. The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria, the main exception being *Streptococcus* spp. A small amount of a culture from NA plate was taken in a clean glass slide and about 2-3 drops of 3% H_2O_2 was put on the surface of the slide. The positive test is indicated by the formation of active bubbling of the oxygen gas. A false positive reaction may be obtained if the culture medium contains catalase (e.g., BA) or if an iron wire loop is used.

B. Oxidase test

This test is performed for the detection of cytochrome oxidase in bacteria which catalyzes the transport of electrons between electron donors. In the presence of redox dye Tetramethyl-*p*-phenylene diamine dihydrochloride, the cytochrome oxidase oxidizes it into a deep purple colored end product Indophenol which is detected in the test. A piece of filter paper was soaked with few drops of oxidase reagent. Then the colony of the test organism was smeared on the filter paper. The positive test is indicated by the appearance of blue-purple color within 10 seconds.

C. Oxidation-Fermentation test

This test is done to determine the oxidative or fermentative metabolism of carbohydrate resulting in production of various organic acids as end product. Some bacteria are capable of metabolizing carbohydrates (as exhibited by acid production) only under aerobic conditions, while others produce acid both aerobically and anaerobically. Most medical

bacteria are facultative anaerobes. The test organism was stabbed into the bottom of two sets of tubes with Hugh and Leifson's media, bromothymol blue being the pH indicator. The inoculated medium in one of the tubes was covered with a 10 mm deep layer of sterile paraffin oil. The tubes were then incubated at 37°C for 24 hours. After incubation the tubes were examined for carbohydrate utilization as shown by acid production. Fermentative organism utilizes the carbohydrate in both the open and sealed tubes as shown by a change in colour of the medium from green to yellow. Oxidative organisms, however, are able to use the carbohydrate only in the open tube.

D. Indole Production test

This test detects the ability of the organism to produce an enzyme: 'tryptophanase' which oxidizes tryptophan to form indolic metabolites: indole, skatole (methyl indole) and indoleacetic acid. A smooth bacterial colony was stabbed on SIM (Sulphide Indole Motility) medium by a sterile stab wire and the inoculated media was incubated at 37°C for 24 hours. After 24 hours incubation, 0.5 ml of Kovac's reagent was added. Appearance of red color on the top of media indicates indole positive. Indole if present combines with the aldehyde present in the reagent to give a red color in the alcohol layer. The color reaction is based on the presence of the pyrrole structure present in indole.

E. Methyl Red test

This test is performed to test the ability of an organism to produce sufficient acid from the fermentation of glucose to give a red color with the indicator methyl red (denotes changes in degree of acidity by color reactions over a pH range of 4.4-6.0). A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of methyl red reagent was added and mixed well. The positive test was indicated by the development of bright red color, indicating acidity.

F. Voges Proskauer (VP) test

This test is employed to detect the production of acetyl methyl carbinol (a neutral end product) or its reduction product 2, 3-butanidiol during fermentation of carbohydrates. A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of Barritt's reagent was added and shaken well for maximum aeration and kept for 15 minutes, positive test is indicated by the development of pink red colour.

G. Citrate Utilization test

This test is performed to detect whether an organism utilizes citrate as a sole source of carbon for metabolism with resulting alkalinity. Organisms capable of utilizing citrate as its sole carbon source also utilizes the ammonium salts present in the medium as its sole nitrogen source, the ammonium salts are broken down to ammonia with resulting alkalinity. A loopful of test organism was streaked on the slant area of Simmon's Citrate Agar medium and incubated at 37°C for 24 hours. A positive test was indicated by the growth of organism and change of media by green to blue, due to alkaline reaction. The pH indicator bromothymol blue has a pH range of 6.0-7.6, i.e. above pH 7.6; a blue color develops due to alkalinity of the medium.

H. Motility test

The motility media used for motility test are semisolid, making motility interpretations macroscopic. Motile organisms migrate from the stabline and diffuse into the medium causing turbidity. Whereas non-motile bacteria show the growth along the stabline, and the surrounding media remains colorless and clear.

I. Triple Sugar Iron (TSI) Agar

The TSI agar is used to determine the ability of an organism to utilize specific carbohydrate incorporated in the medium (glucose, sucrose and lactose in concentrations of 0.1%, 1.0% and 1.0% respectively), with or without the production of gas (indicated by cracks in the media as well as an air gap at the bottom of the tube) along with determination of possible hydrogen sulfide production (detected by production of black color in the medium). The test organism was streaked and stabbed on the surface of TSI and incubated at 37°C for 24 hours. Acid production limited only to the butt region of the tube is indicative of glucose utilization, while acid production in slant and butt indicates sucrose or lactose fermentation. Phenol red is the pH indicator which gives yellow reaction at acidic pH, and red reaction to indicate an alkaline surrounding.

J. Urea Hydrolysis test

This test demonstrates the urease activity present in certain bacteria which decomposes urea, releasing ammonia and carbon dioxide. Ammonia thus produced changes the color of indicator incorporated in the medium. The test organism was inoculated in a medium containing urea and the indicator phenol red. The inoculated medium was incubated at 37°C overnight. Positive organism shows pink red color due to the breakdown of urea to ammonia. With the release of ammonia the medium becomes alkaline as shown by a change in colour of the indicator to pink.

K. Coagulase test

This test is used specifically to differentiate species within the genus *Staphylococcus*: *S aureus* (usually positive) from *S epidermidis* (negative). A positive coagulase test is usually the final diagnostic criterion for the identification of *Staphylococcus aureus.* Free coagulase and bound coagulase are the two types of coagulase possessed by this organism; most strains possess both free and bound coagulase.

Slide Coagulase Test- Bound coagulase (Clumping Factor) is detected by slide test. The bound coagulase is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in alteration of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma. For slide coagulase test, a drop of physiological saline was placed on three places of a slide, and then a colony of the test organism was emulsified in two of the drops to make thick suspensions. Later a drop of plasma was added to one of the suspensions and mixed gently. Then a clumping was observed within 10 seconds for the positive coagulase test. No plasma was added in second suspension. This was used for the differentiation of any granular appearance of the organism from true coagulase clumping. The third drop of saline was used for a known strain of coagulase positive staphylococci.

Tube Coagulase Test- This test is carried out to detect production of free coagulase. Plasma contains coagulase reacting factor (CRF) which activates free coagulase. The activated coagulase acts upon prothrombin thus converting it to thrombin. Thrombin converts fibrinogen into fibrin which is detected as a firm gel (clot) in the tube test. Tube test is performed when negative or doubtful results are obtained in slide coagulase test. In the tube coagulase test, plasma was diluted 1 in 10 in physiological saline. Four small tubes were taken, one for test organism, one for positive control, one for negative control, and one to observe self clotting of plasma. Then 0.5 ml of the diluted plasma was pipetted into each tube and 0.5 ml of test organism, 0.5 ml of positive control (*Staphylococcus aureus*), and 0.5 ml negative control (*Staphylococcus epidermidis*) was added to three tubes, to the fourth tube, 0.5 ml sterile broth was added. After mixing gently, all tubes were incubated at 37^0C on a waterbath for 6 hours and observed for gel formation in every 30 minutes.

L. DNase (Deoxyribonuclease) test

This test is used to identify *Staphylococcus aureus* which produces deoxyribonuclease (DNase) enzyme. The DNase enzyme hydrolyses the DNA. The test organism was

cultured on a medium containing DNA. After overnight incubation, the colonies were tested for DNase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydrolysed DNA. DNase producing colonies are therefore seen as clear areas surrounding colonies due to DNA hydrolysis

Distinguishing reactions of the commoner and pathogenic Enterobacteriaceae

Species	Test/ substrate[a]											
	lac	mot	gas	ind	VP	cit	PDA	ure	lys	H_2S	inos	ONPG
Escherichia coli	+	+	+	+	-	-	-	-	+	-	-	+
Shigella groups A, B, C	-	-	-	±	-	-	-	-	-	-	-	-
Sh. Sonnei	-	-	-	-	-	-	-	-	-	-	-	+
Salmonella (most serotypes)	-	+	+	-	-	+	-	-	+	+	±	-
Salmonella typhi	-	+	-	-	-	-	-	-	+	+	-	-
Salmonella paratyphi A	-	+	+	-	-	-	-	-	-	-	-	-
Citrobacter freundii	±	+	+	-	-	+	-	±	-	±	-	+
C. koseri	±	+	+	+	-	+	-	±	-	-	-	+
Klebsiella pneumoniae	+	-	++	-	+	+	-	+	+	-	+	+
K. oxytoca	+	-	++	+	+	+	-	+	+	-	+	+
Enterobacter aerogens	+	+	++	-	+	+	-	-	+	-	+	+
Ent. Cloacae	+	+	+	-	+	+	-	±	-	-	-	+

Hafnia alvei	-	+	+	-	+	-	-	-	+	-	-	+
Serratia marcescens[b]	-	+	±	-	+	+	-	-	+	-	±	+
Proteus mirabilis	-	+	+	-	±	±	+	++	-	+	-	-
P. vulgaris	-	+	+	+	-	-	+	++	-	+	-	-
Morganella morganii	-	+	+	+	-	-	+	++	-	±	-	-
Providencia rettgeri	-	+	-	+	-	+	+	++	-	-	+	-
Prov. Stuartii	-	+	-	+	-	+	+	±	-	-	+	-
Prov. alcalifaciens	-	+	+	+	-	+	+	-	-	-	-	-
Yersinia enterocolitica[c]	-	-	-	±	-	-	-	±	-	-	±	+
Y. pestis	-	-	-	-	-	-	-	-	-	-	-	±
Y. pseudotuberculosis	-	-	-	-	-	-	-	+	-	-	-	±

[a] lac, inos, fermentation of lactose, inositol; mot, motility; gas, gas from glucose; ind, indole production; VP, Voges-Proskauer; cit, Citrate utilization (Simmons'); PDA, phenylalanine deaminase; ure, urease; lys, lysine decarboxylase; H_2S, H_2S produced in TSI agar; ONPG, metabolism of *o*-nitrophenyl-β-D-galactopyranoside.

[b] Some strains of Serratia marcescens may produce a red pigment

[c] *Yersinia* are motile at 22°C.

{Key: +, ≥85% of strains positive; -, ≥ 85% of strains negative; 16-84% of strains are positive after 24-48 hour at 36°C}

(Source: Collee *et al.,* 1996)

APPENDIX-V

STANDARD METHOD FOR GRAM'S STAINING

First devised by Hans Christian Gram during the late 19th century, the Gram-stain can be used effectively to divide all bacterial species into two large groups: those that take up the basic dye, crystal violet (Gram-positive) and those that allow the crystal dye to was out easily with the decolorizer alcohol or acetone (Gram-negative). The following steps are involved in Gram-stain:

1. A thin film of the material to be examined was prepared and dried.
2. The material on the slide was heat fixed and allowed to cool before staining.
3. The slide was flooded with crystal violet stain and allowed to remain without drying 10-30 seconds
4. The slide was rinsed with tap water, shaking off excess.
4. The slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface.
5. The slide was rinsed with tap water, shaking off excess.
6. The slide was flooded with alcohol acetone decolorizer for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolorizer.
7. Thicker smear requires more aggressive decolorizing.
8. The slide was flooded with counter stain (safranin) for 30 seconds and washed off with tap water.
9. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 1000X.

APPENDIX-VI

METHOD FOR COLLECTION OF MIDSTREAM URINE

It is the responsibility of laboratory to provide patient with sterile, wide mouthed, glass or plastic jars, beakers or suitable receptacles. They should have tight- fitting lids or be covered with papers or foils prior to sterilization by dry heat or autoclaving. Whenever possible, the first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most suitable for culture, microscope and biochemical analysis. Midstream urine (MSU) collection for ambulatory women for microbiological examination is as follows:

1. Wash hands thoroughly with soap and water and dry them with a clean towel.
2. Undress in a suitable room spread the labia and cleanse the vulva and labia thoroughly using sterile cotton gauze pads and warm soapy water wiping from front to rear.
3. Rinse thoroughly with warm water and dry with a sterile cotton gauze pad. During the entire process the patient should keep the labia separated and not touch the cleansed area with fingers.
4. Pass urine, discarding the first part of the stream. Collect the remaining urine in the sterile container, closing the lid as soon as the urine has been collected.
5. Hand the clean- catch midstream urine, in the closed container, to the health personnel for prompt delivery to the laboratory.

For bedridden patients, the same procedure is followed, except that a nurse must assist the patient or, if necessary do the entire cleansing procedure before requesting the patient to pass the urine. In both situations every effort must be made collect a clean- catch urine specimen in a sterile container and to ensure that it is delivered promptly to the laboratory together with information on the patient, clinical diagnosis and requested procedures.

APPENDIX- VII

FEATURES OF THE MOST COMMON TYPES OF VAGINITIS

Basis of diagnosis	Bacterial vaginosis	Vulvovaginal candidiasis	Trichomoniasis
Signs and symptoms	Thin, off-white discharge Unpleasant "fishy" odor, with odor increasing after sexual intercourse	Thick, white, cottage cheese discharge with no odor	Copious, malodorous, yellow green or discolored discharge, Pruritus, irritation, dysuria, 20 to 50% of affected women are asymptomatic
Physical examination	Usually, normal appearance of tissue; discolored discharge with abnormal odor, homogeneous discharge that adheres to vaginal walls	Vulvar vaginal erythema, edema and fissures; Thick white discharge that adheres to vaginal walls	Vulvar and vaginal edema and erythema, Strawberry cervix in up to 25 percent of affected women Frothy, purulent discharge
Vaginal pH (normal=<4.5)	Elevated (>4.5)	Normal	Elevated (>4.5)

Microscopic examination of wet-mount and KOH preparations of vaginal discharge	"Clue cells" (vaginal epithelial cells coated with coccobacilli) and few lactobacilli Occasional motile, curved rods (Mobiluncus species)	Pseudohyphae, mycelial tangles or budding yeast cells	Motile trichomonads Many polymorphonuclear cells
Whiff test (normal = no odor)	Positive	Negative	Can be positive
Additional tests	Amsel's criteria (three of four criteria must be met): provides correct diagnosis in 90 percent of affected women,Criteria of Nugent or Spiegel for Gram stain to diagnose bacterial vaginosis Other tests are controversial	KOH microscopy Gram stain Culture	DNA probe tests: sensitivity of 90 percent and specificity of 99.8 percent Culture: sensitivity of 98 percent and specificity of 100 percent

Information derived from (Carr et al., 1998, Sobel, 1997).

APPENDIX-VIII

DIAGNOSTIC CRITERIA FOR VAGINAL INFECTIONS

Diagnosis	Diagnostic criteria
Bacterial vaginosis	Presence of at least three of the following: a) watery vaginal discharge b) elevated pH (>6) c)positive amine order test d) presence of clue cells in Gram stained vaginal smear
Vaginal candidiasis	Positive culture for *Candida* with presence of clinical signs (red, inflamed tissue and curdy white discharge)
Trichomoniasis	Positive culture of viable *T. vaginalis* or positive wet mount preparation test

(Source: Helen et al., 2005)