BACTERIAL VAGINOSIS
IN MARRIED FEMALE HOSPITAL POPULATION

By

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2014

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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In The Name Of

ALLAH

The most Beneficent, the most Merciful

Read! And your Lord is the Most Generous,
Who has taught (the writing) by the pen,
has taught man that which he knew not.
(Surat Al-Alaq; V.96: 3,4,5)

Dedicated

To my dear
FATHER
Col (R) Muhammad Rafique
(May his soul rest in peace)
His Wish, His Dream
Who has always supported, guided and believed in me

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List of Abbreviations

ATP	Adenosine triphosphate
AM	Ampicillin
BV	Bacterial vaginosis
CAZ	Ceftazidime
CDC	Center for disease control
CFM	Cefixime
CIP	Ciprofloxacin
CLSI	Clinical laboratory standard institute
CRO	Ceftraxone
CTX	Ceftaximine
C trachomatis	Chlamydia trachomatis
DFA	Direct flotrscence assay
EB	Elementry body
E.coli	Escherichia coli
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
ER	Erythromycin
G. vaginalis	Gardenerella vaginalis
GM	Gentamycin
GUM	Genitourinary medicine
HFH	Holy Family Hospital
HIV	Human immunodeficiency virus
HVS	High vaginal swab
H_20_2	Hydrogen peroxide
HPV	Herpes papilloma virus
HS	Herpes simplex virus
V	Horseradish peroxidase
HRP	Heat shock protein
HSP	Immunoglobulin G
IgM	Immunoglobulin M
IUCD	Intrauterine contraceptive device
IMP	Imipenum
KOH	Potassium hydro oxide
LEV	Levofloxacin
LPS	Lipopoly saccharide
NGU	Non gonococcal urethritis
N gonorrheae	Neisseria gonorrheae
NHANE	National health and nutrition examination survey
S NAAT	Nucleaic acid amplification test

PID	Pelvic inflammatory disease
PMN	Polymorphnuclear neutrophils
PCR	Polymerise chain reaction
PAP	papanicolaou smear
QUA	Quaid-i-Azam University
RB	Reticulate body
spp	Species
STI	Sexually transmitted infection
STD	Sexually transmitted disease
S agalactiae	Streptococcus agalactiae
S aureus	Staphylococcus aureus
TET	Tetracyclin
T vaginalis	Trichomonas vadinalis
TZP	Pipracillin/Tazocin
UK	United Kingdom
USA	Unites States of America
WHO	World health organization
>	greater than
<	Less
µL	Micro liter

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AKNOWLEDGEMENTS

In the name of **Allah, Most Gracious, Most Merciful**. I thank my Creator for giving me the strength and the energy to undertake this extremely challenging experience. For that YOU have helped me to gain knowledge, I pledge to spread it and serve till YOU allow me to breathe.

I like to express my profound regards for **Dr Irfan Zai Qureshi** Chairman Department of Animal Sciences for providing me opportunity of research.

My sincere gratitude to my supervisor and chairperson **Professor Dr Samina Jalali,** Department of Biological Sciences, Quaid-i-Azam University Islamabad. She is an institution in herself. Her depth of knowledge and enormous wisdom guided me to reach this far. Her endless guidance, valuable time, sincere advice, critical analysis, encouraging attitude, faith and belief in the work I did and facilitating me throughout my research. Without her supervision and support this thesis would not have come this far.

I am grateful to **Dr S.A.Shami** for his sound advice, keen interest and support for my research endeavors with extraordinary guidance towards statistical analysis. He took me into his world of research, fast driving track of thinking, and never-ending desire for excellence. I thank him for believing in my dreams and helping me to translate them into a scientific thinking process. It would not have been possible without his support.

Special thanks to **Principal** Rawalpindi Medical College Rawalpindi for facilitating me to join the Ph.D. program and to conduct research at Holy Family Hospital Rawalpindi.

Professor Dr Rizwana Chaudhary, of Gynecology and Obstetrics was extremely helpful for allowing me to gather patients and collect samples, for which I am thankful. I am also grateful to all my female patients who agreed to be a part of my study. My gratitude for **Professor Dr Naeem Akhter,** of Microbiology for his guidance in the laboratory work.

A very special thanks to my dear friends **Dr Nuzhat and Dr Homera,** who have always been there at all times and to **Sobia, Iqbal, Mushtaq and Nadeem** for their manual help and assistance in the laboratory work.

My special gratitude is due for my husband **Dr Tariq Mahmood,** for his understanding, continuous encouragement and endless moral/emotional support. My children **Amna, Omer and Kaneez Zahra** for bearing with me during this time period and being compliant with my busy schedule.

My **Mother** whose unconditional love, prayers, support, belief and faith made this difficult task possible.

Last but not the least my **Father** (May his soul rest in peace) who always believed in my capabilities. He was my friend since birth, who never taught me how to live as he was a living example and let me learn from him. It is from him that I learnt the values of perfectionism through dedication, perseverance and honesty in work.

Dr Shireen Rafiq

ABSTRACT

Bacterial vaginosis is a major health issue of females in reproductive age group. The study population comprised of 332 symptomatic married females with vaginal discharge. Patients selected randomly at Gynecology and Obstetrics out-patient department of Holy Family Hospital Rawalpindi. Mean age of 28.01 ± 0.29 years of female patients presented with different symptoms of vaginal discharge. Maximum number of patients presented with low backache (83.73%), lower abdominal pain (81.62%), rash/itching (70.78%), unwell feeling (68.67%), Pain in thighs (68.37%).The lowest symptom observed were dysparunia (50.90%), feverish feeling (22.89%) and intermenstural bleeding in least number of patients (13.85%). Commonest combination of symptoms observed was low backache with lower abdominal pain (72.28%) and along with rash/itching (53.61%). The least observed combination was dysparunia with intermenstural bleeding (8.73%). The patients were clinically ascertained with vaginal examination for the variation in the color, consistency and smell of vaginal discharge. Whitish (41.56%), translucent (21.38%), yellowish (12.95%) and clear (24.09%) color of vaginal discharge was observed. Consistency of discharge varied from thick and homogenous (45.18%), to watery (24.69%) and normal viscous (30.12%) discharge. Along with color and consistency smell was observed as foul smelling (40.66%), pungent (15.05) and no particular odor (44.27%). A non significant negative trend with increase in age was observed on the clinical observations according to age groups. Patients were assessed for the condition of the cervix which appeared as healthy (16.26%), red and swollen (31.32%), red and swollen with ectopy (50%) and healthy with ectopy (2.40%). The condition of the cervix was analyzed for the color, consistency and smell of vaginal discharge which was highly significant ($P<0.0001$) for the smell of discharge. Bacterial vaginosis which is a clinical entity was diagnosed depending on the clinical and laboratory parameters implementing Amsel Clinical Criteria and Nugent Scoring System. According to Amsel criteria 24.69% patients were positive for BV. Parameters of Amsel criteria, Clue cells (37.34%), pH >4.5 (57.22%), homogenous vaginal discharge (45.16%) and Amine odor or whiff test (40.66%) were observed. Patients fulfilling three parameters were 15.96% and all four parameters were 8.73%. According to the age groups all parameters of Amsel analysis observed showed a non significant negative trend. With Nugent scoring system BV was analyzed in 42.16% patients. The bacterial morphotypes, Lactobacillus spp, Gardnerella vaginalis and Mobiluncus spp., were calculated and scored according to Nugent scoring. Patients considered as normal or negative were falling between score 0-3 were 57.83%, whereas patients considered as intermediate as positive were between the score 4-6 were 25% and patients with >7 score were 17.15%. Different parameters which included clue cells, epithelial cells, polymorphnuclear neutrophils, Lactobacilli spp, Gardnerella vaginalis , Mobiluncus spp. and pH of vaginal discharge according to the condition of cervix have important role in the identification of different infections in the patients. These parameters were calculated on direct vaginal smear and even distribution was observed in all conditions of cervix with exception of Mobiluncus spp, significant difference ($P<0.04$) was observed in comparison between healthy and healthy with ectopy. Vaginal, cervical and fundal

characteristics of patients were assessed, in relation to the number of polymorphnuclear neutrophils. It was observed that majority of patients fall in the category of 6-15 and 26-35+ PMN on direct smear gram staining (x1000 magnification) with various vaginal, cervical and fundal conditions which were not significant. High vaginal and endocervical swabs obtained were studied for microorganisms present in the vaginal discharge. All samples were inoculated on different culture media's and different isolates were obtained after incubation under aerobic conditions except for Neisseria gonorrheae which required anaerobic condition. Total single bacterial isolates obtained were 59.03%, Fungal (Candidiasis) 17.16%, Mixed growth (bacterial and fungal) 11.14% and no growth was 12.65%. Single isolates obtained were Escherichia coli (25%), Candida spp. (17%), Klebsiella spp. (10%), Neisseria gonorrheae (9%), Streptococcus agalactiae (7%), Staphylococcus aureus (4%) and Pseudomonas aeruginosa (3%). Among the mixed isolates Escheriachia coli + Candida spp. (6%), Streptococcus agalactiae + Candida spp. (3%), Staphylococcus + Candida spp. (2%). According to the age group 27-31 years of patients had maximum number of various isolates. All isolates identified were tested against various groups of antibiotics for the sensitivity, resistance and intermediate sensitivity. Various groups of antibiotics used were Pencillin (Ampicillin, Tazocin/Pipracillin), Macrolide (Erythromycin), Aminoglycoside (Gentamycin), Tetracyclin, Carbepenem (Imepenum), Quinolones (Ciprofloxacin, Levofloxacin) and Cephalosporins (Cefixime, Cefotaximine, Ceftazadime, Ceftraxone). Sensitivity pattern revealed that all isolates showed good sensitivity to Imepenum, Ciprofloxacin, Levofloxacin and cephalosporins. Sensitivity pattern to all conventional drugs gave more resistance as compared to sensitive effect of drugs. Effect of vaginal discharge in different phases of menstrual cycle, hygienic practices and various related features associated to different bacterial and fungal infection was observed. It became apparent that vaginal discharge increased in 88.55% of patients in the luteal phase mostly in bacterial infection 52.40%. The discharge decreased in follicular phase (51.20%) and ovulatory phase (37.34%). Patients with candidiasis and mixed vaginal infection complained of lesser amount of discharge in all phases of menstrual cycle. The use of various sanitary pads in vaginal infection was an important factor. Bacterial infection was prevalent among patients using different types of sanitary pads. However use of Always showed that bacterial infection was least compared to cotton or cloth. Infections with different organisms was observe due to different bathing and cloth changing habit which was highly significant ($P<0.001$). Majority of patients 77.71% complained of increased discharge due to coitus and standing and strenuous work 76.80%. Lesser number of patients observed to have discharge due to excitement (26.20%) and tension and anxiety (24.36%). Number of episodes regarding recurrent infections was significant ($P<0.03$). Whereas, duration of infection and any previous treatment for the infection was not significant. Sexually transmitted infections Chlamydia trachomatis IgG and IgM was observe in serum in 182 patients and Neisseria gonorrheae on Thayer Martin Media in 332 patients. It was observed as Gonorrheae was 9.33%, IgG was 36.81% and IgM was 39.01%. Combined infection (18%) was observed out of 182 patients. It was also

observed that majority of patients with IgG (17.50%) and IgM (15.93%) in the age group 22-26 years and IgG (11.53%), IgM (9.89%) in the age group 27-31 years respectively. Interestingly majority of patients had matric level education and the second highest percentage was observed in graduates and patients with no schooling. The economics also had important role as the financial level increased the sexually transmitted infections decreased. The most common symptom with which the patient positive with Chlamydial infection presented was low backache followed by un-well feeling and rash/ itching. Least common complaint was dysparunia and intermenstural bleeding. The most common color observed was whitish and translucent and normal clear was observed in lesser number of patients. Majority of patients had thick and homogenous vaginal discharge with red and swollen cervix along with cervical friability. All infections, Chlamydial, gonococcal, bacterial and fungal singly and as mixed infection were assessed on patients educational status and husbands income. It was observed as the economic status increased the educational status increased and was highly significant ($P<0.0001$). Chlamydial infection ($P<0.02$), Bacterial infection ($P<0.0001$) and fungal infection ($P<0.0001$) singly decreased with advancing education and economic status. Gonococcal infection was not significant among the groups. Among co-infections Bacterial + Candida infection ($P<0.0004$), Bacterial + Chlamydial infection ($P<0.006$) and patients with no infection ($P<0.0005$) was observed as the educational status and economic status increased the infection decreased. Patients were calculated for the outcome of conception which live births (64.61%) and pregnancy loss (35.38%). In the pregnancy loss highest percentage was of abortion (22.30%). Maximum percentage of live births 44.61% was observed in patients with bacterial and Candida infection while pregnancy loss was also highest in these patients, which was 22.30%. Among these patients majority had abortions 14.10% (n=110) and miscarriage, still birth and ectopic pregnancy were less in number. Spoken information regarding the husband's symptoms, sexual partners and any addiction was gathered from the females coming to the outpatient department with vaginal discharge. It was observed that females who were suffering from various infections, Chlamydial (36.18%), Gonorrhea (9.33%) and Bacterial vaginosis (42.16%), their husbands had complaints regarding urethral discharge. High percentage of female patients with gonorrhea, their husband (58.06%) had complained of urethral discharge and out of these male partners 35.48% had ulcer on urethra and of these male partners 83.87% had more than one sexual partner. The second highest percentage of urethral discharge (40.12%) and ulcer on urethra (14.92%) was informed in male partners of patients positive with Chlamydial infection, these partners (43.28%) had more than one sexual partner. The lowest percentage of male partner problem was seen in patients with Bacterial vaginosis (37.14%). Of these, 47.14% had more than one sexual partner.

It was concluded that all patient attending the out-patient department with variable complaints of vaginal discharge `had some kind of infection, Bacterial vaginosis, Bacterial vaginitis or a sexually transmitted infection or a combination of infections with resistance to conventional drugs and effect on the conception outcome with higher percentage of pregnancy loss.

INTRODUCTION

The microbiota of the human vagina affects the health of women, their pregnancies, and newborns (Marrazzo et al., 2010). Vaginal discharge being the commonest vaginal symptom prompts women to seek medical care. (Sobel, 1997) The vagina and cervix form a complex and dynamic ecosystem of epithelia, secretions, microbiota and innate immunity factors that depends on the levels of steroidal hormones. Vaginal microflora presents as the most important defense mechanism for the reproductive system, maintaining a healthy environment by preventing the proliferation of microorganisms (Linhares et al., 2010).

Female genital secretion is indicative of various genital tract diseases with different etiologies and prognosis. Bacterial vaginosis (BV) is the most prevalent and least understood problem in women of reproductive age (Ness et al., 2002). It is the most important cause of vaginal discharge and various behavioral factors have been associated with its presence (Rein and Holmes, 1983; Cherpes et al., 2008; Fethers et al., 2008). The vaginal flora is a complicated environment containing variable quantities and proportion of microbiological species. A complex balance of various microorganisms maintains the normal vaginal flora. The actual chain of microbiological events, leading to a change in the normal vaginal flora causing BV remains a mystery (Schwebke et al., 1997). An important genital syndrome, as it affects large number of women of reproductive age group and hence among the commonest reason for women to seek medical help (Biswas, 1993; Morris et al., 2001; Sumati and Saritha, 2009). The etiology of BV remains unknown, is often asymptomatic condition and is still, along with vulvovaginitis candidiasis, most common cause of vaginitis. BV has in the recent years emerged as a global issue due to its association with ascending genital tract infections and with sexually transmitted infections (Kouman and Kendrick, 2001; Nyirjesy, 2008; Verstralen et al., 2010; Kumar et al., 2011).

BACTERIAL VAGINOSIS – A SYNDROME

Vaginal flora is dominated by the genus Lactobacillus. It maintains the acidic environment and has an important role in the main defense mechanism regarding vagina (Morris et al., 2001; Susana et al., 2002; Khan et al., 2004; Hillier, 2008). A

vaginal environment dominated by hydrogen peroxide-producing Lactobacillus species, has been associated with healthy pregnancies and healthy newborns, lack of abnormal vaginal symptoms, and reduced risk for several sexually transmitted pathogens. Bacterial vaginosis – a poly-microbial syndrome, is a clinical entity which is characterized by a change or a shift in the vaginal ecology (Edwards, 2004; Srujana et al., 2010). Normal protective vaginal flora of predominant indigenous lactobacillus is lost and replaced gradually by mixed flora consisting of aerobic, anaerobes and microaerophilic species resulting in symptomatic and asymptomatic vaginitis (Sobel, 2000; Evy et al., 2011). BV first became a concern for women genital health in 1980 while it was first described in 1895. The first publication in 1892 described the normal bacterial flora of the vagina identifying Lactobacillus as constituent of healthy flora by Döderlein (1892). The composition of human vaginal microflora has been extensively studied (Andreu et al., 1995; Gupta et al., 2000; Anderson et al., 2004). Loss of these micro-organisms, play a protective role and other related changes in the vaginal ecosystem. These provide a biological plausibility for increased risk of all kinds of sexually transmitted infection (STI) and are risk factors for developing vaginal infections throughout the world. Bacterial vaginosis renders women vulnerable to Neisseria gonorrhoeae (N gonorrheae), Chlamydia trachomatis (C trachomatis), Herpes simplex virus (HSV-1 and 2), Herpes papilloma virus (HPV) and Human immunodeficiency virus (HIV) (Wiesenfeld et al., 2003; Schewebke, 2005; Hampton et al., 2006; Atashili et al., 2008; Rahkola et al., 2009; Allsworth et al., 2009; Verstraelen et al., 2010). According to center for disease control (CDC) working group, infections which are related to BV can be broadly categorized as opportunistic infections with associated bacteria and infections due to sexually transmitted agents (Kouman and Kendrick, 2001). Schröder, (1921) divided the vaginal discharge into three types. The first type was dominated by Lactobacilli, the second type a mixture of Lactobacilli and other bacteria, and in the third type Lactobacilli was absent. In 1955, Gardner and Duke isolated Gardnerella vaginalis (G. vaginalis), from women with vaginal infection (Totten et al., 1982; Hillier, 2008). The term, bacterial vaginosis in 1984 emerged and gave the definition: a replacement of vaginal Lactobacilli by characteristic groups of bacteria that are accompanied by changes in the properties of the vaginal fluid (Hillier et al., 1995; Hillier et al., 1999; Sobel, 2000; Sumati et al., 2009). There is no known long term therapy which helps in preventing this frequently occurring infection. BV is associated with serious

complications, such as chorioamnionitis, spontaneous abortions, preterm labour, low birth weight, endometritis resulting in increased susceptibility to various sexually transmitted infections including HIV (Gravett et al., 1986; Meis et al., 1995; Hillier et al., 1995; Goldenberg et al., 1997; Taha et al., 1998; Wiesenfeld et al., 2003; Cherpes et al., 2005).

The etiology of BV is not yet clear and is under discussion. It is still debatable whether BV be considered as a sexually transmitted condition or an abnormal colonization with microorganisms or an ecological imbalance of vaginal micro-flora that can arise as a outcome of range of activities or factors. Recurrence after treatment with current therapy protocol signifies inadequate treatment or persistence of infection or can be re-infection from sexual partner. Until this is resolved, preventive measures against BV are not possible and at the same time difficult (Eschenbach et al., 1988; Larsson et al., 1991; Nillson et al., 1997; Padilla et al., 1999; Morris et al., 2001; Fethers et al., 2008; Kumar et al., 2011).

PEVALENCE OF BACTERIAL VAGINOSIS:

BV is considered as one of the most commonly occurring vaginal disorder. The frequency of 3.6-40% has been reported across different population around the world (Forsum et al., 2005). Most of the studies have been conducted in clinic like genitourinary medicine (GUM), STI and in abortion clinics, primary care units (Larsson et al., 2005) with different study populations selected from the gynecology and obstetrics clinics or an STI clinic. Research from the healthy female population is difficult to find even in population based study (Morris et al., 2001, Allsworth et al., 2008). Different categories of patients, like pregnant females, patients coming for abortions, patients with vaginal discharge and sex workers were included in the studies (Larsson et al., 2005). BV seems to be particularly common in Africa. Studies have reported high prevalence rates of 20-49% attending the STI clinic, 21-52% in pregnant women attending the antenatal clinics, and 37-51% in community based studies. These rates are much higher than in the industrialized countries, 13% in GUM clinics UK, 11% in gynecology clinics UK, 15-30% of non-pregnant women in USA (Hay et al., 1992; Ledru et al., 1996; Govender et al., 1996; Thomas et al., 1996;

Mayaund et al., 1998; Fonck et al., 2000; Walraven et al., 2001; Morris et al., 2001; Holzman et al., 2001; Simhanet et al., 2008; Cherpes et al., 2008; Klatt et al., 2010). In 1998 BV reported by GUM clinic in UK was 18.4%, an underestimate of the true burden of disease (Lamagni et al., 1999). The only population based survey took place in Uganda and reported prevalence as 50% (Sewankambo et al., 1997; Wawer et al., 1999) which was typical of rural Africa and cannot be applied on other settings due to the importance of basic hygiene facilities.

Prevalence of BV between 4.9 to 36% had been reported from the European and American studies. First nationally representative study on BV was conducted by NHANES in 2001. It was conducted according to race and ethnicity, higher rates were observed in African Americans (50.3%) and Mexican American women (28.8%) compared to whites (22.4%) (Hampton et al., 2006; Allsworth et al., 2007). Prevalence of BV is consistently two to three times higher among Black women as compared to white women (Culhane et al., 2001; Culhane et al., 2002; Nansel et al., 2006). Sexual orientation is also an important marker for example lesbian populations have shown infection rates between 29% and 52% (Schmid et al., 1999). Young girls, those without any history of sexual activity, up to 33% harbor BV (Shafar et al., 1989). Overall prevalence of BV varies greatly depending on the population. Estimates range from 4% - 60% among asymptomatic college students and among women attending a sexually transmitted disease clinic (Mead, 1993). Evidence against sexual transmission of BV includes similar rates (15%) of bacterial vaginosis in prostitutes and college students in Seattle, also observed in virginal adolescents (Spiegel et al., 1980; Bell et al., 1985; Bump et al., 1988; Vaca, 2010). In the general population the prevalence varies from 10% to 25% of reproductive age women (Nansel et al., 2006).

The overall incidence and prevalence of bacterial STIs, Gonococcal infection and syphilis have declined since World War II. The incidence of STIs is still at a higher side in developing countries. The prevalence and distribution of infection basically depends on the behavior of an individual and his or her sex partner. Globally, an estimated 12 million people are infected every year, and the majority of infections occur in developing countries (Aral et al., 2006; Wellings et al., 2006; Mabey, 2010). The major pathogen causing non-gonococcal urethritus (NGU) is Chlamydia trachomatis, accounts for 30-50% of cases. Chlamydial genital infection is the most

commonly reported infectious disease in STI clinics and highest prevalence in persons less than 35 years as majority of the women remain asymptomatic (Marrazzo et al., 2001; Creighton et al., 2003).

RISK FACTORS

Other risk factors include a low socio-economic status, poor hygiene, cigarette smoking, douching, antibiotic use for other conditions, young age of coitarche, new sex partner or multiple sex partners. A consistent use of condoms is protective against BV (Merchant et al., 1999; Verstraelen, 2008; Fethers et al., 2009; Verstraelen et al., 2010). High risk behaviors are risk factors for acquiring sexually transmitted infections and this suggests that BV could be transmitted sexually (Gardner and Duke, 1955; Verstraelen, 2008). A typical STI usually involves a single etiological agent, BV involves multiple pathogens, majority of these pathogens are detected (in low numbers) in the vaginas of BV-free and sexually inexperienced women. Interestingly, there is no evidence for a decrease in the rates of BV recurrence following antibiotic treatment of partners sexually involved with affected women is another difference between BV and the common STIs (Verstaelen et al., 2010). Race and ethnicity, education, income and age are significant correlates of BV (Allsworth and Peipert, 2007). Other risks for BV include douching for hygiene, which acts to promote loss of hydrogen peroxide-producing lactobacilli spp. and use of an intrauterine contraceptive device (Calzolare et al., 2000; Gray et al., 2009). Comparison of tampon use and napkin (pad) showed that the use of tampon increased the growth of staphylococci during menstruation (Chow et al., 1989).

MICROBILOGY OF BV:

BV is characterized by an alteration of normal vaginal flora, a loss of H_2O_2 producing Lactobacillus species, increase in gram-variable coccobacilli, anerobic organisms, and genital mycoplasma with increase in the vaginal pH. BV has been associated with upper reproductive tract infections and reported to be a strong predictor of Chlamydial and Gonococcal infections. The healthy microbiota of the lower genital tract predominantly consists of Lactobacillus spp. (Pavlova et al., 2002; Wiesenfeld et al., 2003; Zhou et al., 2004; Shi et al., 2009). These Lactobacilli spp. form a line of defense against the potential pathogens. The symbiotic relationship between vaginal

Lactobacilli spp. and the female host is modulated by the hormones circulating in the body. These hormones stimulate the vaginal epithelia to produce glycogen (Hay, 2005). Lactobacilli metabolizes the glycogen secreted by the vaginal epithelia, produces lactic acid that is responsible for maintaining the normal vaginal acidic pH (<4.5) (Donati et al., 2010). The vaginal discharge is the result of degradation of the normal vaginal mucin gel, efficiently performed by mucin-degrading enzymes produced by BV-associated bacteria, particularly Gram-negative anaerobes (Olmsted et al., 2003). The odor, usually described as "fishy," is derived from volatilization of the amines produced by the metabolism of anaerobic bacteria that characterize this disorder.

The acidic environment of a healthy vagina prevents the growth of potential pathogens (Aroutcheva et al., 2001; Donati et al., 2010). Normal vaginal flora consists of both aerobic and anaerobic bacteria, with Lactobacillus spp. being the most predominant microorganism and the bacteria accounts for more than 95% of all bacteria present (Spiegel et al., 1980; Eschenbach et al., 1989). Vaginal lactobacilli keep the pathogens away through formation of biofilms (Domingue et al., 1991) and by the production of antimicrobials like hydrogen peroxide and bacteriocin-like substances (Aroutcheva et al., 2001). BV is characterized by depletion of H_2O_2 producing Lactobacillus spp. accompanied by overgrowth (100 to 1000-fold above normal) of commensal vaginal anaerobic bacteria (Hillier et al., 2008). Women with BV presents with variable flora from vaginal fluid. BV yield spectrum of anaerobic or microaerophilic commensals, Gardnerella vaginalis, Mobiluncus species, Prevotella species, anaerobic gram-positive cocci, Ureaplasma urealyticum, and Mycoplasma hominis. The initial event that leads to this shift is unknown. BV occurs more frequently among women who report new or higher numbers of male sex partners, pattern that invoke the epidemiology of a typical sexually transmitted infection (Avonts et al., 1990; Hawes et al., 1996; Marrazzo et al., 2002; Forsum et al., 2005; Sirinivasan and Fredrick, 2008; Livengood, 2009). Several other microorganisms are found frequently in the vagina like Staphylococcus epidermidus, Streptococcus spp. E coli, Klebsiella spp, Pseudomonas aeruginosa Cornybacterium spp., Peptostreptococci, Bacteroids, Candida spp , Gardenralla Vaginalis, Ureaplasma, Mycoplasma hominis (Sautter et al., 1980; Larsson et al., 2001).

Gardener and Duke (1955) associated vaginal syndrome with isolation of Haemophlis vaginalis, later named Corynebacterium vaginalis and currently named Gardnerella

vaginalis, play an important role in the infection. It has been identified in nearly all women with symptoms of BV but it has also been identified in 40 to 50% of asymptomatic women (Spiegel et al., 1980; Spiegel et al., 1983; Holst, 1990). G. vaginalis, is a Gram-variable or Gram-uncertain microorganism, its reaction to Gram staining varies from gram negative to gram positive (Catlin, 1992). Biochemical tests revealed that G. vaginalis is a catalase negative, oxidase negative and β-glucosidase negative. Ultrastructural investigation conducted by Scott et al. (1989), indicated that the outer fibrillar coat is responsible for the attachment of G. vaginalis to the vaginal epithelial cells (clue cells). G. vaginalis are small, pleomorphic immotile rods, occurring in clumps in vaginal smears (Edmunds, 1960; Greenwood and Pickett, 1980; Taylor-Robinson, 1984; Catlin, 1992). G. vaginalis provides an appropriate environment for colonization by strict anaerobes that are mainly responsible for the clinical symptoms of BV (Gardner and Duke, 1955; Greenwood and Pickett, 1980; Swidsinski et al., 2005; Josey and Schwebke, 2008; Swidsinski et al., 2008; Harwich Jr et al., 2010). BV is not caused by just the presence of the potential pathogens but rather by their marked uncontrolled increase in number (Eschenbach, 1993; Eschenbach, 1994; Forsum et a.l., 2005; St John et al., 2007). However, the exact mechanisms and sequences of the infective processes are mainly unknown. Gardner and Dukes (1955), were the first ones to discover a connection between G. vaginalis and BV. G. vaginalis was isolated from the lower genital tract of females with BV in 92% of cases as compared to a 0% isolation rate from healthy women. Ultimately, with the advance in the formulation of media selective for G. vaginalis allowed for the detection of this microorganism even when present in low numbers and this also helped to observe G. vaginalis in the healthy vaginas (Totten et al., 1982; Hill et al., 1984; Masfari et al., 1986; Eschenbach et al., 1988; Fredricsson et al., 1989; Cristiano et al., 1989; Mikamo et al., 2000).

Physiology of BV

Glycogen, an analogue of starch, is the main source of nutrients for the microbial flora. The metabolism of glycogen in the vagina is controlled by the estrogen hormone via estrogen receptors which are located in the epithelial cells covering the vaginal lumen. The activity of the basic estrogen receptors is mainly dependent on the ovarian hormonal cycle. There is an increase in the estrogen level during the mid-

cycle stage of the menstrual cycle with a resultant increase in the number of the epithelial cells due to increase in the glycogen content (Owen, 1975). As a result of increase in the number of the epithelial cells, there is an increase in the epithelial cell layer thickness (Wagner, 1982; Patton, 2000). The increased level of estrogen results in the decreased viscosity of the mucus with a resultant watery discharge. In the latter half of the follicular phase of menstrual cycle, the production of the mucus increases by 30-folds (Owen, 1975). At the time of menstruation, there is an increase in the vaginal pH to 6 on day two with the subsequent decrease of pH to 4 at day 4 (Eschenbach et al, 2000). With the resultant changes in the environment of the vagina during the menstrual cycle leads to the changes in the vaginal microflora. It is considered that lactobacilli spp. mainly responsible for maintaining the pH of the vagina (Eschenbach et al., 2000; Boskey et al., 2001). The mucus secreted in the vagina is mainly composed of glycoprotein, glycogen, electrolytes, and a larger portion of water. The mucosal layer provides nutrition to the vaginal microflora and also acts as receptors and helps in the adhesion of Escherichia coli (Hawthron et al., 1991; Otero et al., 2007). Lactobacilli undergo physicochemical interaction with the vaginal epithelia, which helps in the colonization and biofilm formation within the mucosal and the epithelial layer of the vagina (Busscher et al., 1987; Otero et al., 2007). The biofilm is composed of the secretary components from the vagina and the bacterial cell layer.

The presence of moisture is required for the proliferation of the microorganisms (Warren et al., 2005). The vagina is kept moist mainly by the vaginal secretion and to lesser extent by the urine (Faergemann et al., 1983). Microorganisms have an optimal pH range in which they show an improved activity. Any intervention with the pH of the system results in the growth of other microorganisms. At fertile age the pH of the normal healthy vagina is within 3.5–4.5 with a typical value of 4.2 (Owen, 1999). The vaginal secretion also contains antimicrobial components of the immune system and leukocytes (Paavonen, 1983; Cole, 2006). Cervical mucus, which has a pH of approximately 8.0, is the main contributor to the vaginal secretion and physically prevents microbes from attaching to the mucosal surface (owen, 1999). The pH is mainly maintained by the production of lactic acid by the Lactobacilli spp. Any compromise with the lactobacilli spp, results in the increase of the pH within the vaginal lumen.

This results in decreased lactobacilli population with a subsequent increase in the growth of other microorganisms (Aroutcheva et al., 2001). With the decrease in lactobacilli count, there is a resultant decrease in the production of lactic acid. Lactic acid has a strong anti-microbial property that has a role in preventing the growth of the pathogenic microbes (Kabara et al., 1972). Lactobacilli also produce antimicrobial products, bacitracin and hydrogen peroxide which prevent the proliferation of the pathogenic microorganisms (Holmes, 1999; Gipson et al., 1999; Brook et al., 1999; Wiggens et al., 2001; Reid, 2002). The metabolic products secreted by the microorganisms influence the availability of the nutrients (Cavallo, 1987). Fatty acids also show antimicrobial activity against Streptococcus pyogenes, Staphylococcus aureus, and Micrococci and helps in adjusting the composition of the microbial flora (Kabara, et al, 1972). The interaction between the host and the microorganisms can create a mutually beneficial relationship (Lactobacillus) or can have a deleterious effect on the host, diseased condition like Candida, Gardnerella, and/or T.vaginalis (Casadevalla, 2000). The adhesion of the pathogenic microorganism to the epithelial cells is one of the important factors for the colonization and biofilm development. The important components of vaginal secretion are (Na+, Ca2+, Cl-), proteins, glycoproteins, lactic acid, acetic acid, glycerol, urea, and glycogen, which vary depending on oestrogen and progesterone, sexual stimulation and the status of microbiocenosis (Huggins and Preti, 1981; Kierzenbaum, 2002).

Vaginal complaints

Infections of the lower genital tract are classified according to the site of infection as vaginitis, and cervicitis. According to the clinical complaints, regarding abnormal vaginal discharge (color and consistency), odor and vaginal itching are classified as Bacterial Vaginosis, Candidiasis, Trichomoniasis, Gonorrhoea or Gonococcal infection and Chlamydial infections in women of reproductive age ranging from 15-45 years. Sexually transmitted infections, Chlamydia and Gonorrhea are screened routinely and considered in females younger than 25-30 years (Holmes, 1999; Edwards, 2004; Anderson et al., 2004; Petersen, 2006; Verstraelen et al., 2010). Furthermore, in a quite high frequency of 20-34% infections, symptoms alone do not allow clinicians to distinguish confidently between the causes of vaginitis (Anderson et al., 2004; Landers et al., 2004). In survey studies which involved symptomatic

patient at health center for gynecological consultation, the number of undiagnosed patients ranged from 7%-72% on complaints only (Carlson et al., 2000). While 30% women with vaginal complaints go without diagnosis even after complete evaluation, explains why many clinicians manage patients without performing pH and microscopy (Mayaud et al., 1998; Wiesenfeld, 1999). Current recommendations for diagnosis of vaginal complaints involve vaginal examination and microscopy as microscopic findings make the picture clear (Bickley, et al., 1999; Mou, 2003; Edwards, 2004; Landers et al., 2004; Nancy, 2010).

Patients with vaginitis complained different combination of discharge, odor, irritation or itch. Discharge is characterized by color (clear, white, grey, green, yellow), consistency (thin, thick, watery or curd like) odor (foul smelling, fishy, pungent) and amount (more or less than normal) which cannot be quantified, bleeding and dyspareunia (Anderson et al., 2004). BV is most often not associated with clinical signs of inflammation thus the term "vaginosis" is used instead of "vaginitis" (Mashburn, 2006). BV is clinically almost identical to candidiasis as grey to white homogenous thin to thick discharge along with erythema and inflammation may be present (Cibley, 1991; Horowitz, 1994; Cerikcioglu and Beksac, 2004). Thick curdy discharge with signs of inflammation and pruritus is indicative of Candidiasis (Nancy, 2010). The cause of the two types of vaginosis is overgrowth of the normal Lactobacillus dominated vaginal flora. Bacterial vaginitis, also called aerobic vaginitis, is sometime confused with BV. The females present with whitish to yellowish color discharge with erythema of vaginal walls and inflammation resulting in vaginal dyspareunia (Donders et al., 2002; Edwards, 2004; Donders, 2007). Aerobic bacterial vaginitis is associated with aerobic microorganisms, Streptococci spp., E. coli and Staphylococcus aureus, Klebsiella spp. (Verstaelen et al., 2010; Mehdinejad et al, 2011).

Cervicitis does not induce pain and becomes apparent only by yellowish opaque mucoid to watery discharge with resultant on contact bleeding and deep dyspareunia. Cervix may be red and swollen with erythema, ectropion and inflammation (Petersen, 2006). Chlamydia trachomatis, Neisseria gonorrhoeae, T. vaginalis, HSV, and HPV are frequent causes of cervicitis (Holmes, 1999). These pathogens invade external stratified squamous epithelium of ectocervix (Holmes, 1999; Stanbery and Bernstein, 2000; Pudney et al., 2005).

Ascending infection from lower to the upper genital tract leads to pelvic inflammatory

disease (PID) (Stanbery and Bernstein, 2000). PID can result in endometritis, salpingitis, tubo-ovarian abscess, pelvic peritonitis or a combination of the above (Holmes, 1999). The most common causes of PID are N. gonorrhoeae, C. trachomatis, BV associated bacteria or highly virulent pathogen, streptococci spp. (Ness et al., 2004; Petersen, 2006).

Effect of age

Bacterial vaginosis demonstrates a striking age profile opposite to what is seen in STIs. BV has a strong association with age as it is more common among females over 25 years. It is unusual for STI where the highest rates are always found in women younger than 25 years (Sewankambo et al, 1997; Morris, 2001; Wilson et al, 2002; CDC 2010). Studies in women undergoing in vitro fertilization treatment have found young females are significantly more prone to have bacterial vaginosis (Ralph et al, 1999). Similar to other studies, there was no association observed between the prevalence of BV and age, as almost equal prevalence was seen in women between 15 to 45 years (Bhalla et al, 2007). However, significant correlation between BV and different age groups was observed (Allsworth et al, 2007; Oliveiria et al, 2007). The causes for the age distribution patterns of BV are difficult to disentangle, as probably various behavioral, physiological, and immunological variables interact (Fang et al, 2007). C.trachomatis is the most frequently reported infection in united states among females age <25 years. Asymptomatic infection is common with the result that annual screening of all sexually active females aged <25 years is recommended (CDC, 2010). Educational status has been found to be associated with BV as lack of education was found related to BV among women in third world countries, whereas certain studies contradict this finding (Fang et al, 2007; Allsworth et al, 2007).

Opportunistic microorganisms associated with Bacterial vaginosis

Aerobic microorganisms

The regulation of the microbiological flora of lower female genital tract which is a dynamic complex example of microbial colonization is not fully understood (Larson and Monif, 2001). Anaerobic bacteria are more prevalent among adolescent subjects, while aerobic bacteria appear to become more common with advancing age, onset of sexual activity and parity. Streptococcus agalactiae (S. agalactiae), E. coli and

Candida spp. are the normal commensals of vagina. These bacteria which are the normal constituents of vaginal flora require some alteration of the micro environment to cause disease (Larson et al., 2001). Candida spp. may be present without any typical symptom. Candida spp. co-colonizes with Lactobacilli spp. as are less susceptible to the effect of hydrogen peroxide as compared to S.agalactiae. When Lactobacilli spp. are eliminated by means of antibiotics the Candida spp. takes over (Hillier et al., 1993). Hillier et al., (1993) also found no difference in the isolation of S.agalactiae, E. coli, S. aureus, Klebsiella spp. in relation to the presence of Lactobacilli spp. According to Donders et al., (2002) vaginal microorganism associated with aerobic vaginitis was mainly S. agalactiae, S. aureus, E.coli. These are more frequent in aerobic vaginitis as compared to normal flora. S. agalactiae, gram positive cocci, are one of the most common colonizers and is an important cause of neonatal sepsis and meningitis (Reid and Bruce., 2003). Presence of these organisms is attributed to the unhygienic bowl practices. These colonizers predispose a female to recurrent urinary tract infection (Tariq et al., 2006). McDonald et al., (1997) found E.coli and S. agalactiae, an important pathogen associated with pregnancy loss and neonatal sepsis. S aureus gram positive cocci is colonized in the vaginal mucous of females, predisposing them to toxic shock syndrome. It is one of the most persistent pathogen of humans and has always remained as one of the most common cause of infection (Veeh et al., 2003; Schlievert et al., 2007).

The fact that the degree of overlap of BV and aerobic vaginitis is possible leading to a mixed infection as women with BV had vaginal leucocytosis (Donders et al., 2002). There is a correlation between aerobic vaginitis and S. agalactiae, S aureus and E.coli. Monif, (1999) provides evidence that S.agalactiae inhibits growth of Lactobacilli and G. vaginalis but not S. aureus. Aerobic vaginitis does not respond to drugs which are used for BV and should be treated with antibiotics according to culture and sensitivity. An optimal treatment plan for aerobic vaginitis includes antibiotics normalizing the vaginal environment (Vigneswaran and McDonald., 1994). As the culture of the sample provides identification of microorganisms, the sensitivity pattern indicates the antibiotics which are effective for treatment. The main conventional drugs such as penicillin group, tetracyclin, macrolides, aminoglycosides have developed resistance while the carbepenams, quinolones and cephalosporins shows sensitivity and effectiveness (Mumtaz et al., 2008). Antimicrobial resistance in E. coli, S. agalactiae, S. aureus, Klebsiella spp has increased worldwide and the

susceptibility patterns show a geographic variation as well as differences in population and environment (von Baum, and Reinhard, 2000). Antimicrobial resistance is a worldwide concern both in the developing and developed countries and has been reported to various microorganisms (Bell et al., 2002). A rise in bacterial resistance to antibiotics complicates treatment of infections.

Hygiene practices

Behavior factors such as vaginal douching or menstrual hygiene practices have been suggested as important factor that influences vaginal flora composition (Bahram et al., 2009). The prevalence of BV/vaginitis is variable among people from different communities. The cofactors effecting BV in various studies were considered and menstrual, personal and coital hygiene was pin pointed as the hygiene related variables. A correlation was observed between different methods of contraception and BV was diagnosed significantly in females with Intra-uterine contraceptive devices (Jindal et al., 2007; Guaschino et al., 2008; Bahram et al., 2009). Use of lubricants and spermicides contribute to the symptoms of BV (Mitchell, 2004). Menstrual hygiene practices in Africa revealed that in females who use sanitary protection reusable cloth incidence of BV was highest as compared to females using sanitary pads and tampons (Demba et al., 2005). Scientists and researchers have associated BV with vaginal douching, before and after coitus. Along with douching use of scented soap or perfumed bubble bath and antiseptic during bath have been the contributing factors (Klebanoff et al., 2010; Kumar et al., 2011). Multiple sex partners and new sex partner are important factors for BV, Chlamydial and infection with gonorrhea (Ryckman et al., 2009).

Most of the genital hygienic measures in women who douched, like tampon use, use of pads and panty when not menstruating, and females usually wearing nylon underwear experienced more BV. It was positively associated with bathing frequency, use of powder and feminine hygiene spray, and usual type of underwear. BV was less common among women experiencing amenorrhea, but type of menstrual protection was not associated with BV (Myer et al., 2004; Morison et al., 2005; Klebanoff et al., 2010). Holzman and colleagues (2001) reported increased chances of BV among women who bathed rather than showered with less chances of BV among women who used tampons. Although Schwebke and colleagues (1999) found tampon use not

associated with BV. Misra et al (2006) found BV in women who douched use feminine spray, wash or toilettes, as well as use powder on their genitals, but the relationship between these behaviors and BV has not been studied.

Recurrent infections

Number of factors increases the susceptibility to vaginal infections. Pregnant females and females on any contraceptives have higher chances of vaginitis (Carr, 1998). Much of the recurrent vaginal infections are those which relapse rather than a new infection (Sobel, 1984). Broad spectrum antibiotics like ampicillin, tetracycline, clindamycin and cephalosporins facilitate vaginal infections by eradicating the normal vaginal flora (McGroarty and Moody, 1993; Swidsinski et al., 2005; Swidsinski et al., 2008). Recurrent vaginal infections which are the marker for immune deficiency and can facilitate HIV, Chlamydia and Gonococci which are the major risk factors for serious complications such as PID, Infertility, tubal blockage resulting in ectopic pregnancy and chronic pelvic pain (Hills et al., 1997; Brunham et al., 2005). Since the treatment results for BV are not very encouraging. Knowledge about the recurrence of BV, its reasons for relapse, the problems which are associated with antibacterial resistance, the possible role of re-infection, and bio-film formation is insufficient (Hay, 2000; Wilson, 2004; Wilson et al., 2005). The situation is made even more difficult by the discovery that the bacteria in the dense biofilm on the vagina temporarily switch to a metabolically latent state during treatment and then returns to an active state after treatment cessation (Swidsinski et al., 2005; Swidsinski et al., 2008). Recurrent BV is troublesome and there are few published studies examining how to handle recurrent BV (Cook et al., 1992; Winceslaus et al., 1996; Hay, 2000; Wilson, 2004; Wilson et al., 2005). As BV is a poly-microbial condition, needs to cover all strains of microorganism to eradicate the issue and majority of these microorganisms are endogenous. Beigi et al., (2004); Austin et al., (2005); Klare et al., (2007); the baseline resistance (before treatment) and after treatment resistance was analyzed, irrespective of treatment, majority of the strains showed high percentage of resistance to the conventional drugs used such as metronidazole and clindamycin. Latest trend towards shorter treatment courses inadequately eradicate the organism and results in higher rates of resistance and recurrent infections (Sobel et al., 1992; Carr et al., 1998). It is considered as sexually transmitted, recurrence by the

male sexual partner or multiple sexual partners has been considered. However treatment of the male partner has not shown to prevent the recurrence (Sobel et al., 1989; Sobel et al., 1992).

Sexually transmitted infections

Sexually transmitted infections are clinical syndrome, which are caused by pathogens that can be acquired and transmitted through sexual activity (Shim, 2011). Studies have associated bacterial vaginosis with an increased susceptibility to STI. Sewankambo and colleagues, (1997) demonstrated an association between altered vaginal flora and HIV-1 infection (Martin et al., 1999). Women with Lactobacillus predominant vaginal flora are less likely to be infected with various STI like Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis than women with altered flora (Hillier et al., 1992; Hillier et al., 1992). Chlamydial genital infections are closely related to infection with gonorrhea in clinical manifestations. Both Chlamydia and Gonococci infect the transitional epithelium of the urethra and extend to the endocervix, the endometrium, salpinx, peritoneum and the rectum (Korenrom et al., 2002; Lyss et al., 2003). They can produce extensive damage by sub-epithelial inflammation, epithelial ulceration and scarring.

Chlamydia trachomatis

Chlamydia trachomatis is a gram negative obligate intracellular bacterium, needs living cells to multiply and infects only the human epithelial cell. It has 18 serotypes, out of which 11 cause STI and neonatal infections. It has incubation period of 7-21 days and a growth cycle of 48 hours (Stamm, 1999; Currie et al., 2007). Chlamydial infection causes major medical, social and economic problems. Its consequences are more damaging to reproductive health of females than men. Worldwide morbidity associated with sexually transmitted Chlamydial infection is enormous (Paavonen et al., 1999). It is now a most common, treatable and notifiable infectious disease in many countries (Adams et al., 2004; McCadden et al., 2005). The World Health Organization (WHO) in 2001 estimated that 92 million cases occur worldwide per year (WHO, 2001). C Trachomatis infection remains asymptomatic in 80% females and serves as a pool responsible for the risk of transmission within the community (Fenton et al., 2001).

C trachomatis has a unique growth cycle in which it exists as infectious elementary body (EB) which can survive outside the host cell and can infect the new host cell. The non infectious reticulate body (RB) is intracellular metabolically active replicating form. A part of reticulate body continues to multiply via binary fission in the cytoplasm of the host utilizing cells adenosine triphosphate (ATP), sugar and aminoacids to form inclusion bodies in the endosomes and the larger part matures to EB. The inclusion body contains upto 1000 infectious EB. The infected cell ruptures after 48-72 hours releasing new extra cellular EB to infect new cells (Stephens et al., 1998; Sharma, 2009). It is assumed that chronic inflammatory response is triggered as bacterial Heat Shock Protein (HSP) reacts with human HSP, an important factor in the immunopathogenesis of female genital inflammation. The proinflammatory and the anti inflammatory cytokines further influence tissue damage with the result Chlamydial infection does not always prevent progressive tubal damage (Kinnunen et al., 2003; Mardh et al., 2004; Tiitinen et al., 2006; Currie et al., 2007). The resultant increase in the number of polymorphnuclear leucocytes (PMN) potential marker of inflammation (Geisler et al., 2004; Culhane et al., 2005).

Various diagnostic methods are available to detect C. trachomatis infection. Endocervical samples are obtained and stained with iodine or geimsa staining to examine for the presence of intra-cytoplasmic inclusion bodies (Chiappino et al., 1995). Cell culture are considered as gold standard for growth and susceptibility testing (Black, 1997) and serological test for IgG and IgM (Mouton et al., 2002), enzyme immunoassay (EIA) (Bakir et al., 1989), direct florescence antibody detection (DFA) (Cles et al, 1988), polymerase chain reaction (PCR) (Currie et al., 2004), nucleic acid amplification test (NAAT) (van der Pol et al., 2000).

Current treatment choice as recommended by CDC (2006) is single dose of azithromycin or a seven day course of doxycyclin, but other drugs like macrolide, quinolones, sulfonamides, rifampicin and clindamycin also have activity against C trachomatis. Antibiotic resistance is rare but inadequate antimicrobial therapy allows persistence of infection (Geisler, 2004; Geisler, 2007).

Neisseria gonorrheae

Neisseria gonorrhoeae is the etiologic agent of gonorrhea, among the most frequently reported sexually transmitted disease in the United States since 1960. The gonococcus is a gram negative diplococcus which causes gonorrhea (Jennifer et al., 2004). According to CDC (2010), it is the major global health problem as 700.000 new infections occur each year in USA and sixty two million cases are reported annually worldwide (Gerbase et al., 1998; CDC, 2007; CDC, 2008). Increased risk is associated with gonorrhea for infection with HIV type 1 (Fleming et al., 1999). Women frequently do not exhibit symptoms, which leads to chronic infection. N.gonorrhoeae readily forms biofilms over abiotic surfaces, primary and transformed cervical epithelial cells, and over cervical tissues in vivo. Biofilms are associated with chronic infection with asymptomatic gonorrhea in women (Falestta et al., 2010).

Up to 80% of infected women do not develop any noticeable symptoms **(**Bozicevic et al., 2006). Undiagnosed infection in women can lead to prolonged or persistent infection (Hansfield et al., 2005). Ascending gonococcal infection occurs in 45% of infected females with persistent infection and developed pelvic inflammatory disease, permanent fallopian tube scarring and blockage with ectopic pregnancy, chronic pain, infertility, and/or disseminated gonococcal infection (Falastte et al., 2010).

Neisseria gonorrhea triggers an inflammatory response that is characterized by the presence of PMN bacteria in the gonorrheal secretion is attached to and within the PMN (Apicella et al., 1996). PMN are the primary innate immune responders and capable of killing the microorganisms (Borregaard et al., 2010). PMN's innate immune response is ineffective at clearing the gonococcal infection. The persistence in PMN facilitates long term colonization, creating opportunity for dissemination and transmission of gonorrhea. Resistance to PMN is a critical aspect of its virulence and replication (Johnson et al., 2011).

Antimicrobial resistance to various drugs is a major determinant for the treatment plan and to evaluate the efficacy as it limits the treatment options (Tapsall et al., 2005; Newman et al., 2007). Resistance of N gonorrhea to pencillin, tetracycline and quinolones is evolving in many European countries (Martin et al., 2006). Resistance to azithromycin at high level has been reported in UK (HPA, 2008). Drugs recommended for gonorrhea by WHO guide lines 2009 are cephalosporins, ciprofloxacin and spectinomycin, although ciprofloxacin is also showing relative

resistance. Clinicians should be aware of the pattern of susceptibility in their community and continuous trials are required to know the latest trend of the organism (Bignell, 2009).

Diagnosis

Laboratory methods for the identification of BV/ vaginitis include wet mount, gram stain, the "Gold standard" of diagnosis and microbiological culture (Mehdinejad et al., 2011). The diagnostic method currently available is the assessment of clinical signs, but the clinical signs are subtle and detection of the signs is basically dependent on the expertise of the clinician performing the test (Nugent et al., 1991). Gram stain laboratory method is the least expensive and requires less time and is the most widely used method (Mohanty et al., 2010). The clinical signs and the laboratory method are the most commonly used methods. Following are the different methods used for the diagnosis of BV /vaginitis.

Amsel clinical criteria

Normal vaginal discharge is clear to white in color, is odorless, and of high viscosity. The Amsel criteria require that atleast three of the following four symptoms be present for the diagnosis of BV (Amsel et al., 1983):

1 Homogenous, white discharge that smoothly coats the vaginal walls and is non-inflammatory.

2 Presence of clue cells on gram stained slide (x1000 magnification).

3 A pH >4.5 of vaginal fluid.

4 Fishy or foul smelling vaginal discharge before or after addition of 10% potassium hydro-oxide (KOH) (positive whiff test).

Though Amsel analysis is used more commonly in clinical settings, the sensitivity and specificity of the criteria used range between 60-70% (Zenilman et al., 2003). Before 1955, nonspecific vaginitis was used to describe patients in whom Trichomonas vaginalis or Candida spp. were not isolated. The term vaginosis has been adapted because inflammatory cells are typically absent in the vaginal discharge (Culhane et al., 2005).

The Nugent scoring system

Nugent et al. (Nugent et al., 1991) developed a more specific scoring system for the diagnosis of BV which is based on the observed bacterial morphotypes on gram stain vaginal smears (x1000 magnification) using oil immersion. The Nugent scoring is the most frequently used laboratory based diagnostic method for detecting bacterial vaginosis. It is considered as the gold standard for the diagnosis of BV.

The scoring is based on the estimation system (0 to 4 points) that is used to measure the amount of different bacterial morphotypes present in the vaginal samples. Average of at least five oil immersion fields are calculated for scoring the bacterial morphotypes. The presence of more than 30 Lactobacilli morphotypes, earns 0 points, whereas the absence of Lactobacilli morphotypes earns 4 points. The amount of small bacteria, Gardenerella vaginalis, present in the sample are also measured on a point system (from 0 to 4 points), but the points for G. vaginalis are assigned in the opposite way. The presence of more than 30 (average) small bacteria oil immersion field earns 4 points and the absence of these small bacteria earns 0 points. The existence of curved rods Mobiluncus spp. earns an additional 1 or 2 points, depending on the amount of curved rods in average oil immersion field of vision. The points are added together and a total score of 0-3 is considered normal; a score of 4-6 is classified as intermediate likely to be positive, and a score of 7-10 is consistent with BV (Nugent et al., 1991). The variable amount of bacteria is categorized according to the Nugent's scoring system which has a high inter- and intra-observer reliability. However, questions still remain that require discussion (Forsum et al., 2002). Forsum et al., (2008) emphasized the need for a standardized interpretation for the basic morphotypes that play a central role in a diagnosis using Nugent's classification. Moreover, the results are influenced by the field size of the microscope (Larsson et al., 2004), issue is of concern. In Nugent's classification, the presence of only 30 Lactobacilli/small bacteria per vision field counts, so both the area of the microscope images and the thickness of the smear make a difference in the interpretation of the results.

Spiegel system

In the Spiegel classification system, the bacterial morphotypes, Lactobacillus spp. and Gardnerella vaginalis are noted and classified as 1+, 2+, 3+ , and 4+ according to the amount of the bacteria observed on a gram stained direct vaginal smears with a magnification of x 1000 under oil immersion. A microscopically detectable change in

vaginal micro flora is observed from the amount of Lactobacillus spp. with or without G. vaginalis morphotypes, to a mixed flora with only few or no Lactobacillus morphotypes. This method is used for the diagnosis of BV. The presence of less number of Lactobacillus morphotypes (1+ to 2+) is interpreted as being consistent with BV. If G. vaginalis morphotypes out-number the Lactobacillus morphotypes even this is also consistent with BV and even if the Lactobacillus morphotypes are present. If only Lactobacillus morphotypes are present, the sample is interpreted as being normal (Spiegel et al., 1983).

The Hay/Ison classification

The Hay/Ison classification or categorization system is used for both PAP smear and gram stained smears (Hay et al., 1992). In the Hay/Ison classification, vaginal flora is divided into the three different categories normal, intermediate, and BV. An estimation of the amount of the bacterial morphotypes is not done in this classification system instead a subjective evaluation between the amounts of bacteria is undertaken. The field size of the microscope does not have an influence on the results (Larsson et al., 2004).

The Ison/Hay classification

In the Ison/Hay classification system, the stained smear is categorized into normal, intermediate, and BV. However, the two categories are added 0 (relatively empty smear) and 4 (dominance of Streptococcus morphotype) (Ison et al., 2002). The categories 0 and 4 are added in an attempt to make the categorization more true to what is observed in clinical practice, as opposed to what might be hypothesized in relation to the concept of BV. The Hay/Ison and Ison/Hay classification systems can be used on slides with different staining methods and also on direct smears with no stains.

Complications

Besides causing unpleasant symptoms, BV/vaginitis is notorious for setting off an entire range of serious gynecological and obstetric complications (Tutovsky et al., 2011). BV is the most common cause of vaginal discharge and malodor, and vaginal infection of females in reproductive age group (Ness et al., 2005; Amsel et al., 1983). BV predisposes to acquisition of STI such as HIV, HSV, HPV, increases the susceptibility to Chlamydia and Gonococcal infection, due to the depletion of the

protective acid producing Lactobacilli (Korn et al., 1995; Hashemi et al., 2000; Van De Wijgert et al., 2008; Gallo et al., 2008; Atashli et al., 2008; Allsworth et al., 2008). Different studies suggest the possibility that females with bacterial vaginosis are at increased risk of acquiring HIV (Sewankambo et al., 1997; Hashemi et al., 1999). The bacterial flora related with bacterial vaginosis increases genital-tract HIV shedding (Sha et al., 2005). Combination of microorganisms associated with BV/vaginitis increase the risk of PID (Hilliers et al., 1995; Ness et al., 2005). In addition to its own morbidity, the microbial flora of the human vagina causes obstetric complications (Holst et al., 1994; Klein, 2004). Studies show that BV/vaginitis and its associated intrauterine infection results in miscarriage and preterm birth (Gravett et al., 1986; Hillier et al., 1995; Thorsen et al., 2006) and are responsible for 70% of neonatal deaths and long-term neurologic morbidity in newborns (Hack, 2000; Benedetto et al., 2004). The microorganisms and their toxins are capable of crossing the placenta and causes brain injury to fetuses. BV is considered as one of the risk factor for neurological complications in children, such as hyperactivity, academic difficulties, and severe handicaps, cerebral palsy and preventricular leukomalacia (Eschenbach, 1997; Grether and Nelson, 2000; Ling et al., 2004). High concentrations of lipopolysaccharides (LPS) found in the vaginas of women with BV causes damage in the dopaminenergic system in neonates (Platz-Christensen et al., 1993; Ling et al., 2004).

In women with a previous history of preterm birth or with a low pre-pregnancy body weight, treatment of BV has been associated with significantly decreased rates of preterm labor, preterm birth, low birth weight, and premature rupture of membranes (Gravett et al., 1986; Morales et al., 1994; McDonalds et al., 1997; Mikamo et al., 1999; Rezeberga et al., 2008). BV is associated with increased obstetrical complications, such as preterm birth, preterm labour, low birth weight, premature rupture of the membranes, miscarriage, spontaneous abortion, chorioamnionitis, intraamniotic infections, postpartum maternal infections and infertility. Gynaecologic complications such as post-operative infections (hysterectomy, legal abortion) have also been associated with BV (Larsson et al., 1989; Persson et al., 1996; Wilson et al., 2002; Leitich et al., 2003; Benedetto et al., 2004; Larsson et al., 2005; Karat et al., 2006; Thorsen et al., 2006; Rezeberga et al., 2008).

BV predispose to STIs which cause ascending infection. Having one STI is a risk factor for another. HPV, gonococcal infection and BV are among the most common

co-infections. BV facilitates the entry of Chlamydia to the upper genital tract. Chlamydia infection has been associated with cervical squamous cell carcinoma (Millier et al., 2004; Kahn et al., 2005; French et al., 2006). Small proportion of women present with symptoms related to infection and majority remain symptom free and untreated. When infection persist for months and years results in salphingitis, tubal factor infertility, ectopic pregnancy, Chronic pelvic pain, PID, reactive arthritis, Prihepatitis. Neonates born to infected mothers are at risk of conjunctivitis and pneumonitis (Paavonen et al., 1999; Honey et al., 2002; Crossman et al., 2006).

Treatment of Bacterial Vaginosis

One of the major issue to effective treatment and prophylaxis of BV is its limited understanding its etiology and condition, which remains mysterious despite decades of research (Forsum et al., 2005; Larsson and Forsum, 2005; Larsson et al., 2005; Nancy, 2010). The US Food and Drug Administration (2010) recommend that clinical cure be defined as the recovery of all four clinical signs of Amsel criteria for BV. Cure rates for BV are about 50% while 85% females do respond to the currently recommended drug regimes. Antimicrobial drugs with broad spectrum activity against anaerobic bacteria are more effective in relieving symptoms of BV. A number of antibiotics (e.g., ampicillin, penicillin, and metronidazole) have been used in the treatment of bacterial vaginitis (Spiegel, 1991). Metronidazole emerged as a drug of choice for the treatment of BV and is the now widely prescribed drug for BV. It is a nitroimidazole derivative which can be administered either orally or locally. Formulations for the intra-vaginal administration of the drug include gels and suppositories (Sobel et al., 2006; Decena et al., 2006; Mitchell et al., 2009). Metronidazole and tinidazole are more preferred and commonly used for the treatment of BV as against ampicillin. Tinidazole has longer half-life with single dose easy to take with better compliance recommendation for the treatment of BV (Dickey et al, 2009). The use of ampicillin is avoided due to the emergence of ampicillin-resistant bacteria as it inhibits the growth of Lactobacilli (Spiegel, 1991). Metronidazole and clindamycin are considered as the mainstays of therapy (Flores Rivera et al., 1997; Hillier et al., 2008). Intravaginal therapies are safer and have fewer side-effects (Marrazzo et al, 2008). Clindamycin-resistant bacteria have been reported among women treated with vaginal clindamycin (Beigi et al., 2004). After

treatment for BV, many females still remain colonized by G. vaginalis or associated anaerobes (Ferris et al., 1995, Boris et al., 1997). The treatment of BV is effective in only 60% of all cases, contributing to the recurrence rate of 30–40% (Colli et al., 1997; Paavonan et al., 2000; Eriksson et al., 2005). These treatments of BV play an important role in the expansion of drug resistance (Lubbe et al., 1999; Bryskier, 2001; Liebetrau et al., 2003). Lactobacilli show a variable susceptibility pattern to cephalosopins but are sensitive to penicillin. On the other hand vancomycin, doxycyclin and metronidazole are not sensitive to Lactobacilli (McGregor et al., 1994; Wilks et al., 2004, Murray., 2003).

More perplexing is the high rate of early recurrence (30% at three months, 50% at six months) reflecting early relapse and more likely late re-infection, for which successful management has not been forthcoming. Although each symptomatic episode usually responds rapidly to conventional antibiotic treatment, rapid recurrence is frequently inevitable. BV can be suppressed with ongoing antibiotic therapy. In women with current BV with at least two prior episodes of BV in the previous year were initially treated with 10 days of vaginal metronidazole gel then, if cured, randomly assigned to receive twice weekly metronidazole vaginal gel for 16 weeks with a follow up therapy for 12 weeks (Sobel, 2006). In a recent study, it has been reported that the mode of administration of metronidazole, either orally or locally, do not have a significant difference in the eradication of the pathogenic bacteria (Mitchell et al., 2009). The gel formulation containing a combination of both lactic acid and metronidazole has shown superior ability to re-colonize the vaginal lumen with Lactobacilli (Simoes et al., 2001; Decena et al., 2006). Studies on the treatment of the BV have also been done with tinidazole, clindamycin, polystyrene sulfonate, and cellulose sulfate and policarbophil-carbopol acidic vaginal gel (Simoes et al., 2002; Nyirjesy et al., 2006; Dickey et al., 2009; Bonferoni et al., 2006). There is an increased number of re-occurrence of BV when the synthetic antimicrobials are used and may be attributed to the development of antimicrobial resistance mechanism within the microbes (Beigi et al., 2004). Hence, the researchers and clinicians are looking for alternative methods for the treatment of BV.

The present study will be conducted on married female patients between 15-42 years to assess the symptoms and complaints of vaginal discharge along with vaginal examination and sampling, high vaginal swabs and endocervical samples. Samples

will be processed to know the prevalence of Bacterial vaginosis along with the associated organism, bacterial and sexually transmitted infection especially Chlamydia trachomatis and Neisseria gonorrheae. BV will be assessed through Amsel criteria and Nugent Scoring System which are considered as 'gold standard'. Various growth media for growth and sensitivity pattern of microorganisms, bacterial and N. gonorrheae will be used. Sensitivity to various groups of drugs in the study population will be undertaken as these bacteria's have a tendency to rapidly change behavior from sensitive to resistant. Change in the effect of drugs in a particular area need to be assessed. The serological markers such as, IgG and IgM for prevalence of C. trachomatis will be done. Associated behavioral factors and hygiene practices of females belonging to this area will be correlated as they are considered important factors for BV and STI. Various studies have been conducted on BV in this area but no set policy and protocol is available, which is followed in the hospitals. Mostly syndromic management is done without patient undergoing required investigations resulting in resistance to conventional drugs, under-treatment of patients and recurrence of infections resulting in complications which increase the economic burden of patients. As the study will be conducted in public sector hospital, only limited information is available so far in general public, poor patients and on STI's. Various studies on sex workers and high risk patients in these areas have been conducted. Due to illiteracy, lack of awareness about the severity of infection and psychological shame, these cases are not reported frequently and are treated by self medication, resulting in worsening of condition of patient and resistance to drugs. Clinically the study will help in improving the reproductive health of patients with early diagnosis through proper diagnostic protocol, early appropriate treatment with selected drugs resulting in prevention of complications. This will also help in educating the hospital professionals.

SUBJECTS AND METHODS

This study was carried out at Holy Family Hospital (HFH) Rawalpindi and Quaid-i-Azam (QAU) University Islamabad. Holy Family Hospital is a tertiary care teaching Hospital of Rawalpindi Medical College. Rawalpindi. Pakistan. It serves as a major health care facility in the public sector of Rawalpindi region and its surroundings.

The study was conducted over a time period of one and a half years for patient selection and sample collection. Patients were selected from the out-patient department of Gynecology and Obstetrics. Only those patients presenting at the out-patient department with the complaint of vaginal discharge were interviewed and selected. Three basic tools were used for the collection of data. These included, conducting a questionnaire which included a complete history of each patient including age, duration of vaginal discharge, symptoms and associated features, sexual history, any history of pregnancy, its loss and treatment history, husband income and occupation along with patients educational level was included. A thorough gynecological examination was conducted which included assessment of genital tract infection. Inspection of genitals, per speculum examination of vaginal cavity and cervix along with the collection of samples for the laboratory diagnosis was done.

Additional **inclusion criteria's** were:
Females with complaints of vaginal discharge
All married sexually active females
Patients with age ranging from 15 to 42 years
Negative history for any antibiotic intake in the recent past
Females not menstruating for 48 hours before their examination

Exclusion criteria's were:
Any surgical procedure on uterus
On any antibiotic or has taken antibiotic in the last two weeks
Presently pregnant, post delivery and post abortion females
Female subjects menstruating or bleeding per vaginal
Females above 42years

Patient selection criteria

Patients presenting in the out-patient department of Gynecology and Obstetrics were selected based on the presenting complaints of vaginal discharge. The symptoms of the patient were important in the selection of the patients for study. Vaginal symptoms were one of the most common reasons for Gynecological consultation. The diagnosis of vaginal discharge was based on history, examination and diagnostic tests as shown in Fig 1.

History

Patient was asked about Itching or rash over the perineum along with, odor, color and consistency of discharge. The patient was inquired about intermenstural bleeding, unwell feeling, painful intercourse (dysparunia), or spotting after intercourse.

A detailed obstetrics history was obtained from the patient regarding number of pregnancies, live births and pregnancy loss. The patient was inquired about the type of pregnancy loss like abortions, miscarriage, still birth and ectopic pregnancies.

Majority of patients coming for the treatment to the public sector hospital belong to low economic status and these patients were accordingly grouped into low (Rs 5000-10,000), middle (Rs 11,000-15,000) and high (Rs 16,000-20,000) groups for description of the study. Literacy level of the patient was judged by asking them about the level of education of the patient. The economic and educational level of the patient plays an important role in the vaginal infections.

Normal vaginal discharge

Normal vaginal discharge appears clear to white in color with variation in the consistency during different phases of menstrual cycle. Consistency varies from thin, viscous to sticky in the mid cycle. The normal vaginal discharge is odorless.

Elicitation of symptoms:

Elicitation of symptoms of vaginal discharge is given in Fig 2. Patients who had vaginal infection generally compliant of discharge having some color, odor and

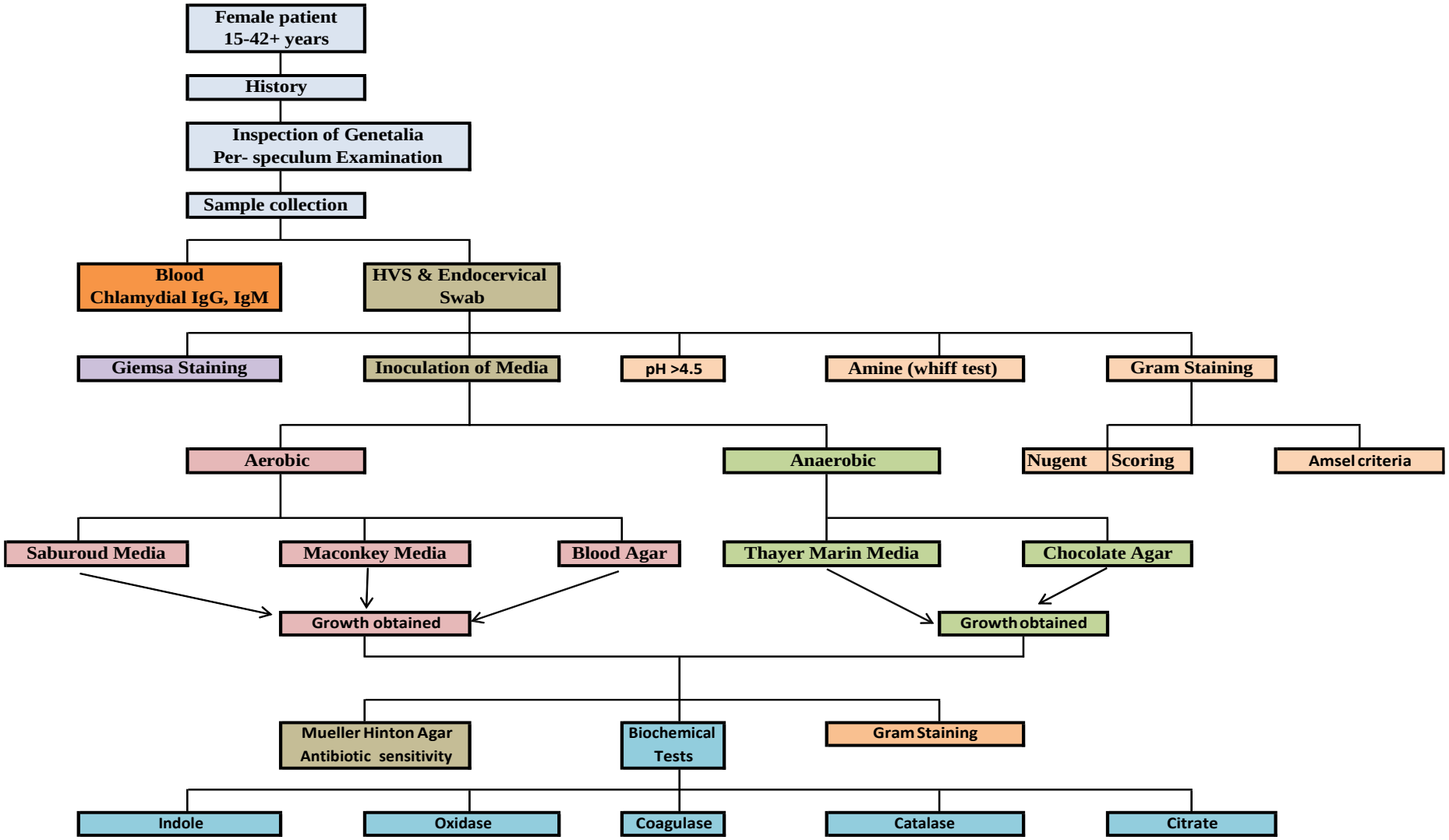


Fig 1: Diagrammatic representation of the methodology used systematically.

consistency, contradictory to the normal appearance of discharge with some irritation or itching in the perineal area. Discharge color was characterized as white, yellow, gray, green or clear. Consistency was found to be thick, homogenous, watery, or curd like. Odor was pungent, foul, or fishy. No scale was available to quantify the amount of vaginal discharge.

Physical Examination

Physical examination was done both externally and internally. For the purpose of vaginal examination patients were asked to lie on the examination coach in the lithotomy position with the facility of good light falling on the pelvis. A dry cuscos speculum was inserted into the vagina, without any lubricant or antiseptic. Externally condition of vulva and perineum were examined for any rash. With the help of speculum internal condition was assessed. Candidasis presents as thick white discharge and red vulva with itching and dryness. Gardnerella has a foul smelling, thin discharge which gets worse after intercourse. Cervicitis has mucopurulent cervical discharge with deep dyspareunia and cervix is tender to touch due to STI, Streptococcus spp., Staphylococcus an overgrowth of bacteria normally found in vagina. Chlamydia causes a purulent discharge with post coital bleeding and deep dysperunia, cervix is friable and bleeds on touching. Gonococcal infection cause purulent vaginal discharge with deep dyspareunia as cervix is tender to touch. Cervical ectropion or erosion is non tender fiery red friable button like, surrounding the os of the cervix which may be due to Chlamydial or Gonococcal infection.

Specimen sampling and preparation

The presenting symptoms of vaginal discharge are usually localized in the vagina and endocervix. High vaginal swabs (HVS), endocervial swabs and blood were obtained in selected patients through history and clinical signs. Cotton swabs and cotton swab with amies transport media (Citotest Transport Swab; Amies with Charcaol GAMMA Sterile; Biomed) were used for vaginal and endocervical sampling. Four samples were obtained, two high vaginal swabs with sterilized cotton swabs and two endocervical swabs in Amies transport media by rotating the swab in anti-clock wise direction.

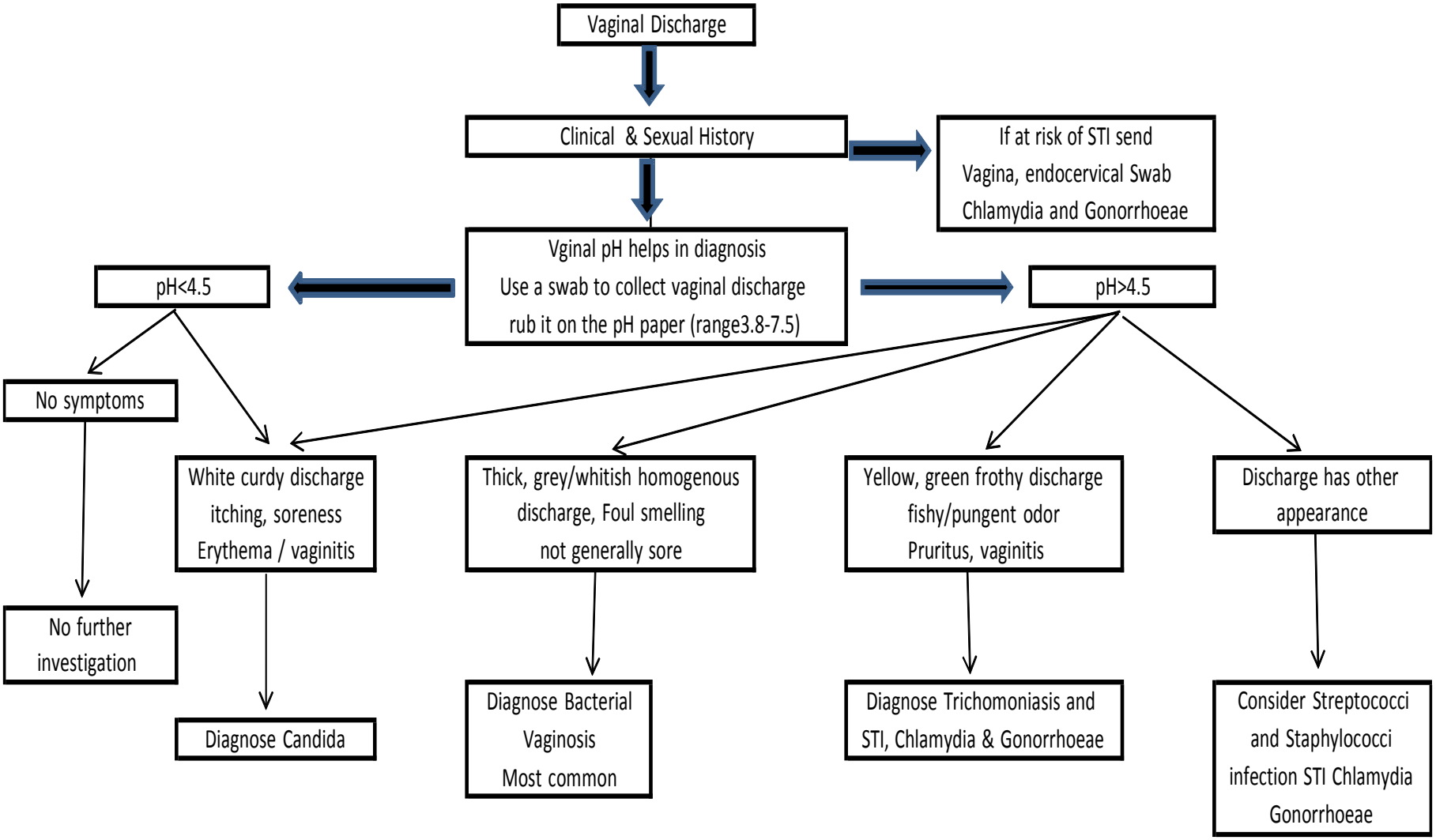


Fig 2: Variations of vaginal discharge, its color and consistency according to pH resulting in different infections

After obtaining the samples the speculum was removed and pH (pH indicator strip; Merck, pH range 3.8-7.4) of the vaginal discharge by placing it in the discharge. Potassium hydroxide (KOH) was poured on any discharge present on the speculum for the observation of Amine odor (Whiff test).

Each swab was properly labeled with patients name, number and date of collection. Endocervical swabs were collected with great care to avoid any contamination with the vaginal wall and vaginal discharge. First swab each of vagina and endocervix for direct smear gram staining and geimsa staining and second one for media plates.

Blood sample was drawn under aseptic measures from the cubital vein of patients who consented to be investigated for Chalmydia trachomatis (STI). The blood was centrifuged at 3000 revolutions per minute and serum was separated in the eppendorf. After proper labeling, name and number, serum was stored at -35ºC.

Gram staining

Gran staining of direct smear was done for the diagnosis of Bacterial Vaginosis according to Amsel Clinical Criteria and laboratory diagnosis through Nugent scoring system. Again gram staining was done after obtaining the isolates from the growth for identification of the isolate.

Stains required for gram staining required were prepared according to the instructions in Koneman's. (Konemans, 2006; Karamat, 2012)

Procedure of gram staining

Reagents

Crystal violet

Lugol's iodine

Acetone-alcohol decolorizer

Neutral red 0.1%

Smear making

A slide was sterilized by passing it over the flame. Vaginal swab was unrolled over the glass slide making a Smear covering at least two-thirds of the surface. It was properly labeled and air dried. Glass Slide was heat fixed by passing over the flame.

Staining

Fixed smear was covered with crystal violet stain for 30 -60 seconds.

Stain was washed with clean tap water.

The smear was covered with lugol iodine for 30 – 60 seconds.

Iodine was washed with clean tap water.

Smear was decolorized (few seconds) with acetone-alcohol.

The slide was washed immediately with clean tap water.

The slide was cleaned and the smear air dried

Evaluation of Gram Stained Slide

Each gram stained slide was evaluated for the following morphotypes under oil immersion (x1000 magnification) and number of each type was calculated

Lactobacilli spp as large gram positive rods, purple in color.

Gardnerella vaginalis as small gram variable rods, purple or pink

Mobiluncus spp as curved gram variable rods, purple or pink

Epithelial cells as pale red

Pus cells PMN

Clue cells as epithelial cells with attached rods covering the whole surface.

Gram negative intracellular Diplococci within PMN.

Reading the stained slides

Slide was scanned using low power objective to locate any clusters of epithelial cells. It was switched to oil immersion lens (x1000 magnification) and four to five representative fields were observed for cell morphology and gram reaction. Bacterial vaginosis score for gram staining was calculated by Nugent method (1991). Average number of lactobacilliary morphotypes were observed per oil immersion field and quantified. These organisms were filamentous, varying from gram positive to gram negative rods often forming chains. Similarly average number of Gardnerella vaginalis observed as small gram variable coccobacilli were quantified. Mobiluncus spp., thin curved faintly stained gram negative rods were also looked and quantified. These bacteria were often absent with other bacterial morphotypes.

The relative amount of the three morphotypes observed, were reported. Each morphotype was quantified from 0 to 4+ according to the number of organism present per oil immersion field. >1 means at least one bacterial morphotype present in any one oil immersion field. All slides were also observed by another microbiologist

Giemsa staining

Giemsa staining for chlamydia trachomatis

Smear making

Slide was passed over flame for sterilization. Endocervical swab unrolled over the slide and was fixed by pouring few drops of ethanol and was air dried.

Giemsa staining

Reagents

Giemsa stain

Buffered water (Phosphate) pH 7 – 7.2

Method

Giemsa stain was diluted in buffered water.

In 19.5 ml of buffered water 0.5ml of giemsa stain was mixed. The slide was vertically placed in a staining jar for 2 hours.

Slide was washed with buffered water.

Evaluation of Giemsa stained slide

Each giemsa stained slide was evaluated under oil immersion lens

Large inclusional bodies of Chlamydia trachomatis stained blue inside the epithelial cells were identified.

AMSEL CRITERIA

Normal vaginal discharge is clear to white and floccular, odorless, thin and viscous.

In clinical practice Amsel clinical criteria is the most commonly used for BV. As it is considered as gold standard for the diagnosis of Bacterial vaginosis which was applied on all the patients selected for this study.

The diagnosis was positive if any three out of the four following criteria's were fulfilled.

1) Thick homogenous, white and smooth discharge
2) Vaginal fluid with pH > 4.5 (pH indicator strip; Merck, pH range 3.8-7.4)
3) Amine odor test also known as whiff test or sniff test. Fishy odor of vaginal discharge before or after addition of 10% KOH.
4) Presence of clue cells on gram stained microscopic examination.

NUGENT SCORING SYSTEM

Nugent scoring the most frequently used authentic standardized laboratory based diagnostic method for detecting bacterial vaginosis was applied.

Each gram stained direct smear was evaluated for the Nugent scoring (1991) under oil immersion lens (x1000 magnification)

Morphotypes are scored as the average number seen per oil immersion field. Total score is lactobaccilli + G. vaginalis + Mobiluncus. Each morphotypes quantitated from 1 to 4+ with regard to the number of lactobacilli mophotype per oil immersion (Table 1a).

0, no morphotype

1+, less than 1 morphotype

2+, 1-4 morphotypes

3+, 5-30 morphotypes

4+, 30 or more morphotypes.

The amount of bacteria (G. vaginalis) present in the sample is also rated on a point system (from 0 to 4 points), but the points are assigned in the opposite way. The presence of more than 30 small bacteria per oil immersion field earns 4 points and the absence of small bacteria earns 0 points. The existence of curved rods (Mobiluncus spp) earns an additional 1 or 2 points, depending on the amount of curved rods in each field of vision (Table 2.2).

When the points are added together, a total score of

0-3 is considered normal.

4-6 is classified as intermediate. 7-

10 is consistent with BV

Table 1a: Nugent scoring system (0 to 10) for gram stained vaginal smear

Score	Lactobacillus spp	Gardnerella vaginalis	Mobiluncus spp
0	4+	0	0
1	3+	1+	1+
2	2+	2+	1+
3	1+	3+	2+
4	0	4+	2+

Table 1b: Laboratory examination of vaginal smear and the determination of the Nugent Score

Lactobacilli	Score	Gardnerella	Score	Mobiluncus	Score	Sum of Score
>30	0	0	0	0	0	0
5-30	1	<1	1	<1	1	3
1-4	2	1-4	2	1-4	1	5
<1	3	5-30	3	5-30	2	8
0	4	>30	4	>30	2	10

INOCULATION OF VARIOUS MEDIAS

Vaginal and endocervical swab collected with cotton swab in amies transport media were immediately transported from the gynecology and obstetrics outpatient department to the microbiology section of the laboratory. The specimen was inoculated on the modified Thayer Martin media, Chocolate agar, Blood agar, MacConkey media and Saburoud media. On the culture media plates, the specimen collected on swab was rolled in Z pattern and cross streaked with bacteriological loop. The bacterium required special conditions for its growth. Blood, MacConkey and Saburoud medias were used for the growth of Gram positive cocci and gram positive rods, gram negative rods and fungal specimen were placed in the incubator at 35ºC under aerobic conditions.

Thayer Martin and Chocolate media are specialized media for the growth of Neisseria gonorrheae which needs special environment for its growth and survival. The plates

were placed in the candle extinction jar with carbon dioxide generating pellets. Immediate incubation at 35ºC for 24 hours under anaerobic conditions was done for the initial growth of the bacterium. If no growth was obtained, it was re-incubated for another 24 hours because N. gonorrheae is a slow growing bacteria and needs 48-72 hours for its growth.

Culture media used

1 Blood agar, an enriched media, for the growth of staphylococcus aureus, streptococcus agalactiae and Escherichia coli was made from Nutrient agar (CM 0003, oxoid UK) as per manufacturer instructions. The addition of sheep or horse blood was done at 50ºC at pH 7-7.2.

2 Chocolate media, a nonselective media for the recovery of Nesseria gonorrhoeae and other non selective genital pathogens, was made from the nutrient agar (CM 0003, oxoid UK), according to manufacturer instructions with the addition of sheep blood at 56ºC at pH 7-7.2.

3 MacConkey media, a differential media (Liofilchem), for the growth of Escherichia coli, Klebsiella spp. and Pseudomonas aeruginosa was made according to the manufacturer instructions at pH 7-7.2.

4 Saborauds dextrose agar (CM 0139, oxoid UK), for fungal growth (Candida spp) according to manufacturer instructions at pH 5.6.

5 Mueller Hinton media (CM 0337, oxoid UK), for antibiotic sensitivity testing, made according to manufacturer instructions at pH 7-7.2

6 Modified Thayer Martin media (selective media) for the selective recovery of Neisseria gonorrhoeae was made from Nutrient agar (CM0003, oxoid UK). Lysed sheep blood at 56ºC with culture media supplement Vitox (SR 0090A, oxide UK) and antibiotics to inhibit the growth of other bacteria's (gram positive, gram negative bacteria's with inhibition of yeast and molds and swarming of proteus) was added. The antibiotics added were namely : Vancomycin hydrochloride , 3µgm/ml (CAT no 195540 Biomedicals LLC), Clostin sulfate salt , 7.5 µgm/ml (CAT no 194157 Biomedicals LLC), Trimethoprim, 3 µgm/ml (CAT no 195527) Nystatin, 1 µgm/ml (CAT no 100417 Biomedicals LLC), Amphoteracin-B, 100µgm/ml (CAT no 195043 Biomedicals LLC) .

After recovery of growth, organisms isolated were identified by gram stained slides under the oil immersion lens for the morphology and arrangement of the organisms. Further confirmation of the isolates was done with the help of various biochemical tests.

Biochemical tests

Catalase

Enzyme Catalase, produced by certain bacteria decomposes H_2O_2 into water and oxygen. It differentiates Staphylococci (positive test) from Streptococci.

Procedure

With an inoculating loop growth colony from center of a media plate was placed on the surface of a glass slide and mixed with 3% freshly prepared hydrogen peroxide and was observed for bubble formation. The rapid and sustained appearance of bubbles confirmed a positive test for Staphylococcus.

Coagulase test

Coagulase causes plasma to clot by converting fibrinogen to fibrin. It was done to differentiate Staphylococcus aureus from other staphylococcus species. Two types of coagulase are produced by most strains of Staphylococcus aureus.

Procedure

A drop of normal saline was placed on the slide. Colony from culture of test organism was emulsified. A drop of plasma was added mixed gently. Clumping within 10 seconds occurred for the test and confirmed a positive test for Staphylococcus aureus.

Oxidase test

The organism producing oxidase oxidised phenylenediamine dihydrochloride (Analar, UK) to a deep purple coloured compound. It identified Neisseria gonorrheae and Pseudomonas species.

Procedure

A piece of filter paper was placed in a clear petri dish and 2-3 drops of freshly prepared oxidase reagent was poured on it. Using a sterile wire loop a colony of the test organism from culture plate was rolled on the filter paper. Development of blue purple colour within a few seconds confirmed the test positive for N.gonorrheae and Pseudomonas aeruginosa.

Indole Test

This test demonstrates the ability of certain organisms to decompose amino acid tryptophan to indole. Indole was detected by putting kovacs reagent to culture media, which formed a pink compound with Indole. Kovacs reagent gave red color and helped in the identification of E. coli.

Procedure

Test organism colony was emulsified in peptone water (Britania, Argentina) on day one and incubated at 37ºC for 24 hours. Few drops of kovacs reagent added (SDL innovative research Pakistan). A change of color in upper layer was observed and red color indicated positive test for E. coli.

Citrate Utilization Test

The test is based on the ability of an organism to use citrate as its only source of carbon and ammonia as its sole source of nitrogen. The test organism was cultured in citrate agar (CM 0155, oxoid UK), which contains sodium citrate , ammonium salts and indicator bromo-thymol blue. Growth in the medium was shown by turbidity and a change in color of the indicator from light green to blue, represented an alkaline reaction, following citrate utilization, it differentiated enterobacteria from other bacteria. It was positive for Klebsiella and negative for E coli

Procedure

With the help of a sterile straight wire, citrate medium was inoculated with the culture of test organism. Incubated at 35-37ºC for about 2-3 days, checked daily for growth and change of color for Klebsiella.

Antibiotic susceptibility pattern

The antibiotic susceptibility pattern of all the isolates obtained was performed on various groups of drugs. Disk diffusion method, modified from the Kirby-Bauer method was used. Clinical and Laboratory Standard Institute (CLSI, 2010) were used to determine the reading zone size according to the standards provided. The isolates were classified as sensitive, intermediate and resistant according to the interpretation of the zone diameter standards, as recommended by CLSI. The concentration of the antibiotic discs (oxoid, Australia) used and the abbreviations of antimicrobial agents used throughout this report are shown in Table 2

Disc diffusion susceptibility test

Disc diffusion method was done for each bacterial isolate on Mueller Hinton agar (CM 0337, oxoid) as a growth medium. Inoculum was spread evenly over the entire surface of the medium by streaking the loop containing the isolate back and forth across the agar in three directions. The plates were allowed to dry before applying the disc, and within 15 minutes discs of given potencies (as shown in table) were applied on the inoculated plates with the help of the forceps. The plates were incubated at 35ºC for 18 hours and zones of inhibition were measured.

Table 2. Discs of antibiotic agents and groups along with symbols used in the study, their potencies and manufacturer.

S.N	Antibiotic Agent	Antibiotic group	Code/ Symbol	Disc Potency
1	Cefixime	Cephalosporin	CFM	5µg
2	Cefotaximine	Cephalosporin	CTX	30µg
3	Ceftazidime	Cephalosporin	CAZ	30µg
4	Ceftraxone	Cephalosporin	CRO	30µ
5	Ampicillin	Pencillin	AMP	10µg
6	Pipracillin	Semi-synthitic pencillin	TZP	110µg
7	Gentamycin	Aminoglycoside	GM	10µg
8	Erythromycin	Macrolides	ER	15µ
9	Tetracyclin	Tetracyclin	TET	30µ
10	Imipenum	Carbepenems	IMP	10µg
11	Ciprofloxacin	Quinolones	CIP	5µg
12	Levofloxacin	Quinolones	LEV	5µg

Spectrum of antibiotics

Spectrum of the group of antibiotics used in this study is as follows

Cephalosoprins

Cephalosporins used were cefixime, cefotaximine, cefttazidime, ceftraxone, all are third generation cephalosporins having activity against gram negative and gram positive bacteria including N.gonorrhea and pseudomonas.

Penicillins

Penicillins used (ampicillin and tazocin) have a wider spectrum of activity against pseudomonas, enterobacteracceae, klebsiella and gram negative organisms.

Aminoglycosides

Gentamycin has activity against staphylococcus, streptococcus, klebsiella, E.coli and pseudomonas.

Macrolides

Erythromycin used have a broader spectrum of activity covering streptococci, staphylococci, gonococci, chlamydia and many other bacteria.

Tetracyclines

It is effective against gram positive and gram negative bacteria. It acts against anaerobes and Chlamydia.

Carbepenems

Imipenum has activity against gram positive cocci, gram negative cocci, gram negative rods and enterobacteraccae.

Quinolones

Levofloxicin a 2^{nd} generation quinolone is more active against gram positive organisms whereas ciprofloxin a first generation quinolone is active against gram negative organisms.

Enzyme linked immunoassay ELISA

Antigen is attached on solid phase for the non competitive assay. The test serum and an enzyme labeled antibody specific for the attached antigen are added together. Chromogenic enzyme substrate is added, the color developed is inversely proportional to the amount of antibody present. Chlamydia trachomatis IgM / IgG ELISA (Product no. CHLM0070 (96 determinations) Nova Tec Immundiagnostica GmbH. Technologic & Waldpark. Germany) is intended for the qualitative determination of IgG & IgM class antibodies against Chlamydia trachomatis in human serum.

Principle of Assay

The qualitative immunoenzymatic determination of IgM / IgG class antibodies against Chlamydia trachomatis is based on ELISA. Microtiter strip wells are pre coated with Chlamydia trachomatis antigens to bind corresponding antibodies of specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labeled antihuman IgM / IgG conjugate is added. This conjugate binds to the captured Chlamydia specific antibodies. Immune complex formed by the bound conjugate is visualized by adding tetramethylbenzidine substrate which gives a blue reaction product. Intensity of this product is proportional to the amount of Chlamydia specific IgM / IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450nm is read using an ELISA microwell plate reader.

Procedure

1. Controls and diluted samples 100 µL were dispensed into their respective wells.
2. Wells were covered with the foil and Incubated for 1 hour± 5 min at 37± 1ºC
3. After incubation contents were aspirated from the wells and each well washed three times with 300µL of washing solution. Chlamydial Trachomatis anti IgM / IgG conjugate was Dispensed 100µL into all wells except .A1 blank well.
4. Incubated for 30±2 minutes at 37±1º C.
5. Repeated step 3.

6. TMB substrate solution was dispensed 100µL in all wells. Incubated for 30 minutes at room temperature in the dark.
7. Stop solution was dispensed 100µL into all wells. Blue color developed during the incubation turned yellow.
8. Absorbance measured of specimen at 450/620nm within 30 minutes after addition of stop solution. Measurement was done by ELISA plate reader

Statistical analysis

All the data was analyzed by using Statistical analysis package, Graph pad prism version 5. Summary statistics are presented as mean and SEM. Chi-square test (for numbers) was used for univariate analysis for the significance of association between categorical variables. The independent potential risk factors, significantly associated with bacterial vaginosis in the univariate analysis were evaluated by linear regression analysis. Differences were considered statistically significant P-value of <0.05 for all tests. Comparisons were analyzed applying students t test with P value of <0.05 considered as statistically significant

PATIENT DATA FORM

PERSONAL PROFILE **Date:**

	WIFE	HUSBAND
NAME		
AGE		
AGE AT MARRIAGE		
YEARS MARRIED		
OCCUPATION		
SMOKER		

SOCIOECONOMIC STATUS ☐ Rural ☐ Urban

Present Address:

Permanent Address: Contact No:

Economic Status: ☐ Low ☐ Middle ☐ High

Monthly Income: ☐ 5000-10,000 ☐ 11,000 – 15000 ☐ >16000

EDUCATIONAL STATUS ☐ Can read ☐ Middle ☐ Matric

☐ Intermediate ☐ Graduate ☐ Post Graduate

OBSTETRIC HISTORY

	ONE	TWO	MORE THAN TWO
Pregnancies			
Children (Alive)			
Abortions			
Miscarriage			
Still Birth			
Ectopic Pregnancy			
Infertility	Primary	Secondary	

Duration of Infertility (yrs):

USE OF CONTRACEPTIVES: ☐ Yes ☐ No

HISTORY OF PRESENT ILLNESS

	YES	NO
Lower abdominal pain		
Backache		
Pain in thighs		
Un-well Feeling		
Fever		
Dysparuniea		
Post-Coital Pain Intermenstural Bleeding		
Ulcer on External Genetalia		
Itching / Rash		

VAGINAL DISCHARGE

Color: ☐ Translucent ☐ Whitish ☐ Greenish ☐ Yellowish

Consistency: ☐ Watery ☐ Thick ☐ Sticky ☐ Frothy

Smell: ☐ Foul Smelling ☐ Pungent ☐ Fishy ☐ None

Associated Features of Increased Discharge:

Coitus ☐ Standing ☐ Excitement ☐ Tension ☐ Anxiety

Phase of Cycle (Discharge): ☐ Follicular ☐ Ovulatory ☐ Leuteal

PAST HISTORY

H/O Similar Infections: ☐ Yes ☐ No

Discharge:

Color: ☐ Translucent ☐ whitish ☐ Greenish ☐ Yellowish

Consistency: ☐ Watery ☐ Thick ☐ Sticky ☐ Frothy

Smell: ☐ Foul Smelling ☐ Pungent ☐ Fishy ☐ None

No of Episodes: ☐ One ☐ Two ☐ > Two

Duration: Treatment:

HUSBAND HISTORY

H/O discharge or Ulcer: Treatment History: Sexual History: No. of Partners:

H/O Addiction: ☐ Drugs ☐ Alcohol

SEXUAL HISTORY

o. of Partners:

H/O Addiction: ☐ Drugs ☐ Alcohol

EXTERNAL GENITALIA

☐ Rash ☐ Redness ☐ Ulcer ☐ VULVA ☐ VAGINA

Discharge

Color ☐ Translucent ☐ whitish Greenish Yellowish

Odor ☐ Foul Smelling ☐ Pungent ☐ Fishy ☐ None

PER SPECULUM

Condition of Cervix

☐ Healthy ☐ Redness ☐ Swollen ☐ Bluish ☐ Ectopy

Discharge

Color ☐ Translucent ☐ whitish ☐ Greenish ☐ Yellowish

Consistency ☐ Homogenous ☐ Frothy ☐ Watery ☐ Sticky

Cervical Friability ☐ Yes ☐ No

BIMANUAL EXAMINATION

	YES	NO
Cervical Motion Tenderness		
Adenexal Tenderness		
Fundal Tenderness		
Abdominal Tenderness		

SAMPLES

☐ Endocervical ☐ Cervical ☐ Post Fornix ☐ Blood

CHLAMYDIA TRACHOMATIS

Geimsa Stain Smear

Epithelial Cells Inclusion bodies

ELISA Negative ☐ Positive ☐ Cut-Off Value Absorbance

NEISSERIA GONORRHOEA

Gram Stain Smear Epithelial cells PMN Lactobacilli G.Vaginalis

Mobiluncus Clue cells Hyphe

Any Other Finding

CULTURE MEDIUM

Thayer Martin Medium Culture

Chocolate Agar

MacConkey

Blood Agar

Sabourads

Gram staining

BIOCHEMICAL TEST

Oxidase Test ☐ Positive ☐ Negative

Catalase test ☐ Positive ☐ Negative

Oxidase ☐ Positive ☐ Negative

Indole ☐ Positive ☐ Negative

Citrate utilization test ☐ Positive ☐ Negative

Antibiotic sensitivity test (Disc diffusion)

S/N	Antimicrobial Agents	Disc Potency	Growth on MHA		
			S	**I**	**R**
1	Cefixime	5 µg CFM			
2	Cefotaximine	30 µg CTX			
3	Ceftazidime	30 µg CAZ			
4	Ceftraxone	30 µg CRO			
5	Ampicillin	10 µg AMP			
6	Tazocin	110µg TZP			
7	Gentamycin	10 µg GM			
8	Erythromycin	15 µg ER			
9	Ciprofloxacin	5 µg CIP			
10	Levofloxcin	5 µg LEV			
11	Tetracyclin	30 µg TET			
12	Imipenum	10 µg IPM			

Sanitary Pads

Cloth ☐

Cotton ☐

Always ☐

BATH and CHANGING CLOTHS

Daily ☐

Twice a week ☐

Once a week ☐

RESULTS

Characteristics of female patients in the study population:

The study population comprised 332 female patients selected randomly at Gynecology and Obstetrics out-patient department of Holy Family Hospital. Rawalpindi. Age of the patients ranged between 17-42+years with mean age of 28.01±0.29 years (n=332). Patients presenting with different symptoms of vaginal discharge are shown in Fig 3. Maximum number of patients presented with low backache (83.73%) and lower abdominal pain (81.62%). The other common symptoms in descending order were rash/itching (70.78%), unwell feeling (68.67%), and pain in thighs (68.37%). The lowest symptoms observed were dysparuniea (50.90%), feverish feeling (22.89%) and intermenstural bleeding in the least number of patients (13.85%). Patients at presentation showed a combination of symptom of vaginal discharge. Most common combination of symptoms observed were backache along with lower abdominal pain (72.28%), backache with lower abdominal pain and rash/itching (53.61%), backache with pain in thighs and unwell feeling (48.79%), rash/itching with dysparunia (40.96%). The least observed combination of symptoms were unwell feeling along with feverish feeling (16.86%) and dysparunia with intermenstural bleeding (8.73%).

Clinical observation of vaginal discharge

Patients were clinically ascertained vaginally with speculum examination regarding changes in the appearance of vaginal discharge (Table 3). The color of vaginal discharge elicited various colors like white, translucent, yellowish and normal (clear). Similarly, variations in consistency from normal, thick viscous and sticky vaginal discharge to homogenous and watery vaginal discharge was observed in these patients. Normally there is no smell in the vaginal discharge but the presence of foul smell or the pungent smell was observed in the discharge of the presenting patients.

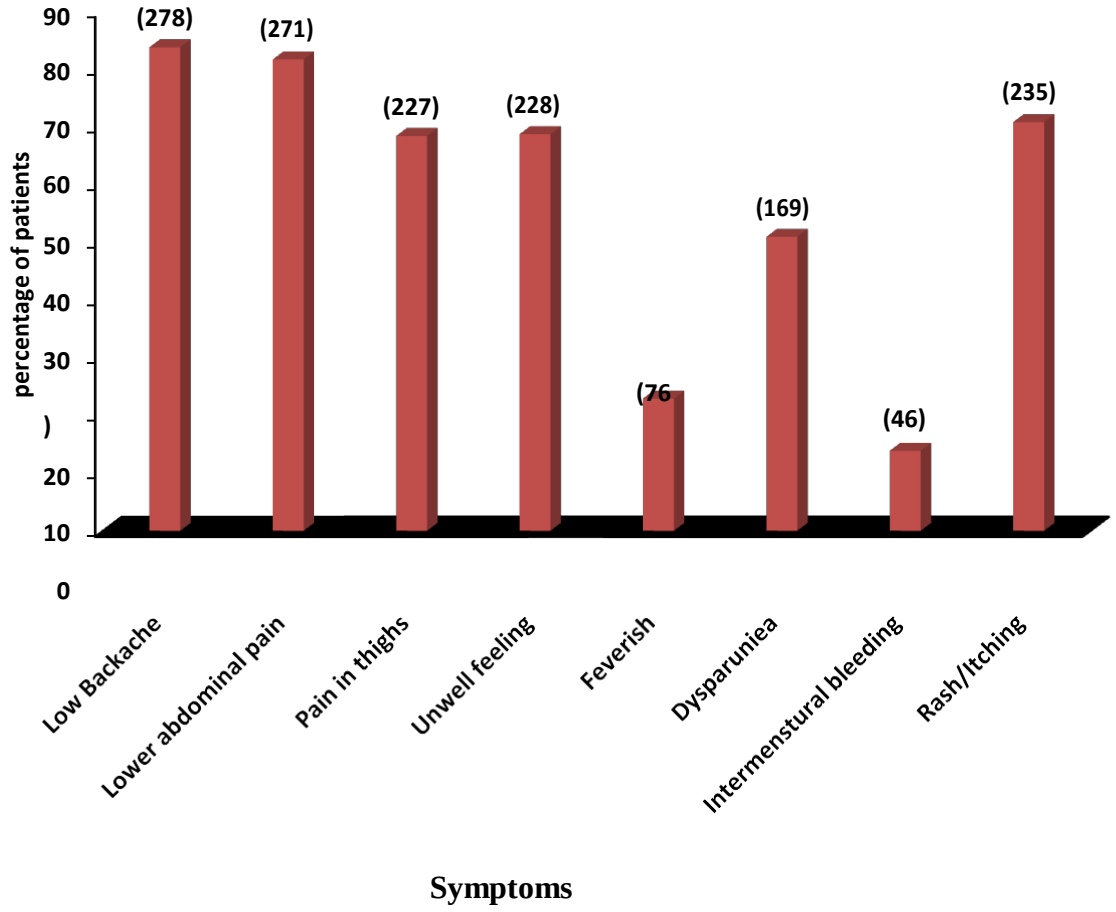


Fig 3: Number and percentage of patients with various symptoms of vaginal discharge. Values in parenthesis ( ) indicate number of patients.

Color of vaginal discharge

A whitish discharge was common among the patients and was observed in 41.56% (n=138) patients (Table 3). Translucent discharge was observed in 21.38% (n=71) and yellowish discharge was seen in the least number 12.95% (n=43) of patients. The vaginal discharge which was clear and whitish similar to that of normal patients was present in 24.09% (n=80) patients.

Consistency of vaginal discharge

Variation in consistency of vaginal discharge from the normal was observed in the patients. In 45.18% (n=150) patients vaginal discharge was thick and homogenous and watery discharge was present in 24.69% (n=82) of patients. Normal viscous vaginal discharge was observed in 30.12% (n=100) patients.

Smell of vaginal discharge

Apart from color and consistency, smell of vaginal discharge also plays an important role in the diagnosis of infection in vagina or cervix. Foul smelling discharge was observed in 40.66% (n=135) of patients while pungent smell was noted in 15.06% (n=50) and no particular odor was present in 44.27% (n=147) patients.

Table 3: Number and percentage of patients with various clinical observations of vagina, for the color, consistency and smell of vaginal discharge.

Clinical observation (n=332)	Patients	
Appearance of vaginal discharge	**n**	**%**
Color of vaginal discharge		
Whitish	138	41.56
Translucent	71	21.38
Yellowish	43	12.95
Clear	80	24.09
Consistency of vaginal discharge		
Thick and Homogenous	150	45.18
Watery	82	24.69
Viscous	100	30.12
Smell of vaginal discharge		
Foul	135	40.66
Pungent	50	15.06
No smell	147	44.27

Distribution of Study Population in different age group

The study population comprised married females with different complaints of vaginal discharge. These patients were grouped in different age category with an interval of 5 years (Table 4). The patient's age ranged between 17-42+ years with mean age of 28.01±0.29 years and these were divided into five groups. Number of patient and their mean age in each age group is shown in Table 4. Majority of the patients were in the age group ranging from 22 years to 31 years, but the least number of patients was observed in the age group 37-42+ years.

Table 4: Age groups, number, percentage and mean age (years) of patients with vaginal discharge complaints.

Age Group	n	%	Mean Age
17-21	45	13.55	19.89 ± 0.17
22-26	99	29.81	24.52 ± 0.12
27-31	103	31.02	29.12 ± 0.11
32-36	67	20.18	33.96 ± 0.18
37-42+	18	5.42	39.11 ± 0.49
17-42+	332	100	28.01 ± 0.29

Characteristics of vaginal discharge according to the patient's age group

Age wise characteristics of vaginal discharge are given in Table 5 indicating the age wise changes in various clinical parameters of vaginal discharge.

Color of vaginal discharge

Color of Vaginal discharge according to different age group showed that whitish vaginal discharge increased with age and was visible in highest number of patients in age group 27-31years (15.96%) which declined with increasing age. Fewer patients were observed in the age group 17-21years (4.21%) and 22-26 years (8.43%). Similarly it was observed that translucent vaginal discharge was more in patients in the age group 22-26 years (9.03%) and declined with increase in age. In case of yellowish discharge very low percentage (1.80%) was observed in the age group 17-21 years and 37-42+ years. Higher percentage of yellowish discharge was observed in 22-26 years (3.61%) and 32-36 years (3.01%) of patients. Clear (normal) vaginal discharge was noted more in the age group 22-26 years (8.73%) and 27-31 years (8.13%) age groups. In older patients whitish color and yellowish color discharge was common than translucent and normal vaginal discharge.

Linear regression analysis of variance for the color of vaginal discharge according to the age showed a negative non significant trend with increase in age for whitish discharge (b= -0.004±1.65; F (1,3) 0.006; P = 0.94); translucent discharge (b= -0.08±1.28; F(1,3) 1.18 ;P=0.35) and Clear (Normal) discharge (b= -0.08±1.19; F (1,3) 1.72; P 0.28). But in yellowish color discharge no effect of age on color was observed (b= -0.07±3.09; F(1,3) 0.04; P=0.84) (Table 6 and Fig 4).

Consistency of vaginal discharge

Change in the consistency of vaginal discharge was observed from normal viscous to thick and homogenous or watery in the different age groups. The highest number of patients with thick and homogenous vaginal discharge was observed in age group 22-26 years (13.85%) and the number of patients declined with increasing age. Low percentage (3.31%) of thick and homogenous vaginal discharge was observed in 17-21 years and 37-

42+ years age group. Watery discharge was noted in highest number of patient in the age group 27-31 years (8.43%) and the number of patients declined with increase in the age. The highest number of patients (9.93%) with normal vaginal discharge was observed in the younger age group (22-26 years) and the patients declined with increasing age. Patients noted in younger age group (17-21 years) were (4.81%). Thick and homogenous discharge consistency was common than in other type of consistencies of vaginal discharge in the study patients.

Linear regression analysis of variance for the consistency of vaginal discharge according to age showed a negative non-significant trend with increase in age for thick and homogenous vaginal discharge (b= -0.007±1.75; F (1,3) 0.02; P = 0.89); Watery discharge (b= -0.01±1.40; F(1,3) 2.01 ;P=0.25) and Viscous (normal) discharge (b= -0.07±1.4; F(1,3) 1.16; P=0.35). (Table 7 and Fig 5).

Smell of vaginal discharge

Vaginal discharge smell which may be foul or pungent was observed, because this is an important factor in determining vaginal infection. Higher percentage of patients with foul smelling vaginal discharge was observed in age group 22-26 years (12.65%) and that of 27-31 years (13.25%). Number of patients with foul smelling vaginal discharge decreased with increasing age showing the least percentage of such patients in age group 37-42+ years (1.80%). Highest percentage of patients with pungent smell was observed in age group 32-36 years (4.81%) smell and lowest percentage in age group 37-42+ years (1.80%) of patients. Patients with no vaginal discharge smell was the highest in age group 22-26 years (14.75%) and 27-31 years (13.85%), the lowest percentage of these patients was observed in age group 37-42+ years (1.80%).

Linear regression analysis of variance for the smell of vaginal discharge according to age showed a negative non significant trend with increase in age for foul smelling vaginal discharge (b= -0.02±1.60; F (1,3) 0.26; P = 0.64); Pungent smelling discharge (b= -0.08±2.21; F(1,3) 0.15 ;P=0.72) and no specific smell of discharge (b= -0.04±1.43; F(1,3) 1.29; P=0.33). (Table 8 and Fig 6).

Table 5: Distribution of characteristics, color, consistency and smell of vaginal discharge according to the age groups

	Age Groups (years)									
Characteristics of Vaginal Discharge	**17-21**		**22-26**		**27-31**		**32-36**		**37-42+**	
n=332	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Color of Vaginal Discharge										
Whitish	14	4.21	28	8.43	53	15.96	33	9.93	9	2.71
Translucent	11	3.31	30	9.03	14	4.21	14	4.21	2	0.60
Yellowish	6	1.80	12	3.61	9	2.71	10	3.01	6	1.80
Clear Discharge	14	4.21	29	8.73	27	8.13	10	3.01	1	0.30
Consistency of Vaginal Discharge										
Thick and Homogenous	11	3.31	46	13.85	44	13.25	37	11.14	11	3.31
Watery	18	5.41	20	6.02	28	8.43	13	3.91	3	0.90
Viscous (Normal) Discharge	16	4.81	33	9.93	31	9.33	17	5.12	4	1.20
Smell of Vaginal Discharge										
Foul	14	4.21	42	12.65	44	13.25	28	8.43	6	1.80
Pungent	7	2.10	8	2.40	13	3.91	16	4.81	6	1.80
No smell	24	7.22	49	14.75	46	13.85	23	6.92	6	1.80

n is number of patients

Table 6: Linear Regression analysis of variance regarding the color of vaginal discharge according to age group

Whitish discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.02	0.02	0.006	0.94
Residual	3	9.97	3.32		
Total	4	10			b=-0.004±1.65

Translucent discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.82	2.82	1.18	0.35
Residual	3	7.17	2.39		
Total	4	10			b=-0.08±1.28

Yellowish discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.14	0.14	0.04	0.84
Residual	3	9.85	3.28		
Total	4	10			b=-0.07±3.09

Clear (normal) discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.64	3.64	1.72	0.28
Residual	3	6.35	2.11		
Total	4	10			b=-0.08±1.19

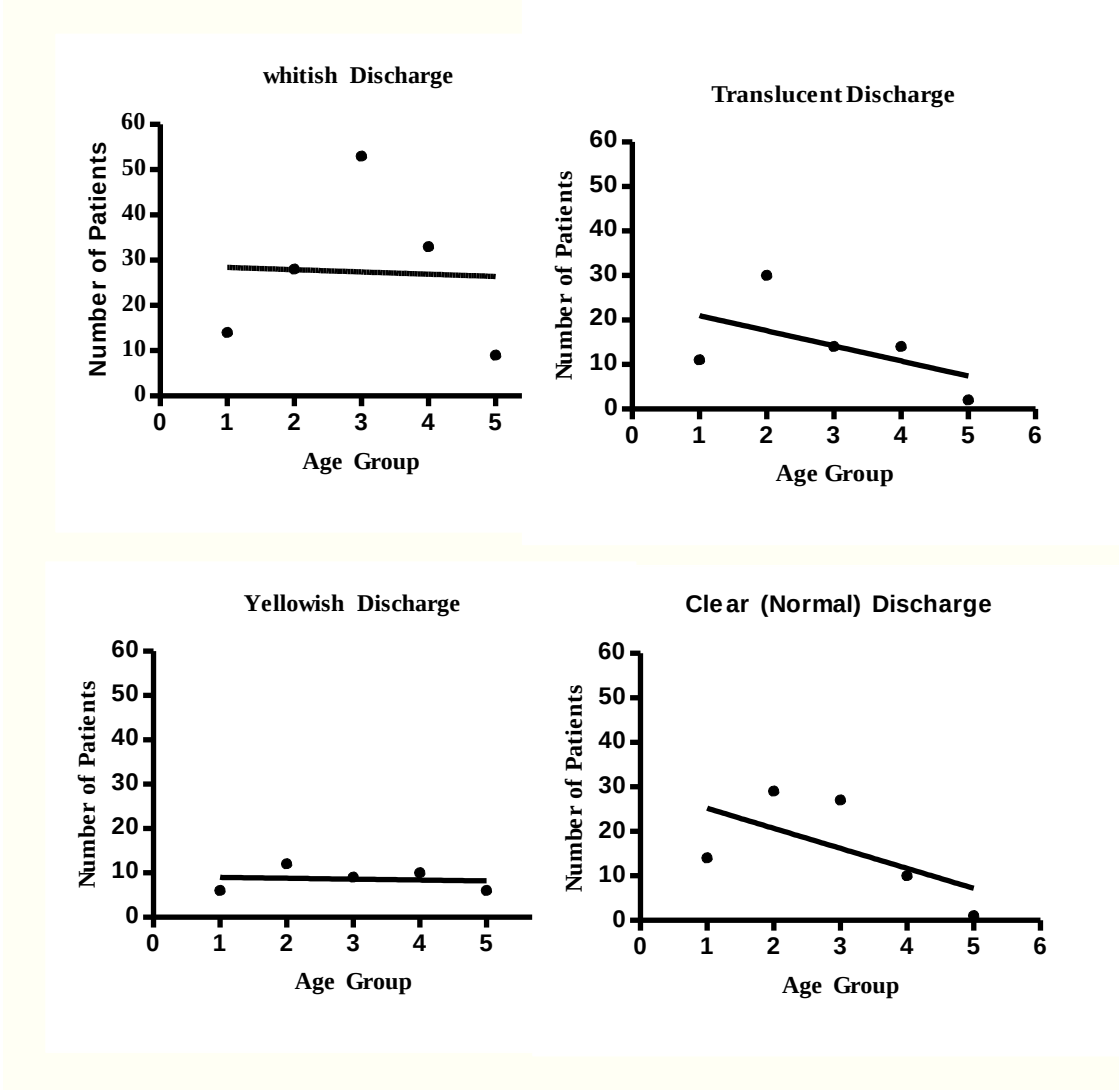


Fig 4: Regression analysis of variance for the number of patients according to age for different colors of vaginal discharge showed a non-significant negative trend with increase in age for whitish, translucent and clear (normal) color vaginal discharge. No relation with age was observed in patients with yellowish color vaginal discharge. Age groups of patients Group 1 (17-21 years); Group 2 (22-26 years); Group 3 (27-31 years); Group 4 (32-36 years); Group 5 (37-42+ years).

Table 7: Linear Regression analysis of variance regarding the consistency of vaginal discharge according to age group

Thick and homogenous discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.06	0.06	0.02	0.89
Residual	3	9.93	3.31		
Total	4	10			b=-0.007±1.75

Watery discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	4.01	4.01	2.01	0.25
Residual	3	5.98	1.99		
Total	4	10			b=-0.01±1.40

Viscous (normal) discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.8	2.8	1.16	0.35
Residual	3	7.19	2.39		
Total	4	10			b=-0.07±1.40

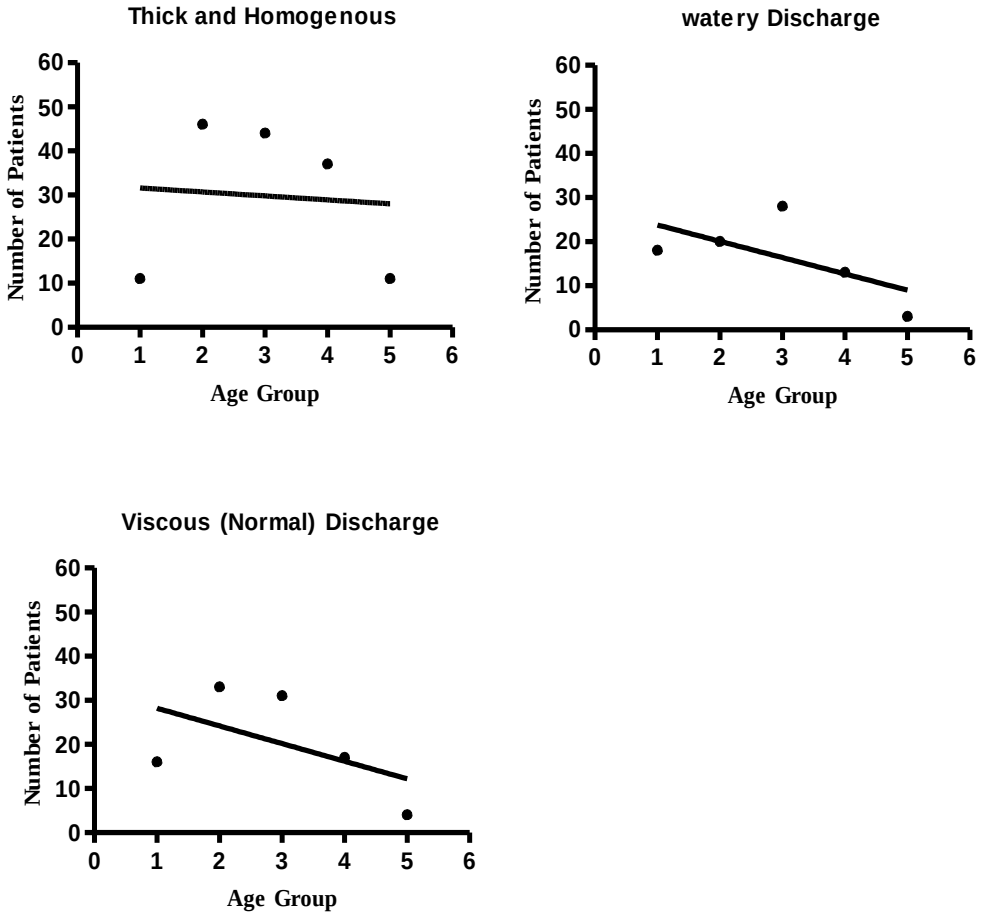


Fig 5: Regression analysis of variance for the number of patients according to age for different consistencies of vaginal discharge showed a non-significant negative trend with increase in age for thick and homogenous, watery and viscous (normal) vaginal discharge. Age groups of patients Group 1 (17-21 years); Group 2 (22-26 years); Group 3 (27-31 years); Group 4 (32-36 years); Group 5 (37-42+ years).

Table 8: Linear Regression analysis of variance regarding the smell of vaginal discharge according to age group

Foul smelling discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.8	0.8	0.26	0.64
Residual	3	9.19	3.06		
Total	4	10			b=-0.02±1.60

Pungent smelling discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.48	0.48	0.15	0.72
Residual	3	9.51	3.17		
Total	4	10			b=-0.08±2.21

No smell of discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	3	3	1.29	0.33
Residual	3	6.99	2.33		
Total	4	10			b=-0.04±1.43

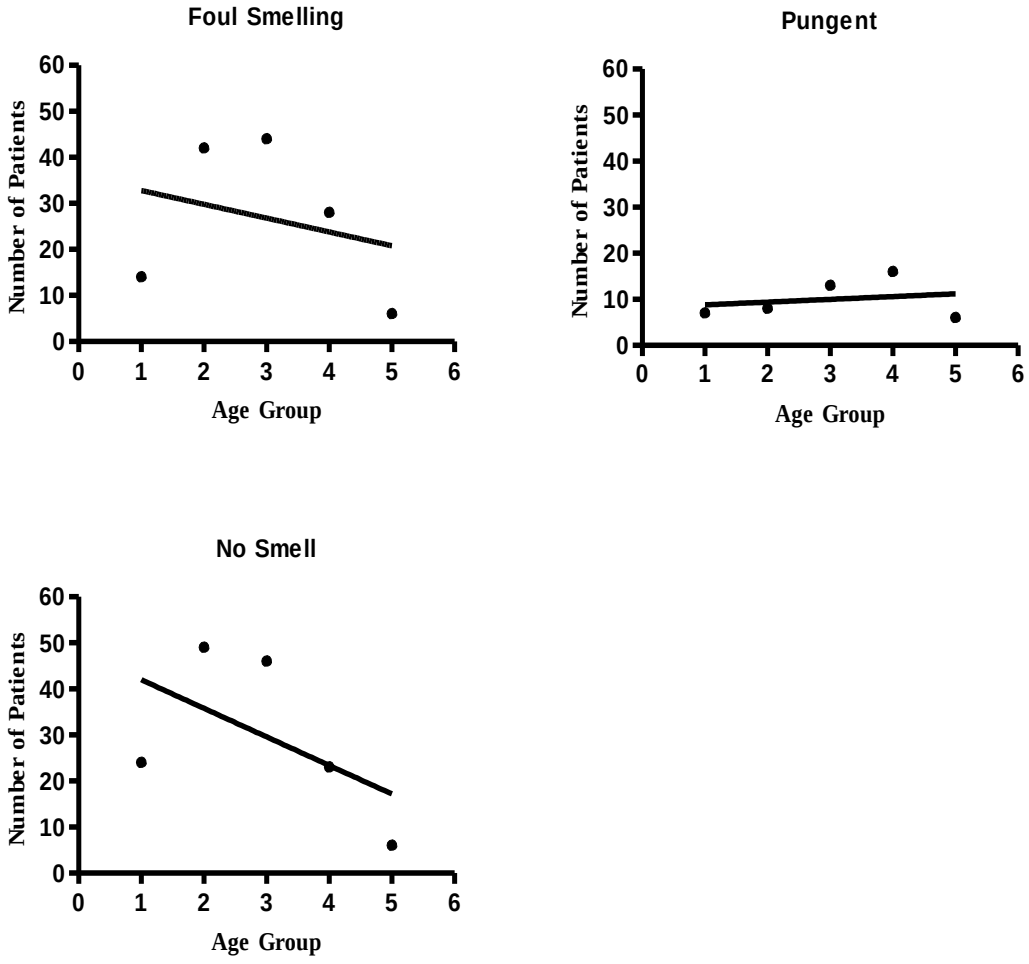


Fig 6: Regression analysis of variance for the number of patients according to age for different smell type of vaginal discharge showed a non-significant negative trend with increase in age for foul smelling discharge and with no specific smell of vaginal discharge. No relation with age was observed in patients with pungent smelling vaginal discharge. Age groups of patients Group 1 (17-21 years); Group 2 (22-26 years); Group 3 (27-31 years); Group 4 (32-36 years); Group 5 (37-42+ years).

Condition of Cervix

Patients with complaints of vaginal discharge at the time of presentation were examined vaginally with the speculum to assess the condition of the cervix (Table 9). The cervix was categorized as healthy, red and swollen, red and swollen with ectopy (erosion) and healthy with ectopy. Of 332 patients, the highest percentage of patients diagnosed with red swollen with ectopy cervix condition and those with red swollen cervix with mean age of 28.30 ± 0.39 years and 28.97 ± 0.53 years respectively. Comparatively younger patients had healthy and healthy with ectopy cervix condition with mean age of 25.91 ± 0.66 years and 23.75 ± 1.83 years respectively.

Table 9: Number, percentage and mean age of patients according to the condition of cervix.

	Patients		Age
Condition of Cervix	**n**	**%**	**Yrs**
Healthy	54	16.26	25.91 ± 0.66
Red and Swollen	104	31.32	28.97 ± 0.53
Red and Swollen with Ectopy	166	50.00	28.30 ± 0.39
Healthy with Ectopy	8	2.40	23.75 ± 1.83

Characteristics of vaginal discharge according to the condition of cervix

Based on cervix condition, characteristics of vaginal discharge like color, consistency, and smell of discharge were recorded. (Table 10)

Color of Discharge

Different conditions of the cervix were observed for different colors of discharge, translucent, whitish, yellowish and clear (normal). All variations in the color of vaginal discharge were observed in greater number of patients with red swollen cervix with ectopy as compared to other conditions of the cervix. Highest percentage of whitish color discharge 21.08% (n=70), translucent color 11.44% (n=38) and yellowish color 7.22% (n=24) of vaginal discharge was observed in patients with red swollen cervix with ectopy as compared with other conditions of the cervix. The second highest percentage of all four color variations of vaginal discharge were observed in patients with red swollen cervix. The distribution of vaginal discharge according to different colors depending on the condition of cervix was not statistically significant ($\sum \chi^2_{(9)}= 10.54$; $P>0.30$).

Consistency of Discharge

Consistency of the vaginal discharge depends on the condition of the cervix. Variation in the consistency of vaginal discharge was observed like thick and homogenous, watery and viscous (normal). Highest number of patients 25.90% (n=86) with red swollen cervix plus ectopy had thick and homogenous discharge. Patients with watery and normal discharge were also observed in greater number in patients with red swollen cervix with ectopy as compared to other conditions of cervix. Highly inflamed edematous cervix depicts change in the consistency of discharge. The variation in the consistency of vaginal discharge according to the condition of the cervix was not statistically significant ($\sum \chi^2_{(6)}= 9.32$; $P>0.20$).

Smell of Discharge

Smell is an important factor in the diagnosis of vaginal infection. Smell was assessed according to the condition of cervix. Variant odors of vaginal discharge were noticed

like foul smell, pungent and no smell. The maximum percentage of patients with red swollen cervix with ectopy as compared with other conditions of the cervix had foul smelling discharge in 18.37% (n=61) and pungent smelling discharge in 12.65% (n=42) respectively along with a high percentage of patients with no specific odor 18.97% (n=63). In patients with red swollen cervix 15.36% (n=51) had foul smelling discharge. Hence smell can be regarded as the definitive criterion for diagnosing Bacterial Vaginosis. Smell of vaginal discharge according to the condition of the cervix was highly significant ($\sum \chi^2_{(6)} = 38.18$; $p<0.0001$).

Table 10: Number and percentage of various characteristics (color, consistency and smell) of vaginal discharge according to the condition of cervix.

	Condition of Cervix												
	Healthy			Red Swollen			Healthy+Ectopy			Red swollen+Ectopy			
Number of patients (n=332)	54			104			8			166			χ^2
	O	**E**	**%**	**O**	**E**	**%**	**O**	**E**	**%**	**O**	**E**	**%**	
Color of Discharge													
Translucent	11	11.54	3.31	20	22.24	6.02	2	1.71	0.60	38	35.51	11.44	
Whitish	21	22.23	6.32	43	42.91	12.95	3	3.31	0.90	70	68.51	21.08	$\sum \chi^2_{(9)}=$
Yellowish	2	6.99	0.60	17	13.46	5.12	0	2.03	0.00	24	21.52	7.22	10.54; P>0.30
Clear (Normal)	20	13.17	6.02	24	25.37	7.22	3	1.95	0.90	34	40.51	10.24	
Consistency of Discharge													
Thick and Homogenous	21	24.39	6.32	41	46.98	12.34	2	3.61	0.60	86	75.01	25.90	
Watery	10	13.33	3.01	32	25.68	9.63	2	1.97	0.60	38	41.01	11.44	$\sum \chi^2_{(6)}=$
Viscous	23	16.26	6.92	31	31.32	9.33	4	2.42	1.20	42	50.01	12.65	3.39; P>0.20
Smell of Discharge													
Foul Smelling	20	21.95	6.02	51	42.28	15.36	3	3.25	0.90	61	67.51	18.37	
Pungent	3	8.94	0.90	5	17.22	1.50	5	1.33	1.50	42	27.51	12.65	$\sum \chi^2_{(6)}=$
No Smell	31	23.09	9.33	48	44.48	14.45	0	3.42	0.00	63	71.01	18.97	38.18; P<0.0001***

O=Observed value; E=Expected value; %=percentage

Bacterial vaginosis

Bacterial vaginosis is the most important cause of vaginal discharge, a clinical entity characterized by a shift of vaginal flora from acidic environment. It depends on the presence of number of clinical and laboratory parameters in a single infected patient. Two separate scoring systems, (1) Amsel and (2) Nugent scoring system which are considered as gold standards were used to diagnose the patients with bacterial vaginosis (Table 11). The Amsel criteria a clinical bedside method, is easy to perform and gives early clue of the problem. However, the Nugent scoring system which is the laboratory method for the fine observation and detailed findings gives accurate results. Of the total 332 patients, those fulfilling all the relevant parameters (Homogenous discharge, pH>4.5, Amine odor and clue cells) for Amsel clinical analysis were 24.69% (n=82). Patients meeting all the scores for Nugent scoring system (Morphotypes of lactobacilli spp, Gardnerella vaginalis and Mobiluncus spp) were 42.16% (n=140). Patients with vaginal discharge but not meeting the parameters for either the Amsel or the Nugent scoring system were 57.83% (n=192).

Table 11: Prevalence of bacterial vaginosis in patients with vaginal discharge according to Amsel clinical criteria and the laboratory Nugent scoring system.

Bacterial Vaginosis	n	%
Not Fulfilling any Criteria	192	57.83
Amsel Clinical Criteria	82	24.69
Nugent Scoring	140	42.16

n = number of patients

Amsel clinical analysis for bacterial vaginosis

Patients presenting with vaginal discharge, were assessed clinically according to Amsel clinical criteria for the presence of clue cells, pH >4.5 (ranging from 4.5-7.2), homogenous vaginal discharge and a positive whiff test of amine odor is shown in Table 12. The highest percentage patients presented with raised pH (57.22%) and 42.77% of patients had pH less than 4.5 thus not fulfilling the Amsel criterion. Homogenous vaginal discharge was observed in 45.18% of patients while vaginal discharge other than homogenous was seen in 54.81% patients. Amine odor (whiff test) was observed in lesser number 40.66% patients. Negative whiff test was observed in higher percentage of patients (59.33%). The least number of patients, 37.34%, with Clue cells (epithelial cells completely covered by gram variable rods) was observed fulfilled the criterion. For

Amsel analysis patient must have any three of the four parameters or all four parameters. Those with two or one parameter were assumed that they were not suffering from bacterial vaginosis. Table 13 presents that majority of the patient 75.30% in the study population with vaginal discharge were not fulfilling the Amsel criteria, 15.96% patients were found fulfilling three out of four parameters. Of 332 patients, a very small percentage i.e. 8.73% (n=29) of the population fulfilled all the four clinical signs required to fulfill the criteria. Majority of the patients presented with two parameters, pH>4.5 and homogenous vaginal discharge.

Table 12: Number and percentage of patients with vaginal discharge fulfilling and not fulfilling all four parameters for Amsel clinical analysis.

No. of Patients (n=332)	Amsel Clinical Analysis			
Parameters	**Fulfilling**		**Not Fulfilling**	
	n	**%**	**n**	**%**
Clue Cells	124	37.34	208	62.65
pH > 4.5	190	57.22	142	42.77
Homogenous Discharge	150	45.18	182	54.81
Amine Odor (Whiff test)	135	40.66	197	59.33

n = number of patients

Table 13: Amsel clinical analysis for bacterial vaginosis in patients with vaginal discharge

Total no. of Patients (n=332)	n	%
Clinical Criteria		
All Four Criteria	29	8.73
Three out of Four Criteria	53	15.96
Not Fulfilling the Criteria	250	75.30

n= number of patients

Amsel clinical analysis according to age groups for bacterial vaginosis

Patients with vaginal discharge were evaluated according to their age groups for prevalence of bacterial vaginosis according to Amsel clinical criteria is shown in Table 14

Clue cells

The gram stained smear observed in different age groups revealed that the number of patients with clue cells increased with increasing age from 2.7% to 12.9% in the age group 17-21 years to 27- 31 years respectively. A decrease in the number of patients with clue cells was observed in the older patients ranging from 32-42+ years. Linear regression analysis of variance was calculated to see age related changes in the parameters of Amsel criteria according to the age group of patients with vaginal discharge. It showed a non significant negative trend with increasing age for the Clue cells (b= -0.01±1.55; F (1,3) 0.05; P = 0.12). (Table 15; Fig 7)

pH >4.5

An increase in the number of patients with pH>4.5 was observed from 17-21 years (8.10%) to 27-31 years (18.60%) and a decrease in the number of patients with pH>4.5 was seen with an increasing age. Linear regression analysis of variance was calculated to see age related changes in the parameters of Amsel criteria according to the age group of patients with vaginal discharge. It showed a non significant negative trend with increasing age for pH of vaginal discharge (b= -0.02±1.51; F(1,3) 0.71;P=0.45). (Table 15; Fig 7)

Homogenous Discharge

Increasing trend in the number of patients with Homogenous vaginal discharge was observed from 17-21 years (3.30%) to 22-26 years (13.80%) and then a decreasing trend was observed from 27 years onwards. Linear regression analysis of variance was calculated to see age related changes in the parameters of Amsel criteria according to the age group of patients with vaginal discharge. It showed a non significant negative trend with increasing age in patients with homogenous

consistency of vaginal discharge (b= -0.007±1.83; F(1,3) 0.02; P=0.89). (Table 15; Fig 7)

Amine Odor (Whiff test)

Presence or absence of amine odor with KOH in the patients was observed (whiff test). There was an increase in the number of patients presenting with amine odor of vaginal discharge in the age group 17-21 years (12.60%) to 27-31 years (13.20%) and with the increase in age the number of patients with amine odor in the vaginal discharge decreased. Linear regression analysis of variance was calculated to see age related changes in the parameters of Amsel criteria according to the age group of patients with vaginal discharge. It showed a non significant negative trend with increasing age for the amine odor (whiff test) (b= -6.3±9.0; F (1,3) 0.48; P 0.53). (Table 15; Fig 7)

Amsel clinical criteria according to age group for Bacterial Vaginosis

Patients presenting with the above parameters were categorized as fulfilling the Amsel criteria according to age groups (Table 14). An increasing trend for all the four parameters was observed in the age group (0.60%) 17-21 years to (3.60%) 22-26 years. And a decreasing trend was observed in the age group 27-31 years onwards. Least percentage (0.30%) of patients was observed in the age group 37-42+ years.

Majority of the patients fulfilling the Amsel criteria (all three parameters) were noted in the age group of 22 to 26 years 6.02%. While a comparatively lesser number of patients were observed between the age groups 27-31 years 3.90%. In the age group 32-36 years similar lesser percentage of patients fulfilling the Amsel criteria were observed (4.20%) and a nominal number of patients were observed in the higher age group. Greater number of patient's not fulfilling the criteria were 25% observed in the age group 27-31 years.

Therefore it was observed that bacterial vaginosis is most pertinent in female patients ranging from 22 to 37 years. Although majority of the sample population was not fulfilling the Amsel criteria.

Table 14: Frequency of clinical signs (four parameters) of Bacterial Vaginosis among different age groups according to Amsel clinical criteria.

	Age Group (years)									
Amsel Clinical Analysis (n=332)	**17-21**		**22-26**		**27-31**		**32-36**		**37-42**	
Parameters	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Clue Cells	9	2.70	36	10.80	43	12.90	30	9.03	5	1.50
pH > 4.5	27	8.10	58	17.40	62	18.60	37	11.10	5	1.50
Homogenous Discharge	11	3.30	46	13.80	44	13.20	37	11.10	11	3.31
Amine Odour	14	4.20	42	12.60	44	13.20	28	8.40	6	1.80
Amsel Criteria's										
All Four Criteria (n=29)	2	0.60	12	3.60	7	2.10	7	2.10	1	0.30
Three out of Four Criteria (n=53)	4	1.20	20	6.20	13	3.90	14	4.20	2	0.60
Not Fulfilling the Criteria (n=250)	39	11.70	67	21.10	83	25.00	46	13.80	15	4.50

n=number of patients

Table 15: Linear regression analysis of variance regarding the clue cells, pH >4.5, homogenous vaginal discharge and amine odor (whiff test) according to age groups of patients with vaginal discharge.

Clue cells

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.17	0.17	0.05	0.12
Residual	3	9.82	3.27		
Total	4	10			b=-0.01±1.55

pH>4.5

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.93	1.93	0.71	0.45
Residual	3	8.06	2.68		
Total	4	10			b=-0.02±1.51

Homogenous discharge

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.06	0.06	0.02	0.89
Residual	3	9.93	3.31		
Total	4	10			b=-0.007±1.83

Amine odor (whiff test)

Source	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.8	0.8	0.26	0.64
Residual	3	9.19	3.06		
Total	4	10			b=-0.02±1.60

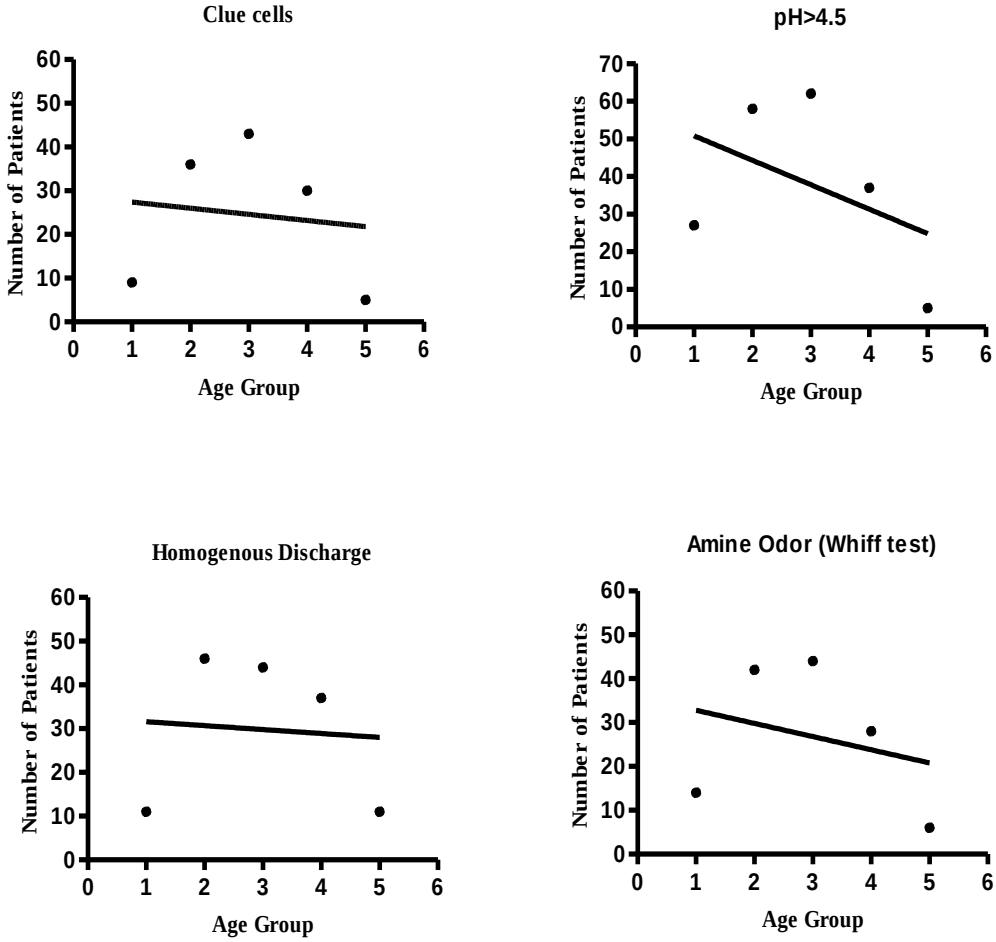


Fig 7: Regression analysis of variance for the number of patients according to age for different parameters of Amsel clinical criteria showed a non-significant negative trend with increase in age was observed for clue cells, pH>4.5, homogenous discharge and amine odor (whiff test) of vaginal discharge. Age groups of patients Group 1 (17-21 years); Group 2 (22-26 years); Group 3 (27-31 years); Group 4 (32-36 years); Group 5 (37-42+ years).

Nugent Scoring for Bacterial Vaginosis

Nugent scoring for the confirmatory diagnosis of bacterial vaginosis was done by standardizing the scoring of bacterial morphotypes identified in vaginal discharge, Lactobacillus spp., Gardrenella vaginalis and Mobilincus spp. on the direct gram stained slides. Nugent scoring on gram stained smear is shown in Table 16.

Lactobacilli spp.

Lactobacilli spp. were calculated on a scale of 0 (>30 bacteria) to 4 (0 number of bacteria) depending on the number of lactobacillus spp. present per microscopic field (x1000 magnification in the oil immersion field) as viewed. Majority of the patients 34.33% fulfilled the criteria on a scale of 4, while 24.69% of patients were noticed at scale 2. A lesser percentage of patients were observed at scale 0, 1 and 3.

Gardnerella vaginalis

As the number of Gardnerella vaginalis increased the score increased likewise, contrary to the lactobacillus spp. the number of organisms increase. Majority of the patients (44.87%) met the scale 0 indicating 0 number of bacteria and a lesser number of patients (17.16%) fulfilled the criteria for scale of 4 indicating >30 bacteria. Hence A decrease in the number of patients was observed with an increase in the score.

Mobiluncus spp

The scoring of Mobiluncus spp resembled to that of G.vaginalis with an even greater decrease in the number of bacterial spp.. As the score increased the number of organisms increased. Maximum number of patients 80.12% were seen at scale 0 indicating 0 number of bacteria and a very small percentage of patients was observed at scale 1 and 2.

Table 16: Criteria for the microscopic diagnosis of bacterial vaginosis according to Nugent scoring system. Number, percentage and score of patients according to the bacterial morphotypes, Lactobacilli spp, Gardnerella vaginalis and Mobiluncus spp.

Lactobacilli spp				Gardnerella vaginalis				Mobiluncus spp				Total	no. of
Score	**N**	**n**	**%**	**Score**	**N**	**n**	**%**	**Score**	**N**	**n**	**%**	**Score**	**Patients**
0	>30	51	15.36	**0**	0	149	44.87	**0**	0	266	80.12	**0**	51
1	5-30	45	13.55	**1**	<1	17	5.12	**1**	<1	22	6.62	**3**	17
2	1-4	82	24.69	**2**	1-4	52	15.66	**1**	1-4	18	5.42	**5**	18
3	<1	40	12.04	**3**	5-30	57	17.16	**2**	5-30	11	3.31	**8**	11
4	0	114	34.33	**4**	>30	57	17.16	**2**	>30	15	4.51	**10**	15

n= number of patients; N=number of bacteria

Score 0= large number of Lactobacilli spp and absent Gardenerella vaginalis and Mobiluncus spp.
Score 1= 5-30 lactobacilli spp and <1 (one in any one field) of G vaginalis and Mobiluncus spp.
Score 2= 1-4 lactobacilli spp. and G. vaginalis and as **score 1** for 1-4 Mobiluncus spp.
Score 3= <1 Lactobacilli spp and 5-30 G vaginalis and as **score 2** for 5-30 Mobiluncus spp.
Score 4= absent Lactobacilli spp and >30 G vaginalis and as **score 2** for >30 Mobiluncus spp

Nugent scoring

Nugent scoring is done by placing the number and score of lactobacillus spp. against the number and score of G.vaginalis and Mobiluncus spp.as shown in the Table 17.

For all the four scores of Lactobacillus spp. the maximum number of patients with G.vaginalis and Mobiluncus spp. was observed at scale 0. There was a drastic decrease in the number of patients in all four score of G.vaginalis and Mobiluncus spp. against the scores of Lactobacilli spp (score=0-4). A deviation from the normal trend was observed in G.vaginalis for score 4 which showed an increased percentage of patients at this score .After the scoring of the three bacteria's cumulative score was made by combining the scores of all the three spp. Lactobacillus spp., Mobiluncus spp. and G.vaginalis spp. as shown in Table 18. The patients falling in the scale range of 0 to 3 (57.83%) were not considered as bacterial vaginosis. Those whose scores fell between 4 to 6 (25%) were regarded as intermediate considered as positive for bacterial vaginosis. Cases that scored more than 7 were considered strongly positive (17%) for bacterial vaginosis.

Table 17: Number and percentage of patients with scoring of full scale morphotypes of G. vaginalis and Mobiluncus spp according to the number and percentage of patients with score of Lactobacilli spp for the microscopic diagnosis of Bacterial vaginosis according to Nugent scoring system in patients with vaginal discharge (n=332)

Lactobacillus spp Score	4+		3+		2+		1+		0	
	n	%	n	%	n	%	n	%	n	%
	114	34.33	40	12.04	82	24.69	45	13.55	51	15.36
G. vaginalis (score)										
0	42	12.65	27	8.13	42	12.65	19	5.72	20	6.02
1+	4	1.21	3	2.41	8	2.41	2	0.61	-	-
2+	14	4.21	7	3.61	12	3.61	9	2.71	9	2.71
3+	16	4.81	-	-	17	5.12	10	3.01	14	4.21
4+	38	11.44	3	0.91	3	0.91	5	1.51	8	2.41
Mobiluncus spp(score)										
0	89	26.80	37	11.14	72	21.68	29	8.73	39	11.74
1+	3	0.91	-	-	2	0.61	9	2.71	8	2.41
1+	8	2.41	-	-	5	1.51	2	0.61	3	0.91
2+	5	1.51	-	-	2	0.61	4	1.20	-	-
2+	8	2.41	3	0.91	1	0.31	1	0.31	1	0.31

n is the number of patients

Score 0= large number of Lactobacilli spp and absent Gardenerella vaginalis and Mobiluncus spp. **Score 1=** 5-30 lactobacilli spp and <1 (one in any one field) of G vaginalis and Mobiluncus spp. **Score 2=** 1-4 lactobacilli spp. and G. vaginalis and as score 1 for 1-4 Mobiluncus spp. **Score 3=** <1 Lactobacilli spp and 5-30 G vaginalis and as score 2 for 5-30 Mobiluncus spp. **Score 4=** absent Lactobacilli spp and >30 G vaginalis and as score 2 for >30 Mobiluncus spp

Table 18: Number and percentage of normal cases, intermediate as positive cases and positive cases according to the Nugent scoring system.

Total Number	Score	n	%
normal cases	0-3	192	57.83
Intermediate as positive cases	4-6	83	25.00
positive cases	>7	57	17.16

n = number of patients

Parameters of Vaginal Discharge

According to the condition of the cervix vaginal smear was gram stained and studied with light microscope at x 1000 magnification with oil immersion. (Table 19). Each slide was studied for different parameter's, which included clue cells, epithelial cells, polymorphonuclear neutrophils (PMN), Lactobacillus spp., G. vaginalis, Mobiluncus spp., and pH. All parameters calculated are major predictors for bacterial vaginosis

Clue Cells

Clue cell identification is important for the diagnosis of bacterial vaginosis. Mean number of clue cells observed in the study patients was 4.05 ± 0.53 (n=332). The maximum mean number of clue cells 5.11±0.70 (n=104) was observed in patients with red swollen cervix and mean number of 4.68±0.60 (n=166) clue cells were noticed in red swollen cervix with ectopy respectively. Mean number of clue cells decreased in healthy cervix with ectopy 2.25±1.21 (n=8). Mean number of clue cell was not significantly different in among cervical conditions.

Epithelial Cell

The mean number of epithelial cells was 22.73±0.90 (n=332) in study population. In all the cervical conditions mean number of epithelial cells did not differ appreciably.

Polymorphnuclear neutrophils (PMN)

Increased number of PMN indicates the inflammatory process and is an important indicator of STI and vaginitis. Mean number of PMN was 19.45±0.82 (n=332) in the whole sample. There is more or less even distribution of PMN in all conditions of cervix.

Lactobacillus spp.

Presence or absence of lactobacillus spp is a major indicator of bacterial vaginosis. The mean number of lactobacillus spp. was 16.64±0.92 (n=332) in study patients. In all conditions of cervix lactobacilli spp. showed even distribution with the exception of red swollen type where mean lactobacilli were 19.26±1.77 (n=166), but this was not significantly different from healthy condition.

Gardnerella vaginalis

G.vaginalis is the most important parameter for BV. Mean number of G vaginalis in this population was 16.29±0.99 (n=332). Uniform distribution of the mean number was observed. Maximum mean number was seen in red swollen cervix 19.64±1.87 (n=166). Compared to healthy cervix all other conditions of the cervix were not significant.

Mobiluncus spp.

Mobiluncus spp alone or in combination with G. vaginalis is also an identification marker for BV. Mean number of Mobilucus spp. was 3.51±0.44 (n=332) in the study population. A significant difference was observed for Mobiluncus spp. in healthy cervix (3.79±1.13; n=54) compared to healthy cervix with ectopy (11.25±5.48; P< 0.04).

pH

The pH of all the patients with vaginal discharge was more than 5, provides condition prone to BV. This provides less acidic environment which is favorable for the growth of bacteria. Mean pH was 5.37±0.49 (n=332).Different parameters of vaginal discharge as well as condition of cervix have important role in the identification of different infections in the patients.

Table 19: Parameters of the microscopic findings of vaginal discharge on direct smear gram staining showing number of clue cells (epithelial cell covered with bacteria's), epithelial cells, polymorphnuclear neutrophils (PMN), along with the bacterial morphotypes, Lactobacillus spp, Gardenerella vaginalis and Mobiluncus spp and pH of vaginal discharge according to the condition of the cervix.

				Bacterial Morphotypes			
Condition of Cervix	**Clue Cells**	**Epithelial Cells**	**PMN**	**Lactobacilli spp.**	**G. vaginalis**	**Mobiluncus spp.**	**pH**
Healthy n=62	3.21 ± 0.90	22.45 ± 2.10	17.84 ± 1.92	15.18 ± 1.98	16.66 ± 2.44	3.79 ± 1.13	5.40 ± 0.17
Red and Swollen n=104	5.11 ± 0.71	22.28 ± 1.58	18.96 ± 1.31	19.26 ± 1.77	19.64 ± 1.87	4.05 ± 0.84	5.42 ± 0.14
Red and Swollen with Ectopy n=166	4.68 ± 0.60	23.13 ± 1.29	20.36 ± 1.25	14.05 ± 1.29	14.05 ± 1.29	3.07 ± 0.56	5.36 ± 0.10
Healthy with Ectopy n=8	2.25 ± 1.21	26.13 ± 6.15	20.00 ± 5.08	12.50 ± 6.19	12.50 ± 6.19	11.25 ± 5.48*	5.37±0.49
Study Population n=332	4.05 ± 0.53	22.73 ± 0.09	19.45 ± 0.82	16.29 ± 0.99	16.29 ± 0.99	3.51 ± 0.44	5.39 ± 0.07

n=number of patients; Mean±SE

*P<0.04 Mobiluncus spp. comparison of healthy cervix and healthy cervix with ectopy

Polymorphnuclear neutrophils with different conditions of vagina, cervix and fundus

Vaginal, cervical and fundal characteristics of the patients were assessed in relation to the number of PMN calculated on direct gram stained smears under light microscope (x 1000 magnification with oil immersion lens). PMN were grouped according to their number (per high power field) (Table 20). Vaginal, cervical and fundal characteristics may be present alone or in combination.

Patients were clinically assessed for vaginal erythema, odor, itching and discharge. It was observed that majority of them were in the category 6-15 PMN had erythema in 10.84% (n=36), odor in 12.65% (n=42), itching in 23.19% (n=77), discharge in 15.06% (n=50) of patients respectively. Similarly patients with 26-35+ PMNs had erythema in 13.85% (n=46), odor in 9.33% (n=31), itching in 19.87% (n=66) and discharge in 12.34% (n=41) of patients respectively. While less percentage for above characteristics was noted in patients (n=40) with 1-5 PMN category and 37 patients showed absence of PMN in this sample study.

The cervical findings which were friability, redness/ swollen, ectopy and cervical motion tenderness were present in majority of patients in category 6-15 PMNs. It was observed in this category patients with friability of cervix were 17.46% (n=58), red swollen cervix 26.80% (n=89), ectopy in 17.77% (n=59) and cervical motion tenderness in 16.56% (n=53) respectively. Similarly patients with 26 -35+ PMN were with friable cervix in 20.18% (n=67),red swollen cervix in 23.49% (n=78), ectopy in 14.75% (n=49) and cervical motion tenderness in 16.56% (n=55) patients respectively.

Fundal, adenexal and abdominal tenderness were observed in majority of patients in category 26-35+ PMNs. Patients with fundal tenderness were 1.80% (n=6), adenexal tenderness were 9.33% (n=31) and abdominal tenderness were 4.51% (n=15) respectively. Similarly in category 6-15 PMN patients with fundal tenderness were 1.20% (n=4), adenexal tenderness were 4.21% (n=25) and abdominal tenderness 1.80% (n=4) respectively. Presence of PMN in accordance with different conditions of vagina, cervix and fundus was non significant.

Table 20: Number and percentage of patients on direct gram stained vaginal smear for the distribution of polymorphnuclear neutrophils (PMN) with different conditions of vagina, cervix and fundus.

Characteristics (n=332)	Polymorphnuclear Neutrophils (PMN) per HPF															χ^2
	PMN (1-5)			PMN (6-15)			PMN (16-25)			PMN (26-35+)			No PMN			
	O	E	%	O	E	%	O	E	%	O	E	%	O	E	%	
Vaginal																
Vaginal Erythema	12	10.27	3.61	36	42.99	10.84	30	28.94	9.03	46	38.58	13.85	14	17.19	4.21	
Vaginal Odour	13	10.05	3.91	42	42.05	12.65	29	28.31	8.73	31	337.75	9.33	20	16.82	6.02	$\sum \chi^2_{(12)}$=
Vaginal Itching	15	17.51	4.51	77	73.21	23.19	47	49.28	14.15	66	65.71	19.87	30	30.01	9.03	7.47; P>0.81
Vaginal Discharge	9	11.17	2.71	50	46.73	15.06	32	31.45	9.63	41	41.94	12.34	18	18.69	5.42	
Cervical																
Cervical Friability	19	19.06	5.72	58	64.98	17.46	38	38.13	11.44	67	62.47	20.18	23	20.32	6.92	
Cervical Redness/Swollen	23	25.21	6.92	89	85.91	26.80	53	50.41	15.96	78	82.59	23.49	28	26.86	8.43	$\sum \chi^2_{(12)}$=
Cervical Ectopy	17	16.16	5.12	59	55.16	17.77	32	32.37	9.63	49	53.03	14.75	17	17.25	5.12	3.83; P>0.80
Cervical Tenderness	17	15.53	5.12	53	52.94	15.96	29	31.06	8.73	55	50.89	16.56	13	16.55	3.91	
Fundal																
Fundal Tenderness	1	1.21	0.30	4	5.71	1.20	7	4.66	2.10	6	8.99	1.80	5	2.42	1.50	$\sum \chi^2_{(8)}$=
Adenexal Tenderness	6	4.34	1.80	25	20.59	7.53	14	16.84	4.21	31	32.45	9.33	7	8.73	2.10	10.37 P>0.30
Abdominal Tenderness	0	0.00	0.00	4	6.69	1.20	6	5.48	1.80	15	10.55	4.51	2	2.84	0.60	

O=Observed number; E=Expected number; %=Percentage

Values in ( ) = number of PMN

Prevalence of vaginal infection

High vaginal swab (HVS) and endocervcial swabs obtained were studied for the microorganisms present in the vaginal discharge. After undergoing gram staining and geimsa staining different vaginal samples were inoculated on various growth media. Vaginal and endocervical samples from 332 patients were inoculated on different culture media. Different isolates were obtained after incubation of media under aerobic conditions except for N.gonorrheae which required anaerobic conditions (Table 21). No growth and no isolates were obtained in 12.65% (n=42) patients. Highest number of patients (59.03%; n=196) were with single bacterial isolates (E.coli, S.agalactiae, klebsiella spp., S.aureus, P.aeruginosa and N.gonorrheae), fungal (candida spp.) infection was 17.16% (n=57), while that of mixed infection (both bacterial and fungal isolates) were obtained in 11.14% (n=37) of symptomatic patients.

Table 21: Prevalence of various isolates obtained after inoculation on different culture media aerobically and anaerobically in patients with vaginal discharge

Type of infections (n 332)	n	%
Growth on Culture Media		
No Growth (no isolate)	42	12.65
Bacterial (total single isolates)	196	59.03
Fungal (Candidiasis)	57	17.16
Mixed Growth (Bacterial and Fungal)	37	11.14

n is the number of patients

Vaginal Isolates

In patients presenting with vaginal discharge (n=332), different microorganisms were isolated which were the source of infection in the symptomatic patients. Prevalence of these isolates is shown in Table 22; Fig 8. Among the single isolates, the most prevalent organism was Escherichia coli in 25% (n=84) of the patients. Second most prevalent organism obtained was Candida spp in 17% (n=58) patients. Others are given in the descending order in Table 3.20. No growth was obtained in 12% (n=41) patients from the total of 332 samples.

Table 22: Number and percentage of vaginal isolates obtained on various culture media after incubation under aerobic condition with the exception of Nesserria gonorrheae (which requires anaerobic environment)

Isolates (n=332)	n	%
Single Isolates		
Escherichia coli	84	25
Candida spp	58	17
Klebsiella spp	34	10
Nesserria gonorrheae	31	9
Streptococcus agalactiae	24	7
Staphylococcus. aureus	14	4
Pseudomonas aeruginosa	9	3
Mixed Isolates		
Escherichia coli + Candida spp	20	6
Streptococci agalactica + Candida spp	11	3
Staphylococcus aureus + Candida spp	6	2
No growth	41	12

n is number of patients

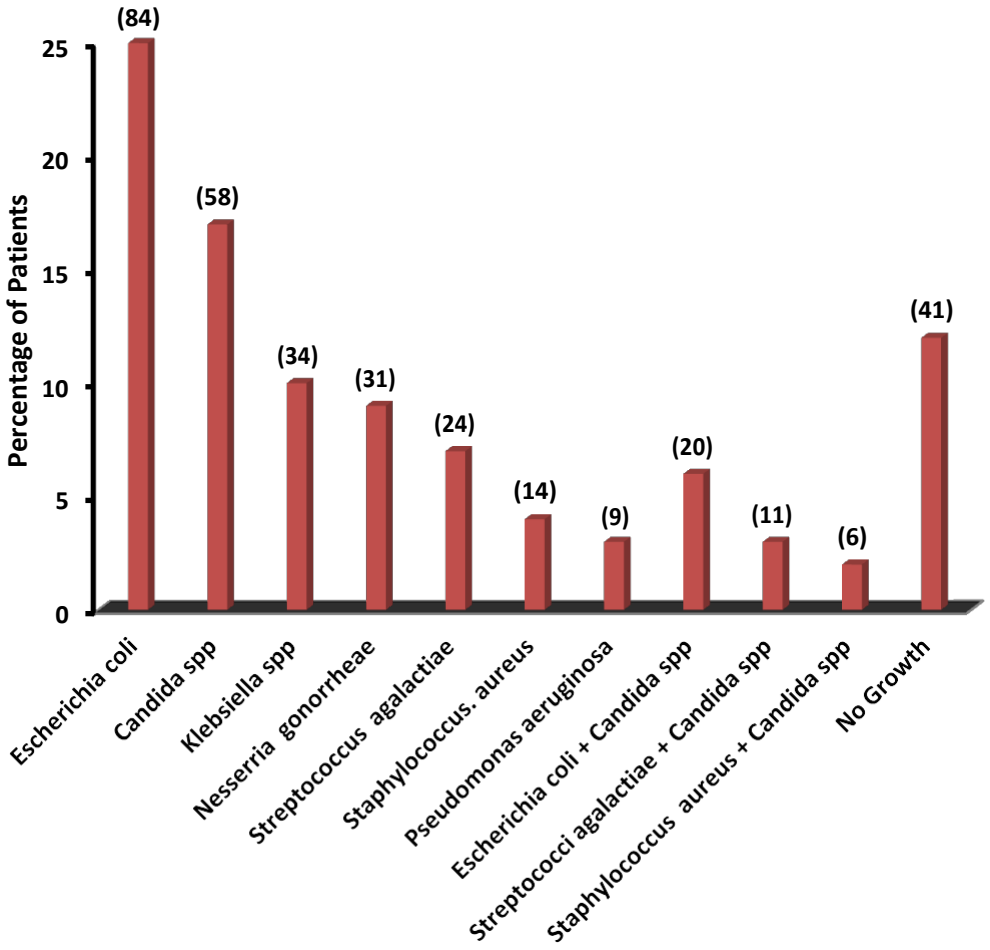


Fig 8: Number and percentage of vaginal isolates. (Values in parenthesis represent number of patients)

Vaginal isolates according to age group

In different age groups number and percentage of vaginal isolates obtained after inoculation are shown in Table 23. The highest number of E. coli isolate 9.63% (n=32) was observed in age group 27-31 years, while 8.13% of the isolates were observed in age group 22-26 years. E. coli decreased with increasing age. Candida was the second highest prevalent isolate. It was observed that the percentage of the Candida isolate increased from 3.01% (n=10) in age group 17-21 years to 6.02% (n=20) in age group 27-31 years and decreased with increasing age groups. The number of patients with Klebsiella spp increased with increasing age to 3.31% (n=10) in 27-31 years and then decreased with increasing age group. N. gonorrheae, a sexually transmitted infection was isolated in 3.91% (n=13) in age group 32-36 years while it decreased with increasing age. S. agalactiae isolates were shown to increase from 1.50% (n=5) in age group 17-21 years to 3.01% (n=10) in the age group 27-31 years and decreased with increase in age groups. S.aureus was observed in 0.90% (n=3) to 1.20% (n=4) except in the age group 36-42+ years as no organism was isolated. P. aeruginosa was less in the hospital population and no organism was isolated in the older age group of study population. Combination of infection as mixed growth of E coli with Candida spp. was the most prevalent with highest percentage 2.71% (n=9) in 22-26 years age group and declined with increasing age. Only small percentage was observed. S. galactiae and S. aureus were isolated in less percentage with no isolate in the youngest and the oldest age group but the percentage of no growth observed increased with increasing age.

Table 23: Number and percentage of different isolates obtained after inoculation of vaginal discharge according to age groups.

	Single Isolates							Mixed Isolates			
Age Group	Escherichia coli	Candida spp	Klebsiella spp	Nesserria gonorrheae	Streptococcus agalactiae	Staphylococcus aureus	Pseudomonas aeruginosa	E.coli+ Candida spp	S.agalactiae+ Candida spp	S. aureus + Candida spp	No Growth
17-21											
n=45	12	10	5	4	5	3	2	1	-	-	2
%	3.61	3.01	1.50	1.20	1.50	0.90	0.60	0.30	-	-	0.60
22-26											
n=99	27	14	7	8	6	4	4	9	5	3	12
%	8.13	4.21	2.10	2.40	1.80	1.20	1.20	2.71	1.50	0.90	3.61
27-31											
n=103	32	20	11	5	10	4	2	6	1	1	11
%	9.63	6.02	3.31	1.50	3.01	1.20	0.60	1.80	0.30	0.30	3.31
32-36											
n=67	10	8	10	13	1	3	1	3	4	2	12
%	3.01	2.40	3.01	3.91	0.30	0.90	0.30	0.90	1.20	0.60	3.61
37-42+											
n=18	3	6	1	1	2	-	-	1	-	-	4
%	0.90	1.80	0.30	0.30	0.60	-	-	0.30	-	-	1.20
n=332	84	58	34	31	24	14	9	20	11	6	41
%	25.30	17.46	10.24	9.33	7.22	4.21	2.71	6.02	3.31	1.80	12.34

Antibiotic sensitivity pattern of study population

All isolates identified were tested against various groups of antibiotics for the sensitivity, resistance and intermediate sensitivity. Various groups of antibiotics used were Penicillin (Ampicillin, Tazocin), Macrolide (Erythromycin), Aminoglycoside (Gentamycin), Tetracycline, Carbepenem (Imepenum), Quinolones (Ciproxin, Levofloxacin) and Cephalosporins (Cefixime**,** Cefotaximine**,** Ceftazadime**,** Ceftraxone**.**).

Antibiotic sensitivity pattern of single isolates

Antibiotic sensitivity pattern of Escherichia coli

Sensitivity pattern of E.coli for various drugs (antibiotics) is shown in Fig 9. E coli showed sensitivity to AMP (18%), TZP (27%), GM (64%), ER (54%), TET (38%), IMP (90%), CIP (65%), LEV (56%), CFM (35%), CTX (48%), CAZ (37%) and CRO (45%).While E coli showed resistance against AMP (56%), TZP (56%), GM (21%), ER (33%), TET (51%), IMP (10%), CIP (30%), LEV (29%), CFM (54%), CTX (46%), CAZ (56%) and CRO (51%) and intermediate sensitivity for AMP (26%), TZP (17%), GM (14%), ER (13%), TET (11%), CIP (5%), LEV (15%), CFM (12%), CTX (5%), CAZ (7%) and CRO (4%).

Marked sensitivity was observed with GM, ER, CIP, LEV, while highest sensitive pattern was noted with IMP. Maximum resistance was observed with AMP, TZP, TET, CFM, CAZ, CRO

Antibiotic sensitivity pattern of Klebsiella spp

Sensitivity pattern of Klebsiella spp for various drugs (antibiotics) is shown in Fig 10. Klesiella spp showed sensitivity to AMP (3%), TZP (35%), GM (47%), ER (68%), TET (62%), IMP (92%), CIP (91%), LEV (82%), CFM (65%), CTX (62%), CAZ (65%) and CRO (62%). While Klebsiella spp showed resistance against AMP (91%), TZP (62%), GM (47%), ER (32%), TET (38%), IMP (6%), CIP (9%), LEV (15%), CFM (26%), CTX (26%), CAZ (21%) and CRO (26%) and intermediate sensitivity was observed with few antibiotics AMP (6%), TZP (3%), GM (6%), LEV (3%), CFM (9%), CTX (12%), CAZ (15%) and CRO (12%).

Marked sensitivity was observed with ER, TET, CFM, CTX, CAZ and CRO while highest sensitive pattern was noted with IMP, CIP, LEV. Maximum resistance was observed with AMP and TZP.

Antibiotic sensitivity pattern of N. gonorrheae.

Sensitivity pattern of N. gonorrheae for various drugs (antibiotics) is shown in Fig 11. E coli showed sensitivity to AMP (32%), TZP (48%), GM (61%), ER (65%), TET (19%), IMP (100%), CIP (84%), LEV (61%), CFM (55%), CTX (48%), CAZ (52%) and CRO (58%).While E coli showed resistance against AMP (65%), TZP (39%), GM (39%), ER (35%), TET (74%), CIP (16%), LEV (29%), CFM (42%), CTX (35%), CAZ (29%) and CRO (23%) and intermediate sensitivity for AMP (3%), TZP (13%), TET (6%), LEV (10%), CFM (12%), CTX (16%), CAZ (19%) and CRO (19%).

Marked sensitivity was observed with GM, ER, LEV, CFM, CAZ and CRO while highest sensitive pattern was noted with IMP and CIP. Maximum resistance was observed with AMP, TZP and TET.

Antibiotic sensitivity pattern of Streptococcus agalactiae

Sensitivity pattern of S. agalactiae for various drugs (antibiotics) is shown in Fig 12. S. agalactiae showed sensitivity to AMP (96%), TZP (75%), GM (83%), ER (63%), TET (25%), IMP (96%), CIP (79%), LEV (75%), CFM (54%), CTX (55%), CAZ (59%) and CRO (67%).While E coli showed resistance against AMP (4%), TZP (25%), GM (17%), ER (29%), TET (75%), IMP (4%), CIP (21%), LEV (21%), CFM (42%), CTX (37%), CAZ (37%) and CRO (33%) and intermediate sensitivity for observed with few antibiotics ER (8%), LEV (4%), CFM (4%), CTX (8%), CAZ (4%).

Marked sensitivity was observed with TZP, ER, CIP, LEV, CFM, CTX, CAZ and CRO while highest sensitive pattern was noted with AMP, GM and IMP. Maximum resistance was observed with TET.

Antibiotic sensitivity pattern of Staphylococcus Aureus

Sensitivity pattern of S. aureus for various drugs (antibiotics) is shown in Fig 13. S.aureus showed sensitivity to AMP (29%), TZP (43%), GM (57%), ER (64%), TET (43%), IMP (86%), CIP (79%), LEV (71%), CFM (43%), CTX (50%), CAZ (57%) and CRO (64%).While S. aureus showed resistance against AMP (71%), TZP (50%), GM (29%), ER (14%), TET (57%), IMP (14%), CIP (14%), LEV (7%), CFM (36%), CTX (29%), CAZ (21%) and CRO (29%) and intermediate sensitivity for TZP (7%), GM (14%), ER (21%), CIP (7%), LEV (21%), CFM (21%), CTX (21%), CAZ (21%) and CRO (7%).

Marked sensitivity was observed with GM, ER, CIP, LEV, CTX, CAZ and CRO while highest sensitive pattern was noted with IMP. Maximum resistance was observed with AMP, TZP and TET.

Antibiotic sensitivity pattern of Pseudomonas aeruginosa

Sensitivity pattern of P. aeruginosa for various drugs (antibiotics) is shown in Fig 14. P.aeruginosa showed sensitivity to AMP (56%), TZP (44%), GM (56%), ER (78%), TET (67%), IMP (100%), CIP (100%), LEV (78%), CFM (33%), CTX (44%), CAZ (56%) and CRO (56%).While P. aeruginosa showed resistance against AMP (44%), TZP (44%), GM (44%), ER (22%), TET (33%), LEV (22%), CFM (56%), CTX (33%), CAZ (33%) and CRO (33%) and intermediate sensitivity for TZP (11%), CFM (11%), CTX (22%), CAZ (11%) and CRO (11%).

Marked sensitivity was observed with AMP, GM, ER, TET, LEV, CAZ and CRO while highest sensitive pattern was noted with IMP and CIP. Maximum resistance was observed with CFM.

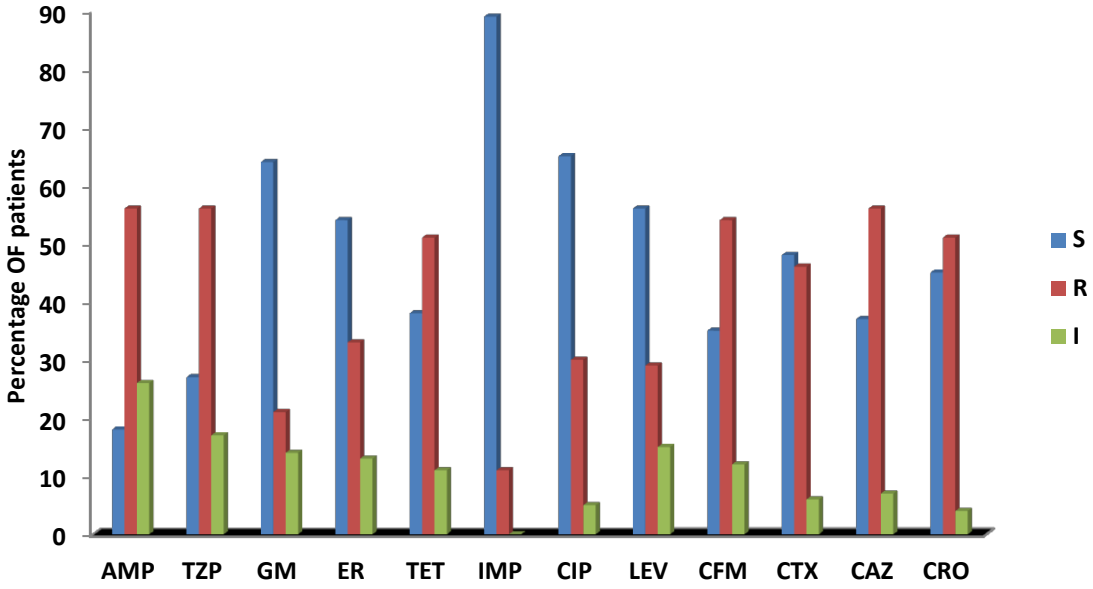


Fig:9 Antibiotic sensitivity pattern to single isolate of E coli indicating sensitivity (S), resistance (R), and intermediate sensitivity (I) pattern to different drugs belonging to various groups, AMP (Ampicillin), TZP (Pipracilli/Tazocin), GM (Gentamycin), ER (Erythromycin), TET (Tetracyclin), IMP (Imepenum), CIP (Ciprofloxacin), LEV (Levofloxcin), CFM (Cefixime), CTX (Cefotaximine), CAZ (Ceftazadime), CRO (Ceftraxone). Greater sensitivity observed with GM, ER, IMP, CIP, LEV, CTX and CRO. Greater resistance was observed with AMP, TZP, TET, CFM, CAZ and CRO.

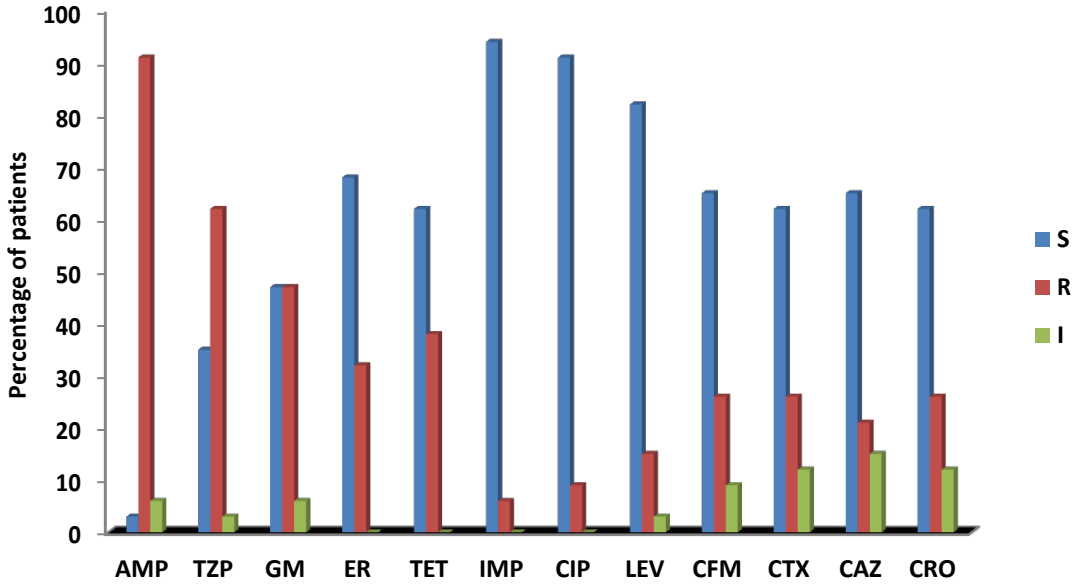


Fig: 10: Antibiotic sensitivity pattern to single isolate of Klebsiella spp indicating sensitivity (S), resistance (R) and intermediate sensitivity (I) pattern to different drugs belonging to various groups, AMP (Ampicillin), TZP (Pipracilli/Tazocin), GM (Gentamycin), ER (Erythromycin), TET (Tetracyclin), IMP (Imepenum), CIP (Ciprofloxacin), LEV (Levofloxcin), CFM (Cefixime), CTX (Cefotaximine), CAZ (Ceftazadime), CRO (Ceftraxone). Greater sensitivity was observed with ER, TET, IMP, CIP, LEV, CFM, CTX, CAZ and CRO. Greater resistance was observed with AMP and TZP.

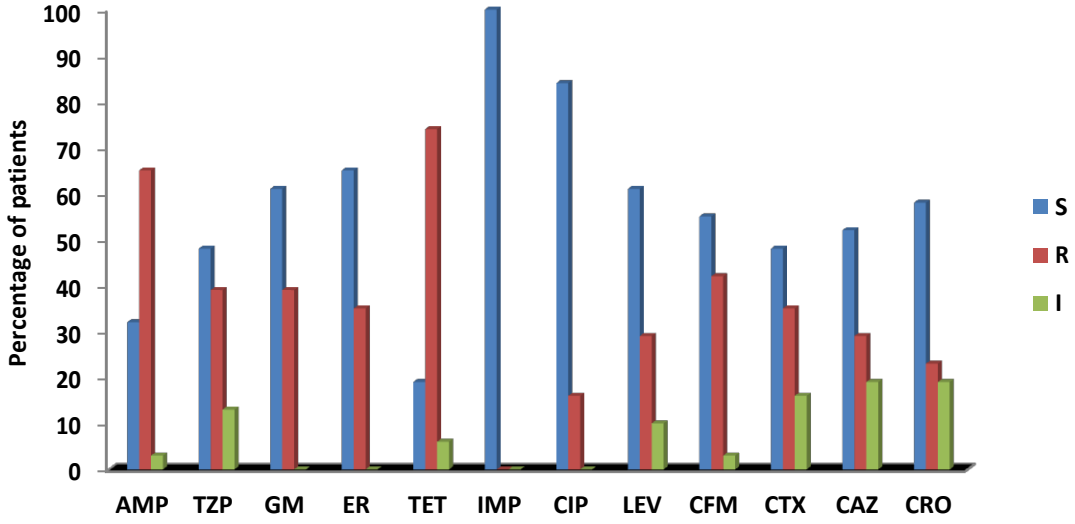


Fig: 11: Antibiotic sensitivity pattern of N.gonorrhea, a sexually transmitted organism to various groups of drugs for sensitivity (S), resistance (R) and intermediate sensitivity (I) pattern. AMP (Ampicillin), TZP (Pipracilli/Tazocin), GM (Gentamycin) ER (Erythromycin), TET (Tetracyclin) IMP (Imepenum), CIP (Ciprofloxacin), LEV (Levofloxcin), CFM (Cefixime), CTX (Cefotaximine), CAZ (Ceftazadime), CRO (Ceftraxone). Greater sensitivity was observed with GM, ER, IMP, CIP, LEV, CFM, CTX, CAZ and CRO. Greater resistance was observed with AMP and TET.

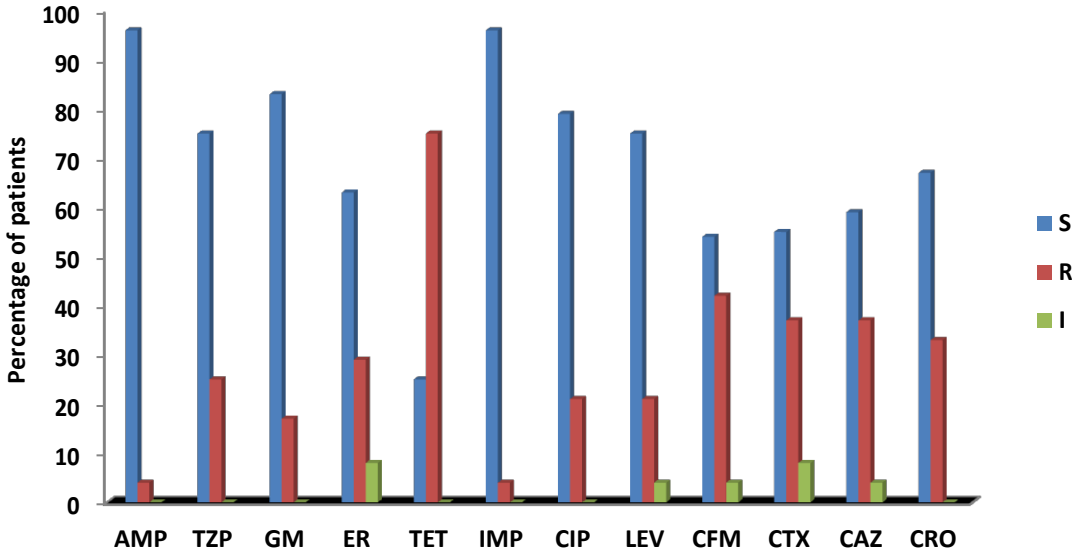


Fig:12: Antibiotic sensitivity pattern to single isolate of S. agalactiae indicating sensitivity (S), resistance (R) and intermediate sensitivity (I) pattern to different groups of drugs, AMP (Ampicillin), TZP (Pipracilli/Tazocin), GM (Gentamycin), ER (Erythromycin), TET (Tetracyclin), IMP (Imepenum), CIP (Ciprofloxacin), LEV (Levofloxcin), CFM (Cefixime), CTX (Cefotaximine), CAZ (Ceftazadime), CRO (Ceftraxone). Greater sensitivity was observed with AMP, TAZ, GM, ER, IMP, CIP, LEV, CFM, CTX, CAZ and CRO. Greater resistance was observed with TET.

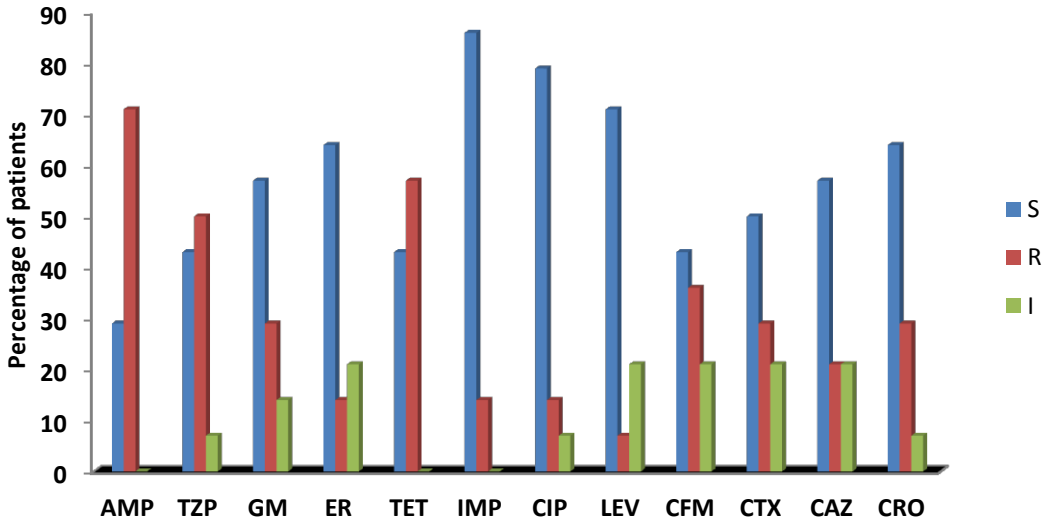


Fig: 13: Antibiotic sensitivity pattern of S. aureus indicating sensitivity (S), resistance (R) and intermediate sensitivity (I) pattern to different drugs belonging to different groups, AMP (Ampicillin), TZP (Pipracilli/Tazocin), GM (Gentamycin), ER (Erythromycin), TET (Tetracyclin), IMP (Imepenum), CIP (Ciprofloxacin), LEV (Levofloxcin), CFM (Cefixime), CTX (Cefotaximine), CAZ (Ceftazadime), CRO (Ceftraxone). Greater sensitivity was observed with GM, ER, IMP, CIP, LEV, CTX, CAZ and CRO. Greater resistance was observed with AMP, TAZ and TET.

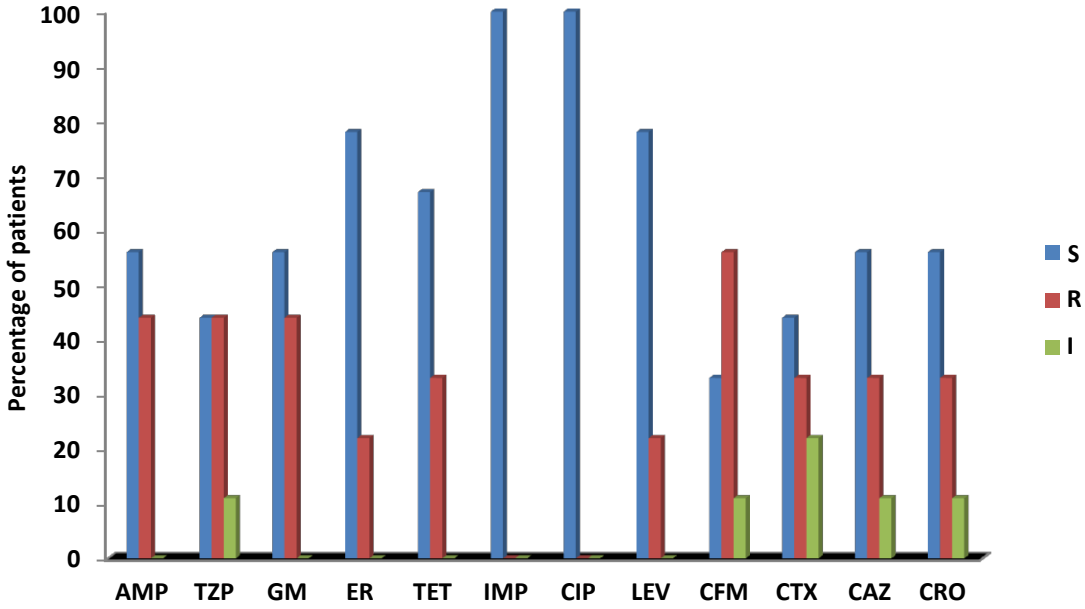


Fig: 14: Antibiotic sensitivity pattern of P. aeruginosa indicating sensitivity (S), resistance (R) and intermediate sensitivity (I) pattern to different drugs belonging to different groups, AMP (Ampicillin), TZP (Pipracilli/Tazocin), GM (Gentamycin), ER (Erythromycin), TET (Tetracyclin), IMP (Imepenum), CIP (Ciprofloxacin), LEV (Levofloxcin), CFM (Cefixime), CTX (Cefotaximine), CAZ (Ceftazadime), CRO (Ceftraxone). Greater sensitivity was observed with AMP, GM, ER, TET, IMP, CIP, LEV, CAZ and CRO. Greater resistance was observed with CFM.

Antibiotic sensitivity pattern of mixed growth.

E coli with Candida spp

Sensitivity pattern of mixed growth of E.coli and Candida spp for various drugs (antibiotics) is shown in Fig 15. The organism showed sensitivity to AMP (65%), TZP (75%), GM (65%), ER (70%), TET (35%), IMP (95%), CIP (100%), LEV (95%), CFM (75%), CTX (75%), CAZ (65%) and CRO (85%).While E coli in combination showed resistance against AMP (35%), TZP (15%), GM (35%), ER (30%), TET (65%), IMP (5%), LEV (5%), CFM (25%), CTX (15%), CAZ (10%) and CRO (15%) and intermediate sensitivity for TZP (10%), CTX (10%), CAZ (25%).

Marked sensitivity was observed with AMP, TZP, GM, ER, CFM, CTX, and CAZ while highest sensitive pattern was noted with IMP, CIP, LEV and CRO. Maximum resistance was observed with TET.

S. agalactica with Candida spp.

Sensitivity pattern of mixed growth of S agalactiae and Candida spp for various drugs (antibiotics) is shown in Fig 16. E coli showed sensitivity to AMP (100%), TZP (91%), GM (82%), ER (73%), TET (45%), IMP (91%), CIP (82%), LEV (73%), CFM (45%), CTX (36%), CAZ (18%) and CRO (27).While E coli showed resistance against GM (9%), ER (27%), TET (36%), IMP (9%), CIP (9%), LEV (27%), CFM (55%), CTX (45%), CAZ (73%) and CRO (55%) and intermediate sensitivity for TZP (9%), GM (9%), TET (18%), CIP (9%), CTX (9%), CAZ (9%) and CRO (18%).

Marked sensitivity was observed with ER, LEV while highest sensitive pattern was noted with AMP, TZP, GM, IMP and CIP. Maximum resistance was observed with CFM, CAZ and CRO.

S. aureus with Candida spp

Sensitivity pattern of mixed growth of S.aureus and Candida spp. for various drugs (antibiotics) is shown in Fig 17. E coli showed sensitivity to TZP (33%), GM (33%), ER (67%), TET (67%), IMP (50%), CIP (100%), LEV (50%), CFM (67%), CTX (67%), CAZ (17%) and CRO (67%).While E coli showed resistance against AMP (100%), TZP (67%), GM (67%), ER (17%), TET (17%), IMP (33%), LEV (33%), CFM (33%), CTX (17%), CAZ (67%) and CRO (17%) and intermediate sensitivity for ER (16%), TET (16%), IMP (17%), LEV (17%), CTX (16%), CAZ (16%) and CRO (16%).

Marked sensitivity was observed with ER, TET, IMP, LEV, CFM, CTX and CRO while highest sensitive pattern was noted with CIP. Maximum resistance was observed with AMP, TZP, GM and CAZ.

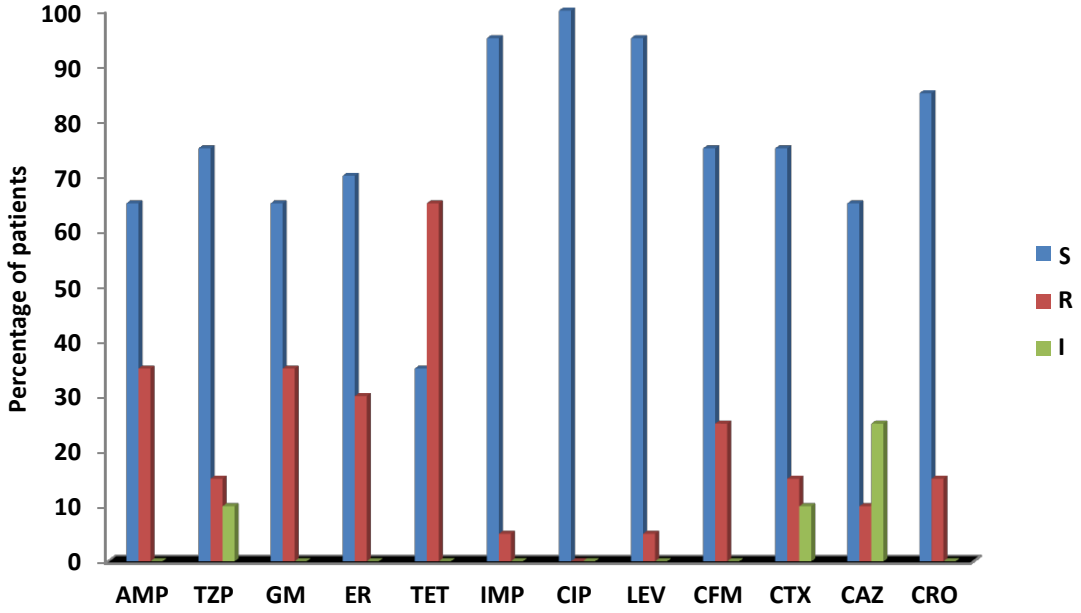


Fig: 15: Antibiotic sensitivity pattern of E. coli with Candida spp., mixed growth indicating sensitivity (S), resistance (R) and intermediate sensitivity (I) pattern to different drugs belonging to different groups, AMP (Ampicillin), TZP (Pipracilli/Tazocin), GM (Gentamycin), ER (Erythromycin), TET (Tetracyclin), IMP (Imepenum), CIP (Ciprofloxacin), LEV (Levofloxcin), CFM (Cefixime), CTX (Cefotaximine), CAZ (Ceftazadime), CRO (Ceftraxone). Greater sensitivity was observed with AMP, TZP, GM, ER, IMP, CIP, LEV, CFM, CTX, CAZ and CRO. Greater resistance was observed with TET.

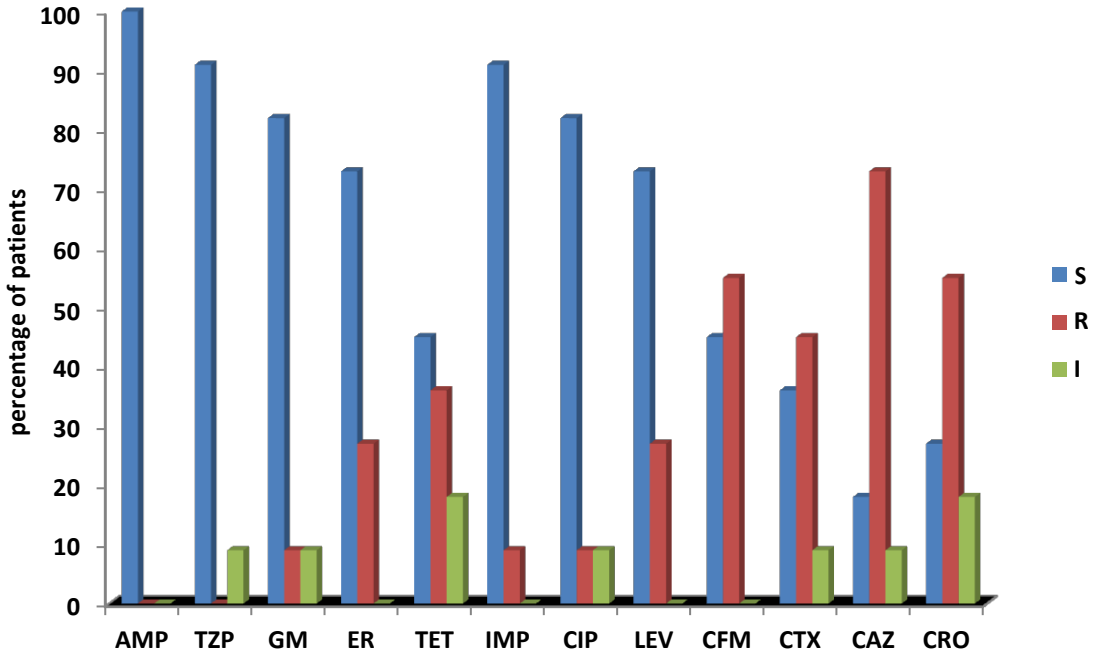


Fig: 16: **Antibiotic sensitivity pattern of mixed infection of S. agalactica with Candida spp. sensitivity (S), resistance (R) and intermediate sensitivity (I) pattren to different groups of drugs, AMP (Ampicillin), TZP (Pipracilli/Tazocin), GM (Gentamycin), ER (Erythromycin), TET (Tetracyclin), IMP (Imepenum), CIP (Ciprofloxacin), LEV (Levofloxcin), CFM (Cefixime), CTX (Cefotaximine), CAZ (Ceftazadime), CRO (Ceftraxone). Greater sensitivity was observed with AMP,TZP, GM, ER, IMP, CIP and LEV. Greater resistance was observed with CFM, CTX, CAZ and CRO.**

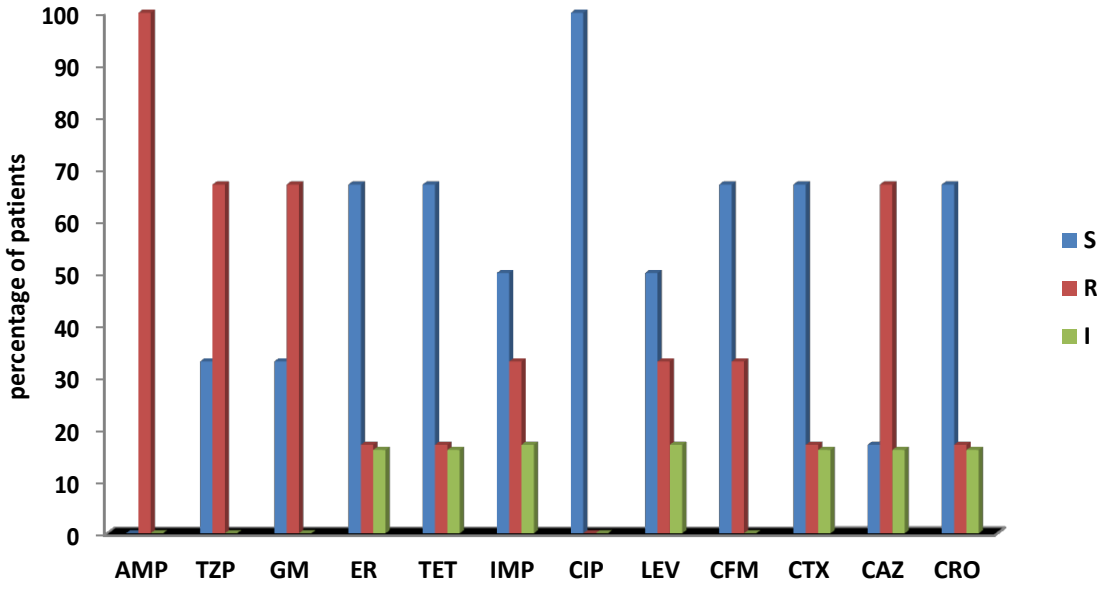


Fig: 17: **Antibiotic sensitivity pattern of mixed growth of S. aureus with Candida spp indicating sensitivity (S), resistance (R) and intermediate sensitivity (I) pattern to different drugs, AMP (Ampicillin), TZP (Pipracilli/Tazocin), GM (Gentamycin), ER (Erythromycin), TET (Tetracyclin), IMP (Imepenum), CIP (Ciprofloxacin), LEV (Levofloxcin), CFM (Cefixime), CTX (Cefotaximine), CAZ (Ceftazadime), CRO (Ceftraxone). Greater sensitivity was observed with ER, TET, IMP, CIP, LEV, CFM, CTX and CRO. Greater resistance was observed with AMP, TZP, GM and CAZ.**

Effect of menstrual cycle, hygiene practices and associated features on vaginal discharge

Effect of menstrual cycle, hygienic practices and various related features associated on vaginal discharge in patients with different bacterial and fungal infection is shown in Table 24.

Phase of cycle (Discharge)

A through history about the increase or decrease of vaginal discharge was assessed in different phases of menstrual cycle. Pattern of Vaginal discharge was recorded in more than one phase of menstrual cycle in each patient. Most of the patients, 88.55% (n=294) complained for excess of vaginal discharge in the luteal phase, of which 52.40% (n=174) patients had bacterial infection. The complaint of excessive vaginal discharge decreased in the follicular phase (51.20%) and the ovulatory phase (37.34%). In patients suffering from Candidiasis and mixed vaginal infection complained of lesser amount of discharge in all three phases of menstrual cycle. The distribution of vaginal discharge due to different bacterial and fungal infections during different phases of menstrual cycle was not significant. ($\sum \chi^2_{(6)}$= 2.56; P>0.90).

Sanitary Pads

The use of various types of sanitary pads in vaginal infection was an important factor. It was observed that highest percentage of patients 52.71% (n=175) who used cloth had high percentage of bacterial 31.32% (n=104) infection and less percentage of fungal 8.73% (n=29) infection. On the other hand those who used cotton 28.61 % (n=95) and Always 18.67% (n=62) as sanitary pads had less amount of infection. Bacterial infection was prevalent among patients using different type of sanitary pads. However, use of Always showed that bacterial infection was in the least number of patients as compared to the use of cloth and cotton. Bacterial infections was not significant due to use of any type of sanitary pads. ($\sum \chi^2_{(6)}$= 5.05; P>0.50).

Hygienic conditions (Bath and Changing cloths)

Bath taking habit among the patients varies. Some were taking bath daily 24.39% (n=81), others twice a week 35.84% (n=119) and still others once a week 39.75% (n=132). Highly significant ($\sum \chi^2_{(6)}$= 18.12; P<0.001) infection with different organism's (candidiasis, bacterial and mixed) was observed due to different bathing and clothes changing habits. The highest percentage of infection was observed in those patients who changed clothes once a week 39.75%. Those who changed clothes twice a week were 35.84% and the lowest percentage was of those who would change clothes along with bath taking daily were 24.39%.

Associated features

In addition to sanitary pads and the hygienic conditions other associated features were related to the infection (Table 24). More than one associated feature causing an increase in the discharge was observed in a patient. Majority of the patients 77.71% (n=258) complained of increased vaginal discharge related to coitus. Standing and strenuous work was another feature related to increased vaginal discharge (76.80%; n=255). Least percentage of patients was observed to have vaginal discharge in excitement 26.20% (n=87) and tension and anxiety 24.36% (n=81). There was no significant difference among associated features ($\sum \chi^2_{(9)}$= 1.58; P>0.9).

Table 24: Effect of menstrual cycle, hygiene practices and associated features on vaginal discharge in patients with no growth, candidiasis, bacterial infections and mixed growth (non sexually transmitted infection).

Number of Patients (n=332)	No Growth			Candidiasis			Bacterial Infection			Mixed Growth			Total Patient		χ^2
	O	E	%	O	E	%	O	E	%	O	E	%	n	%	
Menstrual Cycle															
follicular phase	19	19.01	5.72	32	30.83	9.63	98	99.41	29.51	21	20.74	6.32	170	51.20	
ovulatory phase	10	13.87	3.01	24	22.49	7.22	74	73.18	22.28	17	15.13	5.12	124	37.34	$\sum \chi^2_{(6)}=$
luteal phase	35	32.00	10.54	51	53.68	15.36	174	173.81	52.40	34	36.12	10.24	294	88.55	2.56; P>0.90
Sanitary Pads															
cloth	26	21.06	7.83	29	30.57	8.73	104	101.31	31.32	16	19.51	4.81	175	52.71	
cotton	9	12.31	2.71	19	16.59	5.72	56	56.08	16.86	11	10.58	3.31	95	28.61	$\sum \chi^2_{(6)}=$
always	6	8.03	1.80	10	10.83	3.01	36	36.61	10.84	10	6.91	3.01	62	18.67	5.05;P>0.05
Hygenic Conditions (Bath)															
everyday	8	10.49	2.40	14	14.15	4.21	49	47.81	14.75	10	8.53	3.01	81	24.39	
twice a week	7	15.41	2.10	17	20.78	5.12	82	70.25	15.66	13	12.54	3.91	119	35.84	$\sum \chi^2_{(6)}=$
once a week	26	16.01	7.83	27	23.06	8.13	65	77.92	19.57	14	13.91	4.21	132	39.75	18.12; P>0.001**
Changing Clothes															
everyday	8	10.49	2.40	14	14.15	4.21	49	47.81	14.75	10	8.53	3.01	81	24.39	
twice a week	7	15.41	2.10	17	20.78	5.12	82	70.25	24.69	13	12.54	3.91	119	35.84	$\sum \chi^2_{(6)}=$
once a week	26	16.01	7.83	27	23.06	8.13	65	77.92	19.57	14	13.91	4.21	132	39.75	18.12; P>0.001**
Associated Features															
coitus	32	32.96	9.63	45	44.71	13.55	155	151.91	46.68	26	28.41	7.83	258	77.71	
standing/strenuous work	33	32.57	9.93	45	44.18	13.55	149	150.15	44.87	28	28.08	8.43	255	76.80	$\sum \chi^2_{(9)}=$
excitement	12	11.11	3.61	15	15.07	4.51	48	51.22	14.45	12	9.58	3.61	87	26.20	1.58; P>0.90
tension/anxiety	10	10.34	3.01	13	14.03	3.91	49	47.61	14.75	9	8.92	2.71	81	24.36	

n=number of patients; %=Percentage

O=Observed number; E=Expected number

Recurrent infections

Past history of patient depicted recurrent infections in patients with vaginal discharge is shown in Table 25. A thorough history of the patient for the number of episodes of the vaginal infection, its duration and any treatment taken for the infection was recorded.

3.15.1 Number of episodes

It was observed that majority of the patients presenting with vaginal infection had a predominance of similar bacterial infections 59.03% (n=196) in the past. Number of episodes 1-2 was observed in 21.68% (n=72) and in patients with 3-4 episodes was observed in 21.08% (n=70) with bacterial infection. Greater number of patients in all types of infection had complaint of 1-2 and 3-4 episodes with dominance of bacterial infection. Patients presenting with repeated episodes of infection and those with history of no previous episode of infection were reported in only a small percentage. Highly significant infection with different organisms was observed due to number of previous episodes of infection ($\sum \chi^2_{(9)} = 17.85$; $p<0.03$).

3.15.2 Duration of infection

Most of the patients with vaginal discharge had complaints for last 0-3 years. Majority 30.72% (n=102) had bacterial infection while Candidiasis was observed in 9.33% (n=31) and mixed infection 5.42% (n=18) was seen in a small number of patients. Low percentage of patients 5.72% (n=19) presented with no growth. Patients with a longer duration of infection were less in number. Higher percentage of patients with bacterial infection 15.36% (n=51), who presented for the first time had no history of previous infection. There was no significant difference among patients with variable duration of infection

Previous treatment history

Most of the patients with recurrent episodes of infection under repeated treatment 18.97% (n=63) were suffering from bacterial infection. Patients who had one course of treatment were 13.25% (n=44). Patients who never had any previous course of treatment were 25.60% (n=85). Patient who had received treatment in mixed infection

Table 25: Past history of recurrent infection, number of episodes, duration and any previous treatment in patients with Candidiasis, Bacterial and mixed vaginal infections.

No. Patients (n=332)	No Growth			Candidiasis			Bacterial infection			Mixed Growth			χ^2
No of Episodes	**O**	**E**	**%**	**O**	**E**	**%**	**O**	**E**	**%**	**O**	**E**	**%**	
No Previous Infection	10	9.01	3.01	11	12.75	3.31	45	43.09	13.55	7	8.13	2.10	
One to two	11	14.07	3.31	21	19.91	6.32	72	67.31	21.68	10	12.70	9.01	$\sum \chi^2_{(9)}=$
Three to four	14	13.83	4.21	15	19.56	4.51	70	66.12	21.08	13	12.48	3.91	17.85; P<0.03*
Chronic Repeated	6	4.07	1.80	11	5.76	3.31	9	19.48	2.71	7	3.67	2.10	
Duration													
One month to three yrs	19	20.99	5.72	31	29.69	9.33	102	100.36	30.72	18	18.94	5.42	
Four to six yrs	3	4.32	0.90	5	6.11	1.50	24	20.66	7.22	3	3.91	0.90	
Seven to ten yrs	8	5.55	2.40	10	7.86	9.01	19	26.56	5.72	8	5.01	2.40	$\sum \chi^2_{(9)}=$
First time at Presentation	11	10.12	3.31	12	14.32	3.61	51	48.4	15.36	8	9.13	2.40	7.99; P>0.53
Treatment													
Once	6	9.01	1.80	13	12.75	3.91	44	43.09	13.25	10	8.13	9.01	
Twice	1	0.74	0.30	1	1.04	0.30	4	3.54	1.20	0	0.00	0.00	$\sum \chi^2_{(9)}=$
Repeated	14	11.73	4.21	22	16.59	6.62	63	56.08	18.97	16	10.58	4.81	5.71; P>0.76
No Treatment	20	17.04	6.02	22	24.11	6.62	85	81.46	25.60	11	15.37	5.76	

O=Observed number; E=Expected number; %=Percentage

and Candidiasis were also less in number. Majority of patients with history of recurrent infections (candidiasis, bacterial and mixed infection) had not had any treatment. There was no significant difference among infection and previous treatment.

Sexually transmitted infections

Prevalence of sexually transmitted infection, based on the symptoms and clinical observations, in the hospital study population who gave their consent to give blood to undergo tests for sexually transmitted infection is shown in Table 26. Sexually transmitted infections in the study group showed very high prevalence of Chlamydia trachomatis IgG in 36.81% and IgM in 39.01% patients. Gonococcal infection was observed in only 9.33% of patients.

Table 26: Distribution of sexually transmitted infections number and percentage of patient with vaginal discharge.

Sexually transmitted Infections	n	%
Gonorrhoea	31/332	9.33
Chlamydia trachomatis IgG	67/182	36.81
Chlamydia trachomatis IgM	71/182	39.01

Mixed sexually transmitted infection

After evaluating the results obtained, a mixed pattern (Chlamydia IgG and IgM and Chlamydia with Gonococci) of infections was observed in the patients (Table 27). Chlamydia trachomatis IgG and IgM both were present in 20.87% (n=38) of patients with vaginal discharge. It was noticed that infection with Gonorrhoeae and Chlamydia trachomatis IgG and IgM in combination was present in less number of patients 5.49% (n=10) and 7.69% (n=14) respectively. Least number of patients were observed 4.94% (n=9) with combination of all three infections.

Table 27: Number and percentage of mixed sexually transmitted infections in hospital study population (n=182).

Mixed Infection	**n**	**%**
Chlamydia trachomatis IgG + IgM	38	20.87
Chlamydia trachomatisIgG + Gonorrhoeae	10	5.49
Chlamydia trachomatis IgM + Gonorrhoeae	14	7.69
Chlamydia trachomatis IgG + IgM + Gonorrhoeae	9	4.94

Chlamydia trachomatis (STI) IgG and IgM

Chlamydia trachomatis, a sexually transmitted infection, was assessed for IgG and IgM in serum of the patients with vaginal discharge, which is shown in Table 28.

Age group

IgG and IgM in the serum was observed according to age groups of the patients. It became apparent from the results that majority of patients positive with IgG 17.50% (n=32) and IgM 15.93% (n=29) were in the age groups of 22-26 years and IgG 11.53% (n=21) and IgM 9.89% (n=18) in the age group 27-31 years respectively. With increasing age, the number of patients who presented with Chlamydia trachomatis, positive for IgG and IgM in the serum decreased dramatically. No patient was positive in the older age group of the study population. Similarly in the younger age group less number of positive patients was observed. IgG and IgM among various age groups was not significant.

Educational status

According to educational status majority of patients positive for IgG 10.43% (n=19) and IgM 11.53% (n=21) had matric level education. Second highest percentage was observed in patients positive for IgG 11.96% (n=21) and IgM 8.79% (n=16) were graduates and patients with no schooling presented with IgG 7.69% (n=14) and IgM 8.79% (n=16) respectively. The less percentage of patients with middle and intermediate level education were observed. In post graduate patients only 1 or 2 were positive for IgG and IgM. IgG and IgM in patients with various levels of education was not significant

Economic Status

It was observed that patients with low income group were affected more both with IgG 16.48% (n=30) and IgM 17.50% (n=32) with sexually transmitted Chlamydial infection. The infection decreased with an increase in the financial level, and lesser number of infected patients were observed in the high income group IgG 9.34% (n=17) and IgM 7.14% (n=13). The economic status according to various groups was not significant.

Table 28: Distribution of serum IgG and IgM of Chlamydia trachomatis infection (sexually transmitted infection) in patients with discharge according to the age group, educational and economic status.

	IgG			IgM			
no.of Patients (n=182)	**67/182**			**71/182**			**χ^2**
	O	**E**	**%**	**O**	**E**	**%**	
Age Group							
17-21	9	11.65	4.94	15	12.34	8.24	
22-26	32	29.61	17.50	29	31.38	15.93	$\sum \chi^2_{(4)}$=
27-31	21	18.93	11.53	18	20.06	9.89	3.01; P>0.40
32-36	5	6.79	2.74	9	7.21	4.94	
37-42+	0	0.00	0.00	0	0.00	0.00	
Educational Status							
No Schooling	14	14.56	7.69	16	15.43	8.79	
Middle	7	9.22	3.84	12	9.77	6.59	$\sum \chi^2_{(5)}$=
Matric	19	19.42	10.43	21	20.57	11.53	2.49; P>0.70
Intermediate	5	4.36	2.74	4	4.63	2.19	
Graduate	21	17.96	11.53	16	19.03	8.79	
Post Graduate	1	1.45	0.54	2	1.02	1.09	
Economic Status							
Low (5000-10,000)	30	30.11	16.48	32	31.89	17.50	$\sum \chi^2_{(2)}$=
Middle (10,000-15,000)	20	22.33	10.98	26	23.66	14.28	1.27; P>0.50
High (> 16,000)	17	14.56	9.34	13	15.43	7.14	

O =Observed value; E=Expected value; %=percentage

Symptoms and clinical observations in patients with Chlamydia trachomatis infection

Symptoms and clinical observations were correlated in patients positive for Chlamydial trachomatis IgG, IgM and elementary bodies is shown in Table 29. Chlamydial elementary bodies were observed on giemsa stained direct smear and IgG and IgM in patient serum. Elementary bodies were observed in 29.12% (n=53) patients and serum IgG was positive in 36.81% (n=67) and IgM in 39.01% (n=71) patients with Chlamydia trachomatis.

Symptoms

Patient positive for IgG and IgM presented equally with the symptoms of discharge. The common Chlamydial infection symptoms shown by most of the patients was low back ache was observed in 31.31% (n=57) patients with IgG and 31.86% (n=58) patients with IgM while 31.31% (n=57) patients with elementary bodies. While generalized unwell feeling was observed in 29.12% (n=53) with IgG, 30.21% (n=55) with IgM and 20.87% (n=38) with elementary bodies in patients respectively. Rash/itching was commonly observed in patients with IgG 28.57% (n=52), IgM 25.82% (n=47), and elementary bodies 21.97% (n=40) respectively. Dysparunia and intermenstural bleeding was the least commonly observed symptom. No symptoms were observed in 5-7% patients. The symptoms were evenly distributed among the patients.

Clinical observations

Clinical observation included the color and consistency of vaginal discharge and the condition of the cervix was examined for appearance, friability and tenderness.

Color of vaginal discharge

Whitish color of the discharge was the most frequent observation amongst patients positive for IgG 14.83% (n=27), IgM 13.18% (n=24) and elementary bodies 11.53% (n=21). Patients with translucent and normal discharge were observed in a lesser number while the least number of patients were observed with yellowish discharge. Color of vaginal discharge was not significant.

Consistency of vaginal discharge

Thick and homogenous discharge was most frequent amongst the majority of the patients IgG 17.03% (n=31), IgM 14.28% (n=26) and elementary bodies 13.73% (n=25). Watery and normal vaginal discharge was observed in less percentage. Consistency of vaginal discharge was not significant for IgG and IgM.

Appearance of cervix and speculum examination

Majority of patients positive for IgG 27.47% (n=50), IgM 25.82% (n=47) and elementary bodies 23.62% (n=43) presented with a red and swollen cervix. Patients with ectopic cervices were found in a lesser number while patients with a healthy cervix were in least number. Cervical friability was also observed in patients with IgG 21.97% (n=40), IgM 24.72% (n=45) and elementary bodies 19.23% (n=35). Cervical motion tenderness was 18% in IgG and IgM positive patients. IgG, IgM and elementary bodies for various cervical conditions was not significant.

Table 29: Number and percentage of patients with Chlamydia trachomatis infection for serum IgG, IgM and elementary bodies in epithelial cells in relation to the symptoms and clinical observation regarding vagina and cervix.

	Chlamydia trachomatis									
	IgG			IgM			Elementry body			
Patients Characteristics	**67/182**			**71/182**			**53/182**			χ^2
Symptoms	**O**	**E**	**%**	**O**	**E**	**%**	**O**	**E**	**%**	
Low Backache	57	57.67	31.31	58	58.20	31.86	44	43.12	24.17	
Un-well Feeling	53	52.95	29.12	55	53.44	30.21	38	39.59	20.87	
Dysparunia	33	34.26	18.13	34	33.31	18.68	24	24.68	13.18	$\sum \chi^2_{(10)}=$
Intermenstural Bleeding	13	12.33	7.14	13	12.44	7.14	8	9.22	4.39	1.25; P>0.99
Rash/Itching	52	50.41	28.57	47	50.88	25.82	40	37.69	21.97	
No Symptom	10	11.60	5.49	13	11.71	7.14	9	8.67	4.94	
Clinical Observation										
Color of Vaginal Discharge										
Whitish	27	25.25	14.83	24	26.76	13.18	21	19.97	11.53	
Yellowish	10	10.17	5.49	9	10.78	4.94	10	8.04	5.49	$\sum \chi^2_{(6)}=$
Translucent	15	15.78	8.24	19	16.72	10.43	11	12.48	6.04	2.27; P>0.89
Normal	15	15.78	8.24	19	16.72	10.43	11	12.48	6.04	
Consistency of Vaginal Discharge										
Thick and Homogenous	31	28.76	17.03	26	30.48	14.28	25	22.75	13.73	
Watery	17	18.59	9.34	21	19.70	11.53	15	14.70	8.24	2. $\sum \chi^2_{(4)}=$
Normal	19	19.64	10.43	24	20.81	13.18	13	15.53	7.14	20; P>0.69
Appearance of the Cervix										
Healthy	5	6.13	2.74	9	6.55	4.94	4	5.31	2.19	$\sum \chi^2_{(4)}=$
Red and Swollen	50	65.23	27.47	47	50.95	25.82	43	41.30	23.62	2.03; P>0.72
Ectopy	34	35.12	18.68	39	37.49	21.42	30	30.38	16.48	
Speculum Examination										
Cervical Friability	40	41.49	21.97	45	43.73	24.72	35	34.76	19.23	$\sum \chi^2_{(2)}=$
Cervical Motion Tenderness	34	32.50	18.68	33	34.26	18.13	27	27.23	14.83	0.20; P>0.90

O=Observed number; E=Expected number; %=percentage

Unfolding of vaginal infections

Vaginal infections which were characterized as single infection, sexually transmitted infection (STI), bacterial infection, fungal infection and co infection, were correlated to the patients literacy level and husband's monthly income (Table 30 and 31).

Data was arranged in priority of patient's husband income in relation to their educational level which was school education and college education. School education was further characterized as no schooling (can read), middle (upto grade 8) and matric (upto grade 10). College education was further characterized as intermediate (grade 12), graduate and post graduate level.

Economic status

Patients were divided into three groups based on their husband's economic status. The study has been conducted in a public sector hospital, all of the patients fall under the category of low income. For the purpose of description patients have been divided into the following three categories according to their financial levels. Patients whose husband's monthly income was Rs 5000-10,000 were 42.16% (n=140) categorized as low income group. Patients with husband's monthly income Rs 11,000-15,000 were 31.32% (n=104) categorized as middle income group and patients with husband income Rs 16,000-20,000+ were 26.50% (n=88) categorized as high income group.

Educational status

Patients categorized according to their husband's financial level were further divided according to their own level of education. Majority of patients 20.78% (n=69) falling in low income group had no schooling. As the education level increased, the number of patients in low income group decreased. Patients falling at the educational status of college level were only 1.80% (n=6) with intermediate level and 2.40% (n=8) at graduate level.

In the middle income group patients with no schooling were 4.81% (n=16), with middle level education were 7.53% (n=25) and matric level were 9.63% (n=32). The number of patient's with college level education was more as compared to previous low income group. Patients with intermediate level education were 5.72% (n=19),

graduate level education were 5.42% (n=18) and even post graduate level was observed in 1.20% (n=4).

As the income of the patients increased, as seen in the high income group, the number of patients falling under the category of only school level education decreased and patients with college level education increased. Patients with no schooling were only 0.90% (n=3), and the number patients with schooling was also less. At college level education majority of patients fell under the graduate level 18.67% (n=62) and few fell under the intermediate level education, their percentage being 3.01% (n=10). Only one post graduate patient was observed. School and college level education in accordance with economic status was highly significant ($\sum \chi^2_{(2)}$= 121.4; P<0.0001).

Single vaginal infections

Chlamydial infection

Patients were calculated for the Chlamydial infection, a sexually transmitted infection, according to the husband's economic status and patient's educational level (Table 30). Majority of patients with Chlamydial infection were observed in low income group. Patients with no schooling were 1.20% (n=4) and matric level were 1.80% (n=6) and only one patient was with middle level education. While amongst college level education only one graduate patient was positive for Chlamydial infection. Amongst the middle income group, again, more infection was observed in patients with no schooling and school level education, while at college level education only two patients , one with intermediate and one with graduate degree were observed. In high income group, a decrease in sexually transmitted infection was observed. Only one patient with school level education and 0.90% (n=3) patients at graduate level were identified with Chlamydial infection. Highly significant Chlamydial infection in accordance with educational level and income status was seen ($\sum \chi^2_{(2)}$= 7.11; P<0.02).

Gonococcal infection

Gonococcal infection, a sexually transmitted infection, as a single infection was observed in a small number of patients. Majority of patients infected with gonococci were observed in low income group with school level education. Patients with no

schooling were 1.80% (n=6) and with middle level education were 0.60% (n=2). No patient with infection was observed at college level education. Similarly patients in middle income group patients were observed only in no schooling 0.60% (n=2) and middle level education 0.90% (n=3). In the high income group again a small percentage of 0.60% was positive in the school and college level education. It was observed that as the economic and educational level increased the number of patients presenting with gonococcal infection decrease. Even distribution of patients was observed according to educational and economic status.

Bacterial infection

Bacterial infection was the commonest among various infections. It was observed that more bacterial infections were present in the low income group patients with 9.63% (n=32) with no schooling. With college level education small number of patients was observed. In the middle income group equal number of patients was observed in the school level and college level education. The number of infected patients decreased as the level of education increased. In the high income group the percentage of patients with bacterial infection decreased except in the graduates where more patients were observed 6.62% (n=22). Bacterial infection was highly significant ($\sum \chi^2_{(2)}$= 46.58; P<0.0001) in accordance with the economic status and the educational status.

Fungal (Candida spp) infection

Candidiasis presented in a similar manner in both the low income and the middle income groups. Candidiasis was observed in 2.71% (n=9) patients with no schooling, this percentage decreased at college level education and only one graduate was observed. Similarly in the middle income group number of patients observed 1.80% (n=6) with middle level education and small number was seen with no schooling and matric level education. In the higher income group infection was observed more 3.91% (n=13) in graduates. Candida infection was highly significant among economic and educational status groups ($\sum \chi^2_{(2)}$=24.82; P<0.0001).

Table 30: Unfolding of single vaginal infections according to the economic status and educational status.

		Single Infection														
Economic Status		**Educational Status**			**Chlamydia**			**Gonococci**			**Bacterial**			**Candida**		
		O	**E**	o/t)	**O**	**E**	o/t)	**O**	**E**	o/t)	**O**	**E**	o/t)	**O**	**E**	o/t)
	No Schooling	69	67.50	20.78	4	3.66	1.20	6	6.00	1.80	32	31.01	9.63	9	8.43	2.71
n=l40	**Middle**	25	29.70	7.53	I	1.83	0.30	*2*	2.00	0.60	12	13.68	3,61	3	3.75	0.90
5000 to	**Matric**	32	28.80	9.63	6	5.50	1.80	0	0 00	0 00	8	7.29	2.4	3	2.81	0.90
10,000	**lntermidiate**	6	7.50	1.80	0	0.33	0.00	0	0.00	0.00	*2*	2.98	0.60	0	0.56	0.00
	Graduate	8	3.30	2.40	1	0.16	0.30	0	0.00	0.00	3	1.31	0.90	▮	0.25	0.30
	Post Graadute	0	3.20	0 00	0	0.50	0 00	0	0 00	0 00	0	0.7	0 00	0	0.18	0 00
	No Schooling	16	21.2	4.81	1	1.50	0.30	*2*	2.22	0.60	5	7.3	1.50	▮	2.66	0.30
n=I04	**Middle**	24	25.44	7.22	*2*	2.25	0.60	3	1.66	0.90	5	6.82	1.50	6	5.33	1.80
10,000 to	**Matric**	23	16.35	6.92	3	2.25	0.90	0	1.11	0.00	9	4.87	2.71	3	2.00	0.90
15,000	**Intermidiate**	19	13.79	5.72	1	0.50	0.30	*2*	1.77	0.60	10	7.69	3 01	3	1.33	0.90
	Graduate	18	16.55	5.42	1	0.75	0.30	0	1.33	0.00	9	7.17	2.71	*2*	2.66	0.60
	Post Graduate	4	10.64	1.20	0	0.75	0 00	*2*	0.88	0.60	1	5.12	1.20	0	1.00	0 00
	No Schooling	3	2.21	0.90	0	0.00	0.00	0	0.50	0.00		1.33	0.30		0.13	0.30
n=88	**Middle**	4	11.25	1.20	0	0.75	0.00	*2*	1.50	0.60	1	5.11	0.30	0	0.86	0.00
16,000 to	**Matric**	8	1.53	2.40	I	0.25	0.30	0	0.00	0.00	6	1.55	1.80	0	0.00	0.00
20,000	**Intermidiate**	10	10.78	3 01	0	0 00	0 00	1	0.50	0.30	5	4.66	1.50	1	1.86	0.30
	Graduate	62	54.75	18.67	3	2.25	0.90		1.50	0.30	22	17.88	6.62	13	12.13	3.91
	Post Graduate	▮	7.46	0.30	0	0.75	0.00	0	0.00	0.00	▮	5.44	0.30	0	0.00	0.00
	x2	LX^2 = 121.4; P<O. 000 I***			LX^2 = 7.11; P<0.02*			LX^2 = 5.21; P>0.07			LX^2 =46.58; P<O. 000 I***			LX^2 =24.82; P<O. 000 I***		

O=Observed number; E=Expected number; %=Percentage

Combination of vaginal infections (Co-infection)

Bacterial + Candida spp

Bacterial and Candida spp. infection in combination were more in the low and middle income groups (Table 31). Infection was observed more in the patients with only school level education in the low and middle income group. Patients with no schooling were 1.50% (n=5), matric level was 1.80% (n=6) and only one patient with middle level education in low income group was observed. In the middle income group less number of patients was observed in both school level and college level education. While only one or two patients were observed with college level education category. In the higher income group the percentage of infection was even less 1.80% (n=6) in graduates with no school level patient. Combined infection of bacterial and Candida spp. was highly significant in accordance with educational status and husband income ($\sum \chi^2_{(2)}$=15.87; P<0.0004).

Bacterial + Chlamydia trachomatis (STI)

Percentage of patients with a combination of bacterial and Chlamydial infection was equally distributed in lesser percentage among the school and college level education in all the economic groups with the exception of graduates, 3.61% (n=12) in the higher income group. Combined bacterial and Chlamydial infection was highly significant ($\sum \chi^2_{(2)}$=10.07; P<0.006) among the educational and economic groups.

Chlamydial and Gonococcal

It was present in a comparatively higher percentage in the low income group patients with no schooling 0.90% (n=3) and school level education 0.60% (n=2) in both middle and matric level education. As the level of education and economic status increased, the infection rate decreased. Hence no patient in the higher income group was observed positive for the infection.

No infection

Majority of the patients without any infection were observed in the lower income group and majority with no schooling 2.71% (n=9). While in the middle and higher

income group lesser number of patients presented equally. Patients with no infection among economic and educational groups was highly significant ($\sum \chi^2_{(2)} = 15.13$; $p<0.0005$).

Hence the present result revealed that as the level of education and the financial status of the population increased, the level of awareness also increased. This in turn lead to a decrease in the percentage of population presenting with any type of infection.

Table 31: Unfolding of vaginal co-infections according to the economic status and educational status.

Economic Statns		Co-Infection											
		Bacterial+ Candida			Bacterial+ chlamydia			Chlamydia+ Gonococci			No infection		
		O	E	%	O	E	%	O	E	%	O	E	%
	No Schooling	5	4.28	1.50	I	1.55	0.30	.)	3.88	0.90	9	9.28	2.71
n=140	Middle	I	2.57	0.30	I	1.55	0.30	2	1.55	0.60	.)	2.78	0.90
5000 to	Matric	6	5.14	1.80	5	3.88	1.50	2	1.55	0.60	I	0.92	0.30
10,000	Tntermidiate	0	0.71	0.00	I	0.44	0.30	2	I.II	0.60	I	0.71	0.30
	Graduate	2	0.42	0.60	1	0.44	0.30	0	0.44	0.00	0	0.21	0.00
	Post Graadute	0	0.85	0.00	0	I.II	0.00	0	0.44	0.00	0	007	0.00
	No Schooling	3	3.00	0.90	2	1.16	0.60	0	0.00	0.00	2	3.00	0.60
n=104	Middle	4	4.50	1.20	I	2.91	0.30	1	1.00	0.30	2	1.50	0.60
10,000 to	Matric	2	1.50	0.60	4	2.91	1.20	0	0 00	0 00	2	1.50	0.60
15,000	lntermidiate	1	1.00	0.30	0	0.83	0 00	0	0 00	0 00	2	1.00	0.60
	Graduate	2	1.50	0.60	4	2.08	1.20	0	0.00	0.00	0	0.50	0.00
	Post Graduate	0	0.50	0 00	1	2.08	0.30	0	0 00	0 00	0	0.50	0 00
	No Schooling	0	0 00	0 00	1	0.14	0.30	0	0 00	0 00	0	0.25	0 00
n=88	Middle	0	0.00	0.00	0	1.71	0.00	0	0.00	0.00	1	0.75	0.30
16,000 to	Matric	0	0.00	0.00	1	0.14	0.30	0	0.00	0.00	0	0.00	0.00
20,000	lntermidiate	1	1.00	0.30	0	0.85	0.00	0	0.00	0.00	2	1.75	0.60
	Graduate	6	6.00	1.80	12	10.28	3.61	0	0.00	0.00	5	5.25	1.50
	Post Graduate	0	0.00	0.00	0	0.85	0.00	0	0.00	0.00	0	0.00	0.00
		k^2	=15 87;	k^2	=10 07;			k^2	=15 13;				
	XZ	P<0.0004***			P<0.006**			NA			P<0.0005***		

O=Observed number; E=Expected number; %=Percentage

Conception outcome

A thorough history of previous conception and the out-come of pregnancy was recorded from 332 patients. It was observed from the history that 258 (77.71%) patients conceived and 74 (22.28%) had no history of conception. Patient number was calculated for the outcome of conception which can be live births or pregnancy loss (abortion, miscarriage, still birth and ectopic pregnancy) as shown in Table 32. The number and percentage of patients in each category was calculated on the basis of the number of patients who conceived (n=258) and the number of total conceptions (n=780). Percentage of live births and the percentage of pregnancy loss was the main objective of this calculation. Of total patients conceived with live birth were 78.68% (n=203) with 64.61% (n=504) conceptions for live births and 35.38% (n=276) conceptions with pregnancy loss with highest percentage of abortions 22.30% (n=174).

There was not much difference observed between the live births 1.66% (n=13) and pregnancy loss 1.28% (n=10) in Patients with Chlamydial infection. In patients with Gonococcal infection percentage of live births 7.05% (n=55) were more than the pregnancy loss 4.61% (n=36) with highest percentage of abortions 3.07% (n=24). All the above infections play an important role in the conception outcome. Chlamydial and Gonococcal infection in combination presented with 2.69% (n=21) live births with 1.92% (n=15) pregnancy loss with majority of abortions.

Patients with combined infection, sexually transmitted (Chlamydial or gonnococal) with bacterial and fungal (Candida) infections presented with 8.58% (n=67) live births and 5.25% (n=41) pregnancy loss. Maximum percentage of live births 44.61% (n=348) was observed in patients with bacterial and Candida infection while pregnancy loss was also highest in these patients which was 22.30% (n=174). Among these patients majority had abortions 14.10% (n=110) and miscarriage, still birth and ectopic pregnancy were less in number.

Table 32: Number and percentage of conception and its outcome : in patients with Chlamydial, Gonococcal infection alone, in combination and along with Bacterial and Candida infections and combination of Bacterial and Candida infection.

Conception Outcome	Total				Chlamydial (STI)				Gonococcal (STI)				Chlamydial+ Gonococcal (STI)				STI+Bacterial+ Candidia				Bacterial+ Candida			
	Patient		Conception		Patient		Conception		Patient		Conception		Patient		Conception		Patient		Conception		Patient		Conception	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Total Conceptions	258		780		6	2.32	23	2.94	21	8.13	91	11.70	10	3.87	36	4.61	44	17.05	108	13.84	177	68.60	522	66.92
Live births	203	78.68	504	64.61	5	1.93	13	1.66	14	5.42	55	7.05	9	3.48	21	2.69	33	12.79	67	8.58	142	55.03	348	49.23
Pregnancy Loss																								
Abortions	104	40.31	174	22.30	3	1.16	5	0.64	13	5.03	24	3.07	5	1.93	9	1.15	22	8.52	26	3.33	61	23.64	110	14.10
Miscarriage	24	9.30	29	3.71	2	0.77	2	0.25	1	0.38	1	0.12	1	0.38	1	0.12	2	0.77	2	0.25	18	6.97	23	2.94
Still Birth	26	10.07	40	5.12	2	0.77	2	0.25	2	0.77	2	0.25	2	0.77	2	0.25	4	1.55	9	1.15	16	6.20	25	3.20
Ectopic Pregnancy	29	11.24	33	4.23	1	0.38	1	0.12	7	2.71	9	1.15	3	1.16	3	0.38	4	1.55	4	0.51	14	5.42	16	2.05
Total Pregnancy Loss	183	70.93	276	35.38	8	3.10	10	1.28	23	8.91	36	4.61	11	4.26	15	1.92	32	12.40	41	5.25	109	42.24	174	22.30

n=number ; %=percentage

Verbal information regarding husbands History

Verbal information regarding the husband's symptoms, sexual partners and any addiction was gathered from the females coming to the outpatient department with vaginal discharge (Table 33). It was observed that females who were suffering from various infections, Chlamydial (36.18%), Gonorrhea (9.33%) and Bacterial vaginosis (42.16%), their husbands had complaints regarding urethral discharge. High percentage of urethral discharge (40.12%) and ulcer on urethra (14.92%) was informed in male partners of patients positive with Chlamydia infection. Of these (22.38%) partners had some kind of treatment for the urethral discharge or ulcer. Of these partners (43.28%) had more than one sexual partner. Majority of female patients with gonorrhea, their husband (58.06%) had complained of urethral discharge and out of these male partners 35.48% had ulcer on urethra and all had treatment for the problem. Of these male partners 83.87% had more than one sexual partner. The lowest percentage of male partner problem was seen in patients with Bacterial vaginosis (37.14%). Of these, 47.14% had more than one sexual partner. Addiction of any kind was not seen in many partners. It was revealed from the verbal information that sexually transmission plays an important role in the three infections, which are considered as sexually transmitted.

Table 33: Verbal information regarding husbands of female patients with different reproductive tract infections

	Chlamydia		Gonorrheae		Bacterial Vaginosis	
	n	%	n	%	n	%
Female patients (Number)	**67/182**	**36.18**	**31/332**	**9.33**	**140/332**	**42.16**
Husbands Symptoms						
Urethral Discharge	27	40.29	18	58.06	52	37.14
Ulcer on Urethra	10	14.92	11	35.48	6	4.28
No Infection	30	44.77	2	9.67	82	58.57
Husbands Treatment						
Urethral Discharge and Ulcer	15	22.38	11	35.48	44	31.42
Husbands Sexual Partner (Two or More)	29	43.28	26	83.87	66	47.14
Addiction (Husband)						
Cigarette	23	34.32	9	29.03	44	31.42
Drugs	2	2.98	2	6.45	-	-
Alcohol	5	7.46	3	9.67	8	5.71

n=number ; %=percentage

DISCUSSION

The present study was conducted on married females in a public sector hospital. The age of participants (332) ranged from 17-42+ years with a mean age of 28.01 ± 0.29 years. They were all symptomatic females with complaints of vaginal discharge. Vaginal discharge in sexually active females is a worldwide problem and is one of the most common reasons for gynecological consultation. Symptomatic women's health care visits in United States estimated were 6-10 million annually, recorded during 2004 and 2006 (Kent et al., 1991; Owen and Clenney, 2004; Nancy et al., 2009). In women with vaginal discharge bacterial vaginosis is the most frequent cause of vaginitis and has been associated with severe complications (Schmidt, and Hansen, 2000; Hilmarsdottir et al., 2006).

Symptoms and clinical observations

Patients generally complained of a combination of vaginal discharge with irritation, odor, increased amount, color and consistency which do not match the normal conditions. (Anderson et al., 2004). The most frequent complaints compelling a patient to report to Health Care Facility is vaginal discharge and malodor. Only patient with vaginal discharge were included in the study and 55.72% were with mal-odor vaginal discharge. Various studies conducted showed variable results depending on the type of population selected like married females, college students, pregnant females and sex workers. Taylor-Robinson et al., (2003) and Anderson et al., (2004) reported 50% patients with mal-odor vaginal discharge and with increased discharge in 76.5%. The most frequent symptom reported by Donders et al., (2002); Manavi et al., (2004) and Geisler et al., (2004) was vaginal discharge in 87% of patients and with vaginal odor in 28-40% patients. Complaint in this study of ma-lodor is strongly associated with BV, this was also confirmed by whiff test ($P<0.0001$). Absence of mal-odor vaginal discharge increases the likelihood of candidiasis and STI (Anderson et al, 2004). Frequently reported symptom among the study patients was low backache 84%, lower abdominal pain 82% along with rash and itching 71% and combination of these complaints 53.61%. Investigators (Steinhandler et al., 2002; Geisler et al., 2004; Manavi et al., 2004) showed abdominal pain in 13-54% and genital itching in 32% patients but no information regarding low backache is available. Dysparunia in 51% and intermenstural bleeding 14% patients and

combination of these two in 8.73% of patients was noted. These finding indicate vaginitis (57.83%) and cervical friability (21.97) as sign of inflammation. These symptoms were observed in lesser percentage 4-13.8% by Brunhamn et al., (1984); Sellors, (2000); Marrazzo et al., (2002); Geisler et al., (2004). They further considered that the previous symptoms were diagnostic for STI and bacterial vaginitis If the diagnosis of vaginitis/vaginosis is based only on the patient complaints, history, symptoms and clinical examination the accuracy of the diagnosis will be unacceptably low (Holmes et al., 1999; Ledger, 1999; Mardh et al., 2002). In present investigation various colors of vaginal discharge were noted in the outpatient department as whitish, translucent, yellowish and clear along with thick, homogenous, watery and viscous consistency. According to different investigators (Fule et al., 1990; Chandeying et al., 1998; Sellors et al., 2000; Anderson et al., 2004) a thick, curdy white discharge is predictive of candidasis. Females with clear (normal) but increased discharge are less likely to have bacterial vaginosis than the females with moderate to profuse discharge. A white discharge makes bacterial vaginosis less likely. They also observed that bloodstained green, clear, purulent and frothy discharge as uncommon with bacterial vaginosis. A yellow homogenous discharge increases the likelihood of bacterial vaginosis and STI. All patients with STI had homogenous yellow to opaque blood stained discharge. Present investigation showed that patients with bacterial vaginosis were found to have white to translucent, homogenous, foul smelling vaginal discharge. Similar result was also observed with candidiasis presenting a whitish thick, homogenous foul smelling discharge with signs of cervical inflammation and friability. The present investigation observed post-coital bleeding and friability of cervix to bleed on touch in bacterial vaginosis, candidiasis, chlamydial and Gonococcal infection. Which has also been shown by other scientist (Livengood et al., 1990; Ryu et al., 1999; Anderson et al., 2004), has also been suggested by ACOG (2006) to make testing options cost effective so that the diagnosis could be made on the basis of patient ‘s history and physical examination. Signs of inflammation were observed in the females attending the hospital as red swollen cervix alone, cervix with ectopy and healthy cervix with ectopy. Similar findings were also observed by Geisler

et al., (2004) on examination as vaginal erythema and cervical tenderness, friability with inflammation of cervix.

Difficulties do arise when diagnosis is based only on patient's symptoms and clinical findings. Anderson et al., (2004) are of the opinion that precision of vaginal symptoms refers to the degree to which an observer finds the same results when a relevant test is applied. Mostly basis of diagnosis is through symptoms and clinical observations of the patients. However, Gutman et al., (2005); Landers et al., (2004) have highlighted inaccuracies in the clinical diagnosis based on the symptoms and clinical observations of these common clinical problems when compared to traditional gold standards for both symptomatic and asymptomatic women. Patients selected were from the lowest range of socioeconomic level, in spite of this all required tests as planned were undertaken which usually are not done in the Out-patient Department. However symptoms and examination findings from the patients were also considered for diagnosis. In the investigation diagnosis is based both on clinical tests as well on symptoms and clinical examination.

Bacterial vaginosis: Amsel criteria and Nugent scoring system

Prevalence

The accuracy of the clinical signs/ observations does not help distinguishing between the various conditions of vaginitis/ bacterial vaginosis. More than 60% of the patients with vaginitis/bacterial vaginosis have vaginal discharge. Determining the prevalence of bacterial vaginosis is difficult because one third to three quarters of affected women are asymptomatic (Sobel, 1990; Hay, 1998; Koumans et al., 2007). In addition, reported prevalence varies based on the population studies. Accurate epidemiologic data on the reproductive tract infections (RTI) are scarce and existing information varies regarding prevalence that ranges from 20% to 70% (Goto et al., 2005; Bahram, et al., 2009). In comparison with similar studies, the results from this study were 42% bacterial vaginosis and 17% Candiadiasis confirmed above authors findings. In a study conducted in the rural area of Shandong province in China, the prevalence of BV and candidiasis were 6.6 and 3.9% respectively significantly low as

compared to the present study prevalence (Fang et al., 2007). In a study performed in Hamedan province (Iran), the prevalence of candidiasis and BV was 17.2, and 28.5%, respectively (Shobeiri et al., 2006). Among women referred to hospital in Vientiane, the capital of Laos, the prevalence of BV and candidiasis were 24.5 and 39.5% respectively (Sihavong et al., 2007). While, in the rural area of Northeast Brazil, 20% of women had BV and 12.5% candidiasis (Oliveira et al., 2007). Bacterial vaginosis has been found in 15 to 19 percent of ambulatory gynecology patients, 10 to 30 percent of pregnant patients and 24 to 40 percent of patients in sexually transmitted disease clinics (Hill, 1983; Hill, 1993). This clinical condition has a high and varied prevalence, depending on the surveyed population in 2007, varying from 4% in developed countries to 61% in the third world countries, with a mean prevalence of 14% considering developed and developing regions (Bahram et al., 2009). In USA prevalence of BV is 26-37% while in European countries 4-37% of BV cases in general population were observed (Numanovic et al., 2008). National and international comparisons are hampered because of the different methodology of studies. The majority of scientists investigated the prevalence of each organism separately (Hart et al., 1993; Mead, 1993; Konje., et al., 1991), while others, only the high risk population groups (Gerting et al., 1997). Okonko et al., (2012) in a recent study in Nigeria showed that in young females with the complaint of vaginal discharge the incidence of bacterial vaginosis was 11.5% followed by vaginal candidiasis 27%. Study conducted in Military Hospital Pakistan (Azaz et al., 2005) the frequency of BV using Amsel's criteria (Amsel et al.,1983) was found low 11.3% as compared to the present observation. While a similar kind of study conducted in Railway Hospital, Pakistan had prevalence of BV similar to the study population. (Khan et al., 2009). The reason for very low BV in Military Hospital could be due to the effect of higher education and higher socioeconomic status, a different class of patients. Contrast is obvious in Railway Hospital where mostly patients with low educational level with lower range of economic level were tested However, in both hospitals only Amsel criteria was applied and other methods were not used. Educational and economic level plays an important role and this was also observed in this study. However, survey conducted by National AIDS control program (2005) revealed 47% BV among sex workers in Pakistan.

Amsel and Nugent scoring for BV

The sequence of events concludes with the development of BV causing alteration in the physiological vaginal flora, still need to be determined (Linhares et al., 2010). The goal of this study was to describe the vaginal flora related with bacterial vaginosis/vaginitis, both by clinical signs, (Amsel criteria), and laboratory methods (Nugent scoring). The traditional diagnosis of bacterial vaginosis has been clinical, requiring three of four criteria in the evaluation of vaginal discharge (Amsel., 1983). These criteria include pH > 4.5, an amine or fishy odor (positive Whiff test), homogeneous discharge and clue cells on microscopy (Krohn et al., 1992). A clue cell is a squamous epithelial cell with obscured border by adherent bacteria (Sweet, 1985). In the present study all patients with symptoms and clinical findings were followed by the application with Amsel clinical analysis. Thick and homogenous discharge was observed in (45.18%) females. While the presence of clue cells (37.34%) indicated greater numbers of organisms, including Gardnerella, which is indicative of vaginal infection (Hans et al., 2010). PH>4.5 was observed in 57.22% and whiff test was positive in 40.66% patients. Considering the different parameters of Amsel criteria, the diagnosis of BV by vaginal pH and whiff test showed a 100% sensitivity which was considered the best criteria in Amsel clinical analysis for the diagnosis of BV (Coppolillo et al., 2003). Considering this the use of vaginal pH and whiff test was also observed in the study patients and about 40.66% were suffering from BV which is nearly equivalent to Nugent scoring where the positive patients were 42%. If all four criteria are considered then 8.73% patients showed BV and by using three parameters then 15.96% positive BV patients were observed. This is quite low for the symptomatic patients. Clinical signs are difficult to standardize between clinicians and impossible to interpret (Nugent et al., 1991). Amsel clinical criteria are often misdiagnosed as the components are subjective. However, microscopy of a Gram stained smear has been both sensitive and specific in the diagnosis of BV/ vaginitis. In the present study the results of Nugent scoring were more accurate as compared to Amsel clinical criteria as the slides were confirmed by an expert microbiologist. Microscopy results, using standard diagnostic method (Nugent scoring) which is widely used are comparable with other studies in demonstrating the dynamic nature of the microbial population of the vaginal flora. (Hay et al., 1992). Nugent et al, (1991)

the gram stain appears to be better and widely used method to diagnose bacterial vaginosis. The nugent scoring system appears to be reliable, convenient and cost effective and requires least time and most interpretative method for laboratory evaluation of patients with bacterial vaginosis. Additionally the gram stained method helps to identify associated finding such as presence of yeast or PMN seen in acute vaginitis or STI (Joesoef et al., 1991; Numanovic et al., 2008).

Notably, in the study data approximately 76% by Amsel criteria, 58% by Nugent scoring and 12% by culture sensitivity method, symptomatic women remained undiagnosed after clinical evaluation. According to Landers (2004), more than 26% and 21% respectively of the women were found free of any laboratory identifiable BV or infections despite the presence of clinical symptoms. These findings were also consistent with those of others who have reported normal flora by laboratory standard (Gutman et al., 2005), and the absence of a diagnosis for a significant percentage (30-35%) of symptomatic women (Anderson et al., 2004). Laboratory findings in both the current study (42%) and that by Landers et al, 2004 (46%) showed the highest incidences for BV, followed by Candidiasis 28% and 29% respectively. Similarly in India prevalence of 20-48% BV on Gram stained method was diagnosed. The laboratory, interpretation of the gram stain by Nugent scoring, is considered standardized method (Tohill et al., 2004). Various studies conducted in which the diagnosis of bacterial vaginosis (BV) was made in 22 to 50% of symptomatic women and candidiasis vaginitis in 17 to 39% women and the reproducibility with which gram stained slides were interpreted showed excellent results (Mazzulli et al., 1990; Joesoef et al., 1991; Forsum et al., 2002). Some investigators found discrepancies in Nugent Scoring which may be influenced by variation in the method of fixation, different sampling devices used, various methods to collect sample, variance in the sample collection site of vagina, variation in the homogeneity and thickness of smear, and tendency of old lactobacilli to appear gram variable. (Forsum et al., 2002; Mohanty et al., 2010)

Age Factor

BV demonstrates a striking age profile as there is a strong association of BV with age over 25 years (Sewankambo et al., 1997). In the present study BV was 9.80% in the age group 22-26 years all four and all three Amsel criteria combined while it

decreased with increasing age to 6% in the age group 27-31years and 32-37 years. A very low percentage of BV was observed in older group 37-42 years (0.90%). However, other studies showed a significant correlation between BV and different age groups. (Allsworth et al., 2007; Oliveira et al., 2007). The present study does not show significant relationship between age and decrease in BV ($b=-0.57\pm1.29$; $F(1,3)=0.17$;$P=0.70$. Larsson et al., (2007) observed frequency of BV 16% in age group 18-21years and 8% in age group 31-35 years. The argument that BV increases with age comes from STI clinics as BV is not sexually transmitted and STI usually occur in younger age groups (Larsson et al., 2005). Bartolomeo et al., (2002) found 23.8% BV and 17.8% candidiasis in adult women but in adolescents 17.8% BV and 29.7% Candidiasis were observed. However there was no association between prevalence of BV and age (Bhalla et al, 2007). Interestingly young women undergoing in vitro fertilization treatment had significantly higher BV infection (Wilson et al., 2000). The present study Indicates that females in reproductive age group are more prone to BV infection, the reason could be multiple pregnancies and poor hygienic conditions.

Polymorphnuclear neutrophils

Bacterial vaginosis was considered as a non inflammatory syndrome. Several studies have demonstrated the presence of vaginal leucocytes (white blood cells, PMN) in women with bacterial vaginosis (Eschenbach et al., 1988; Sturum-Ramirez et al., 2000; Steinhandler et al., 2002; Hakakha et al., 2002; Yudin et al., 2003). The discharge usually contains very few white cells although the presence of greater number of white cells does not exclude the diagnosis. Detection of PMN on gram staining is a simple inexpensive mean to assess inflammation of vagina and cervix and an important marker for vaginitis (Geisler et al., 2004). This study demonstrated

that greater number of PMN were related with cervical changes as cervical redness and swelling with ectopy and friability as it bleeds to touch along with its tenderness. Vaginal symptoms were not very marked except for vaginal erythema. Lesser number of PMN can be seen with BV as it stimulates the inflammatory cytokines (Sturum-Ramirez et al., 2000). The cervical signs with PMN are strong predictor of infection, Candidiasis, Chlamydia and gonococcal and aerobic bacterial vaginitis (Sellor et al.1998; Geisler et al., 2004). If PMN is observed on gram stained slide a persumptive diagnosis of gonococcal and Chlamydial infection can be made and presumptive single dose treatment can be given to prevent complications. This can result in re-infection (Sellor et al., 1998). The bacterial interaction with the epithelial cells promotes the PMN influx (Jennifer et al., 2004). In the present study foul smelling copious discharge was observed. A mucopurulent whitish to yellowish vaginal discharge is indicative of STI or candidiasis or BV (Geisler et al., 2004). Pate, (2001) report that mucopurulent discharge and PMN on endocervical swab does not predict Chlamydial infection as it induces only the inflammatory response.

Bacterial infection and drugs

Further Research is going on to determine the occurrence of co-infection of Gardnerella spp. and Streptococcus spp. This co-infection confirmed that it might be due to sexual transmission as the prevalence of Gardnerella spp. in male with women sexual partners with BV have been reported (Schwebke et al., 2009). Meanwhile, it is therefore essential for women to embrace sanitary prophylaxis that prevents the entrance of the E. coli into the vagina from anus. It is also important to avoid indecent sexual habits that contribute to vagina's bacterial load and can in turn lead to difficult-to treat bacteria. Resistant bacteria should be properly included into the routing and diagnostic laboratory. When the patient is not`infected, medical prophylaxis should be avoided as it results in bacterial resistance to infections. When already infected however, antibiotic sensitivity testing should always precede the administration of any antibiotic therapy to avoid abuse (Adegoke et al., 2011). Isolates of E. coli showed prevalence of 22.9% as recovered, while other bacterial species and their frequencies of occurrence include Micrococcus spp. (2.1%), Staphylococcus aureus (12.5%), Streptococcus spp. (2.1%), Gardnerella spp. (20.8%), Lactobacillus spp. (62.5%), and

they exhibited resistance to various antibiotics. It is consistent with the present results as 25% E coli were isolated but the percentage of Staphylococcus was less 4% and streptococcus spp. were 7%. Streptococcal and staphylococcal infections may require treatment, but only if associated with significant leucocytosis. (Margaret, 2001) Streptococcus agalactiae is the most common cause of neonatal sepsis, but vaginal colonization by this organism and its connection with vaginal symptoms is still

controversial. According to Maniatis et al., (1996) Streptococcus agalactiae in symptomatic women with evidence of inflammation are considered a causative agent of vaginitis. Other scientists were of the opinion that patients should not be treated with antibiotics on routine isolation of Streptococcus agalactiae from vaginal swabs in female patients (Shaw et al., 2003; Casari et al., 2010). Moreover, some authors associated Escherichia coli with symptomatic vaginal infections (Gonzales Pedraza et al., 2004). Even the low carriage rate indicates that it is not part of normal indigenous vaginal flora. Antimicrobial resistance in E coli has been reported worldwide and increasing rates of resistance among E coli is a growing concern in both developed and developing countries (Kibret and Abera, 2011). E. coli exhibited 22 to 78% resistance to ampicillin, cotrimoxazole, gentamycin, nitrofurantoin, colistin, tetracycline, nalidixic, ciprofloxacin, ofloxacin were resistant to third generation cephalosporins as treatment options (Aboderin et al., 2009; Ullah, et al., 2009). Similarly results of this study demonstrated that E coli isolates showed greater resistance against conventional drugs. It may be due to the recommendation of drugs to patients without investigations in the laboratory. Due to use of such drugs against bacteria ultimately results in the development of resistance against antibiotics. In the presently studied population profile showed sensitivity to gentamycin (64%), ciprofloxacin (65%), Levofloxacin (56%). Other antibiotics used showed high sensitivity for E coli to imipenum (92%) and erythromycin (54%). Antibiotics are used even where not required along with non compliance of the patients. Due to the easy availability of antibiotics resulting in self medication is also a major problem in our population. Results of antibiotics profile resistance of Streptococcus spp. isolates showed that all isolates (100%) were resistant to Ampicillin and Amoxicillin , whereas resistance in a lesser degree was observed to tetracycline and Gentamicin

(87.5%) and to Cefotaxime and Ciprofloxacin (75%) and to Erythromycin (62.5%). However Sharat, (2004) and Jebur, (2012) observed that all isolates showed high sensitivity (100%) to Amoxiclave, Ampiclox and Tetracyclin antibiotics. Antibiotics which are not commonly used gives higher sensitivity in treatment of vaginitis (Culebras et al, 2002).

Hygiene practices

The composition of the vaginal ecosystem is not static but changes with time and in response to endogenous and exogenous influences. (Schwebke et al., 1999; Eschenbach et al., 2000). Variables include ethnicity, smoking, stage of the menstrual cycle, pregnancy, use of contraceptive agents, frequency of sexual intercourse, specific sexual partners, vaginal douching, use of panty liners, vaginal deodorants, and utilization of antibiotics or other medications with immune or endocrine activities (Eschenbach et al., 2001; Witkin et al., 2007; Amaral et al., 2007; Fethers et al., 2008; Yudin and Money, 2008). The observation showed some of the external factors which potentially altered the environment of the vagina and cause BV seemed to be related to frequent use of scented soap. There appeared to be an additive effect of bathing and changing clothing and other hygienic factors like use of cloth, cotton and Always as sanitary pads for menstrual protection. Patients who were changing cloths and taking bath less frequently (40.36% once a week and 35.84% twice a week) along with the use of cloth (52.71%) and cotton (28.61%) as sanitary pads were more affected with BV. Obviously no firm conclusions can be drawn in view of the small numbers studied. However, the study emphasizes the multi-factorial etiology of BV, and these factors warrant further investigation. This study shows significant ($P<0.001$) correlation between BV and hygiene behaviors. In comparison to other similar studies, it was evident that the lack of hygiene practices was significantly associated with BV in this study population as well as among women in Zanjan (Bahram et al., 2009). Whereas, certain studies contradict this finding (Fang et al., 2007; Allsworth et al., 2007). Demba et al., (2005) also found no association of menstrual hygiene practices, washing, douching, use of soap and water with BV.

Exposure to an altered condition will cause a fluctuation in the local environment and increase or diminish the selective advantage of specific vaginal microbes. The theory that semen is one of a number of factors that alters the environment of the vagina, possibly by raising the pH, and trigger off a change in the flora due to the loss of Lactobacilli is in accordance with the present study as 77.17% complained of increased vaginal discharge after sexual intercourse (coitus) (Priestley et al., 1997; Schwebke et al., 1999). It has been found that sexual intercourse without a condom had no effect on vaginal Lactobacilli but led to elevated levels of Escherichia coli and facultative Gram-negative bacilli (Eschenbach et al., 2001).Present study revealed highest percentage of E coli (25%) along with Klebsiella spp (10%). Based on the previous knowledge it was found that BV is more common in women having unprotected sex, as all females included in this study were married and were not practicing any type of contraceptives. This provides evidence that BV is sexually transmitted (Preistley et al., 1997; Witkins et al., 2007). BV seems to be closely related to sexual intercourse, although not defined as a sexually-transmitted infection, and the explanation behind its high prevalence among sexually inactive women remains vague (Giraldo et al., 2007; Holmes, 1999; Morris et al., 2001). Sexually inactive women are rarely affected. The treatment of male sexual partners has also not shown any beneficial effects on the occurrence of BV (Workowski and Berman, 2006).

Observation showed that the change to the BV type flora is preceded by a rise in pH, suggest that the pH increase is a cause of this condition. Over the course of the menstrual cycle, vaginal levels of hormones and glycogen vary, and menstrual blood alters vaginal pH and provides an environment favorable for many microorganisms. Nevertheless, levels of vaginal Lactobacilli appear to remain constant throughout the cycle. Non-Lactobacillus species increase during the proliferative phase. In this study 89% patients complained of increased vaginal discharge during luteal phase and 57% in follicular phase of the menstrual cycle. Both the Candida and the bacterial infection observed increased towards the menstruation. It was also observed by Eschenbach et al., (2000), that the Candida spp. concentrations are highest towards menstruation. Candidiasis is tolerant of the acidic vaginal environment and is present in the vagina of approximately 10-20% reproductive age group women. The concentration of this

microbe is usually low, and carriage is typically asymptomatic. However, under conditions such as frequent sexual intercourse or induction of a local allergic response causes proliferation of Candida spp. (Witkin et al., 1987; Witkin et al., 2007) resulting in the development of a symptomatic vaginitis.

Recurrent infection

According to Castelleno Filho, et al., (2010), approximately 80% of the treated patients will have another BV episode within one year and 20% within thirty days of treatment. In this study patients with recurrence rate of infection was high (59%) with bacterial infections and 16% with candidiasis and mixed infections. Treatment trials with relevant antibiotics for one week report cure rates of 80-90% but with recurrence rate of 15-30% within three months. Most relapses occur during first year and are related with sexual contacts (Wilson, 2004). Although there is no consensus on the causes of recurrence of BV which explains why some women, even after receiving adequate treatment, do not respond well to drugs effective against anaerobic bacteria (Giraldo, et al., 2007). However, the recurrence of infection was observed even if the sexual partner or partners have undergone treatment. There can be other reasons for recurrence of infection like usage of broad-spectrum antibiotics like ampicillin, tetracylin, cephalosporins and clindamycin as they eradicate the normal vaginal flora, non compliance of drugs and short course of antibiotics (McGroarty et al., 1993; Carr et al., 1998). Recurrent infections can also be due to hygiene habits, vaginal douches, frequency of sexual intercourse, spermicides, IUCD, lack of vaginal immune response, and even lactobacilli contamination with bacteriophages, with the consequent death of protective microbiota and biofilm formation (Ugwumadu, et al, 1997). In candidial infection the recurrence could be due to adherence to epithelial cells, wrong choice of drugs, metronidazole with tetracyclin and local pessaries which have 2-5% absorption (Spiegel et al., 1980; Hilliers et al., 1985; Carr et al., 1998)

Complications

The patients in this study showed a variable picture of complications. A very high pregnancy loss of 35.38% was recorded from the history in all the patients attending the outpatient with vaginal infection. High percentage of 22.30% pregnancy loss was observed in patients with combined infection (bacterial and fungal). Among these patients rate of abortions was as high as 14.10%. The benefits of therapy for females are relief of signs and symptoms of vaginal infection along with the resultant reduction of complications and reduction of other prevalent infections, such as HIV

and other STI. (Workowski and Berman, 2006). However, there is conflicting evidence regarding the benefit of BV treatment in asymptomatic pregnant women for premature delivery (Leitich et al., 2003; Nygren et al., 2008). Nevertheless, several investigators indicated that treatment of high-risk pregnant women reduce the risk for prematurity (Workowski and Berman, 2006). Many scientists studied the role of bacterial vaginosis on conception and miscarriage (Hay PE et al., 1994; Mc Gregor et al., 1995; Hillier SI et al., 1995), and a predisposition to preterm labour, postpartum endometritis and low birth weight infants was demonstrated. In this study of 332 patients 258 (78%) conceived. Total number of pregnancies of conceived patients was 780 (3.02/patient). Total live births were 504 (65%) and pregnancy loss was 276 (35.38%). Number of abortions 174 (22%) was highest among pregnancy loss. The influence of bacterial vaginosis on in vitro fertilization and embryo implantation during assisted reproduction treatment is controversial (Wittemer et al., 2004; Burrello et al., 2004). There is no doubt about the role of Chlamydial infection in the tubal-factor infertility (Paavonen et al., 1999; Akande et al., 2003), but the role of other intracellular microrganisms remains unclear (Imudia et al., 2008; Grzesko et al., 2007).

Syndromic management

Empirical treatment is based not on the laboratory results but on the disease category with which a patient presents. Based on the findings of the clinical trials and surveys the Malawi Government adopted the Syndromic Management Approach to RTI

(Chilongozi et al., 1996) and revised them till 2007 (Reproductive Health Unit, 2007). Syndromic management has its own draw back. The rate of resistance of the causative organisms to various drugs in use is likely to increase rapidly making these drugs ineffective after a short time of use. The increase in resistance due to over-diagnosis and over-use of antibiotic means that there is need to find new and usually more expensive drugs. Syndromic approach remains the first choice in the management of BV and STIs in resource poor settings. In public sector hospitals even drugs are mostly not available and a good laboratory setup is required to meet the demands. Even when it is available free of cost laboratory investigations are not possible. Even in communities where laboratory support is either inadequate or non-existent, the syndromic approach is considered cost-effective. Syndromic management cannot be used to find asymptomatic cases, and thus asymptomatic cases will not be treated (Costello et al., 1998). Similarly in our settings where the patient is poor and the resources are limited, syndromic management is used to treat the patients resulting in the resistance of conventional drugs which are considered cheap and effective.

Economic and educational factors

The educational and economic status of female has strong association with BV and STI. A significant correlation between BV and educational status was evident in Zanjan, Iran (Culhane et al., 2005; Bahram et al., 2009). Similarly, in the present study carried out in public sector hospital population a strong relationship was observed among patients belonging to low income group ($P<0.0004$). It was observed that as the economic status and the educational levels increased the number of females decreased regarding vaginal infections. Similar findings were observed among African population and in public sector health care facility in Argentina where it was observed that loss of job and economical crisis increases BV and STI. (Holzman et al., 2001; Bartolomeo et al., 2002; Demba et al., 2005). Lack of education was found related to BV among women in third world countries, whereas certain studies contradict this finding (Fang et al., 2007; Allsworth et al., 2007).

Sexually transmitted infections

Chlamydia trachomatis and Nesserria gonorrheae are the most common bacterial sexually transmitted infection (STI) worldwide. The World Health Organization estimated in 2001 that per year 92 million new cases of Chlamydia trachomatis infection occur worldwide (WHO, 2001). The World Health Organization has estimated an incidence of 340 million new cases of curable STIs among adults in 1999. Globally STI are on the rise and prevalence rates of C. trachomatis infection in asymptomatic women vary from 0-37% depending on the study population, setting and test methods used. Unexpectedly, high prevalence of up to 17% has been documented for asymptomatic women in Europe (Wilson et al., 2002). Result of this study points towards a high prevalence of C. trachomatis which was 37% but the patients included were all symptomatic and seeking treatment for vaginal discharge.

However prevalence of sexually transmitted infections was nearly 50% positive in South African asymptomatic women and pregnant women (Wilkinson et al., 1999; Rours et al., 2006; Rours et al., 2010). Chlamydial and gonococcal rates detected were 12% and 9%, respectively. National AIDS control program survey in Pakistan (2005) revealed 12% Chlamydial and 11% gonococcal infection among sex workers.

Highest prevalence of STI was shown in young women in their late teens (19%) and early twenties (22%) with a significant decline after age 30 (Rours et al, 2010). In the present symptomatic patient's investigation revealed 17.50% in the age group 22-26 years and 11.53% in 27-31 years age group while the number of positive patients decreased drastically. A similar age distribution has been shown elsewhere and can be explained in part by the natural course of STI infection (Wilkinson et al, 2000; Rollins et al, 2002). Chlamydial infection rates also vary widely among different populations as the patients investigated belonged to public sector and poor patients with low affordability for various tests. Sexual activity with multiple partners and unprotected sex is known to occur more in younger age groups. Gonorrhoea was the least prevalent STI, but the rate detected 9% among the study patients was consistent with those previously recorded by various scientists. Gonorrhoea was also most prevalent in women less than 20 years of age (13%), but no significant differences were recorded between age groups. (Rours et al., 2006; Rours et al., 2010)

The WHO recommends a syndromic approach to the management of STIs in developing countries (WHO, 1991). However, in the study patients typical symptoms for an STI appeared to be insufficiently specific to estimate the risk for an STI. Chlamydial and gonococcal infections 60-70% in women are known to be asymptomatic and detection and subsequent treatment is routinely achieved by antenatal screening (Rours et al 2006). Young women under 25 years of age are mainly at risk for STIs, also poor women are more likely to have numerous sexual partners and therefore to be at increased risk for STIs (Matambo et al., 1999). In the present investigation it was noted as the age and economic status increased the percentage of infected patients also decreased. Rours et al., (2006) also found that socio-economic risk factors, unemployed status, lack of regular income, all are associated with STIs. Co-infection of Chlamydia with gonorrhoea was frequently recorded in women less than 20 years of age. (Donders et al., 1993). In the study patients 3.91% of co-infection (Chlamydia and Gonococci) was observed with majority of younger patients, less educated and with low income status.

Overall, this study confirms the high rate of infection with C. trachomatis and N. gonorrhoeae among symptomatic women. Approach of symptomatic patients is based on socio-demographic factors which prove more effective than mass treatment or dependence on syndromic management principles in women. Basic approaches to decrease the burden of STIs would be early recognition of symptoms associated with chlamydial and gonococcal infections, safe sex, antenatal care and routine screening. Since treatment of chlamydial and gonococcal infections is one of the most cost-effective health interventions available in developing countries in terms of cost, additional screening for chlamydial and gonococcal infection is required at least for women at highest risk (Donders et al., 1993; Rours, 2010). Asymptomatic chlamydial and gonococcal infections result in neonatal infection, complicated pregnancy outcome, post-partum pelvic inflammatory disease and transmission to sexual partners (Rours et al., 2006).

CONCLUSIONS

It can be concluded from the present study, that of the selected symptomatic female patients with vaginal discharge; all patients were found positive either with, bacterial vaginosis / vaginitis or STI (Chlamydial or Gonococcal infection). A correlation between the symptoms, clinical observations and laboratory findings revealed the type and intensity of vaginal infection. The concentrations of various bacterial morphotypes Lactobacilli spp, Mobiluncus spp and Gardnerella vaginalis along with pH, Amine odor and homogenous discharge when correlated revealed BV in 25% patients with Amsel clinical criteria and 42% patients with Nugent's scoring, the most widely used methods. The composition of the vaginal microbiota was analyzed by culturing and sensitivity testing with various antibiotics. Both aerobic and anaerobic cultures revealed most commonly E.coli, Candida spp, Klebsiella spp and N. gonorrheae. Less common were Streptococcus agalactiae, Staphylococcus aureus and Pseudomonas aeruginosa. The sensitivity pattern with various groups of drugs gave better sensitivity with Imepenum, Ciproflloxacin, Levofloxacin and cephalosporins. While less sensitivity with the conventional drugs was observed. It is important to culture the vaginal discharge for the prevalence of frequently involved microorganisms in symptomatic females in a particular area with particular hygiene practices and self medication resulting in alteration in the behavior of microorganisms to antibiotics. The reduction of Lactobacilli and the presence of polymorphnuclear neutrophils increase the chance of sexually transmitted infection. Chlamydia and gonococci are major threat to reproductive health of females. Chlamydial IgG in the serum was positive in 37% patients. Detecting the above vaginal infections at an early stage helps to tackle the fertility problems by improving the re-productive health. This could be an important approach to controlling both vaginal infections and their associated complications, which are costly. Adverse pregnancy outcome in the study population was very high as the total pregnancy loss was 35.38%, with a high percentage of abortions 22.30%.

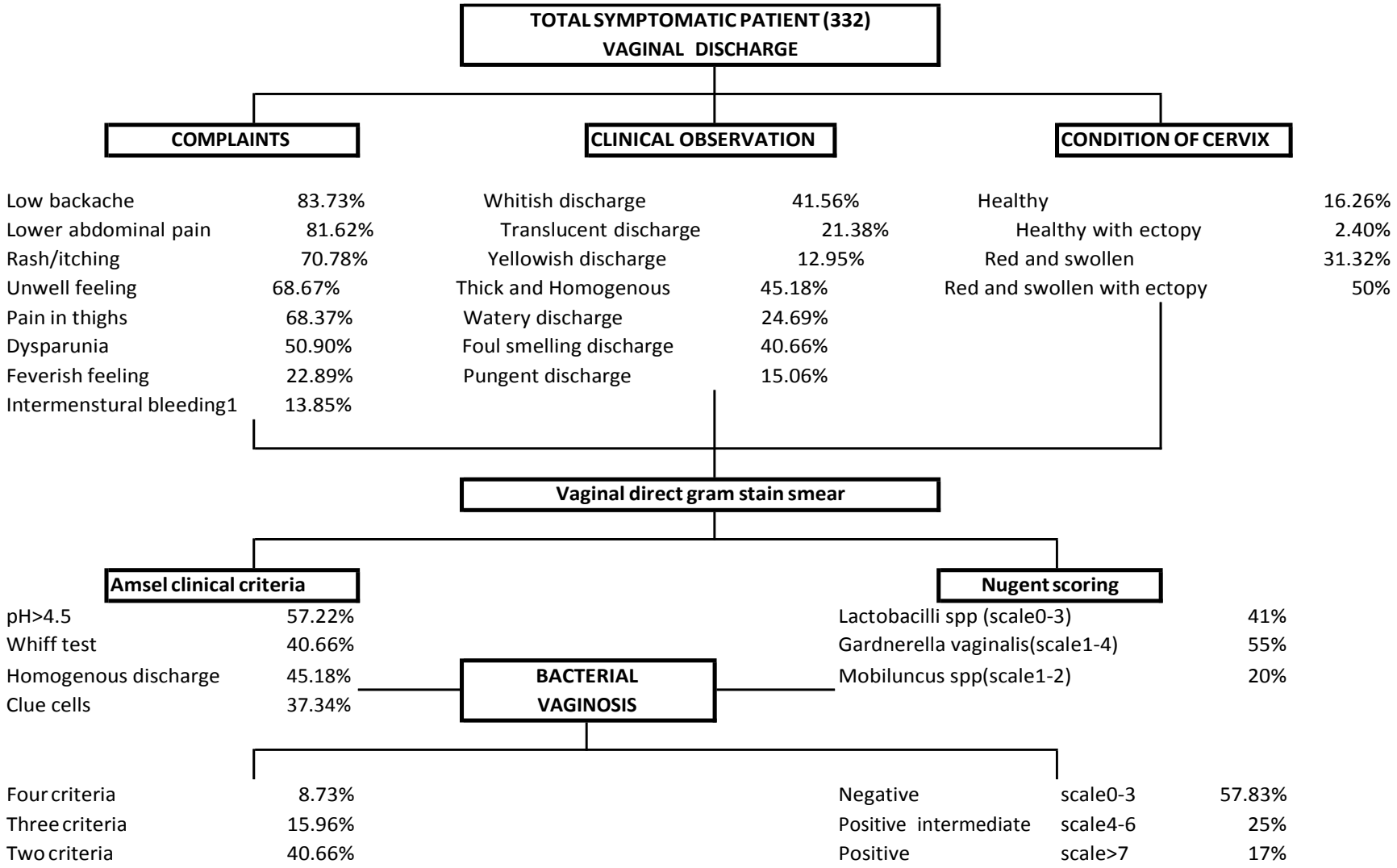


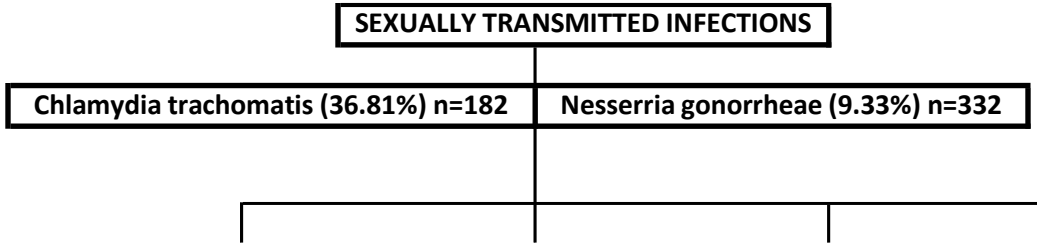
Fig 18: Patients presenting with vaginal discharge presenting with the complaints and their clinical findings assessed by direct vaginal discharge gram staining for the diagnosis of Bacterial Vaginosis with Amsel clinical criteria and Nugent scoring system.

AEROBIC VAGINITIS ORGANISMS WITH ANTIBIOTIC SENSITIVITY PATTERN

- Fungal (Candidiasis) 17.16%
- Bacterial 59.03%
- No growth 12.65%
- Mixed growth 11.14%

DRUGS	Staphylococcus aureus 4%		Escherichia coli 25%		Klebsiella spp 10%		Neisseria gonorrh / Anaerobic 9% (S		Streptococcus agalactiae 7%		Pseudomonas aeruginosa 3%	
	S	R	S	R	S	R	S	R	S	R	S	R
AMPICILLIN	29%	71%	18%	56%	91%	6%	32%	65%	96%	4%	56%	44%
TAZOCIN	43%	50%	27%	56%	35%	62%	48%	39%	75%	25%	44%	44%
GENTAMYCIN	57%	29%	64%	21%	47%	47%	61%	39%	83%	17%	56%	44%
ERYTHROMYCIN	64%	14%	54%	33%	68%	32%	65%	35%	63%	29%	78%	22%
TETRACYCLIN	43%	57%	38%	51%	62%	38%	19%	74%	25%	75%	67%	33%
IMEPENUM	86%	14%	92%	11%	94%	6%	100%	0%	96%	4%	100%	0%
CIPROXIN	79%	14%	65%	30%	91%	9%	84%	16%	79%	21%	100%	0%
LEVEFLOXACIN	71%	7%	56%	29%	82%	15%	61%	29%	75%	21%	78%	22%
CEFIXIME	43%	36%	35%	54%	65%	26%	55%	42%	54%	42%	33%	56%
CEFOTAXIMINE	50%	29%	48%	46%	62%	26%	48%	35%	55%	37%	44%	33%
CEFTAZADIME	57%	21%	37%	56%	65%	21%	52%	29%	59%	37%	56%	33%
CEFTRAXONE	64%	29%	45%	51%	62%	26%	58%	23%	57%	33%	56%	33%

Fig 19: Growth and sensitivity pattern of various organisms isolated. Percentage of sensitivity (pink) and resistance (blue) according to the organism to different group of drugs in the symptomatic patients with vaginal discharge in public sector hospital population.



		n=332	Chlamydia trchomatis	Nesserria gonorrheae	Chlamydia+ gonorrheae	Chlamydia + Bacterial
	School	126	11	8	7	7
Low (Rs 5000-10,000)		**37.95%**	**6.04%**	**2.40%**	**3.84%**	**3.84%**
n=140	**College**	14	1	0	2	2
		4.21%	**0.54%**	**0%**	**1.09%**	**1.09%**
	School	69	6	5	1	7
Middle (Rs 11000-15000)		**20.78%**	**3.29%**	**1.50%**	**0.54%**	**3.84%**
n=104	**College**	41	2	4	0	5
		12.34%	**1.09%**	**1.20%**	**0%**	**2.74%**
	School	15	1	2	0	2
High (Rs 16000-20000)		**4.51%**	**0.54%**	**0.60%**	**0%**	**1.09%**
n=88	**College**	73	3	2	0	12
		21.98%	**1.64%**	**0.60%**	**0%**	**6.59%**

Fig 20: Percentage of sexually transmitted infections, Chlamydia trachomatis and Neisseria gonorrheae , in patients presenting with vaginal discharge at out-patient department of Gynecology and Obstetrics in a public sector hospital.

RECOMMENDATONS

- Patients with vaginal discharge should be investigated before start of treatment.
- Syndromic management should be avoided as it is the cause of resistance to conventional drugs, which are cheap and affordable to ensure compliance.
- The prevalence and causes of vaginitis are uncertain in part because the condition is often self diagnosed and self treated. The common practice of empirically treating all patients of suspected vaginitis with oral and/or vaginal pessaries is not a rational approach.
- The organisms involved in causing infection have tendency to change the sensitivity pattern relevant test, culture and sensitivity should be undertaken on routine basis to ensure effectiveness of drugs used.
- All these findings raise the need for health, educational program through different media to educate women about the difference between normal and abnormal vaginal discharge and whom to consult.
- Healthcare providers' training should be an on-going process, and should be through a social marketing initiative.
- Further research with larger sample size is needed to study the known risk factors, for example, screening of women at highest risk for adverse pregnancy outcome
- Future studies are required in our surroundings, setup, poor resource conditions, and different economic and educational groups for better results.
- A large health system or multi-site university-based research clinic or network collaboration might create an opportunity for significant benefits for adverse re-productive health outcomes .

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