

**CLINICO-INVESTIGATIVE PROFILE OF
CERVICO-VAGINAL DISCHARGE IN HIGHRISK
WOMEN ATTENDING SEXUALLY
TRANSMITTED INFECTION CLINIC IN
TERTIARY CARE HOSPITAL**

Dissertation Submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

*in partial fulfillment of the regulations
for the award of the degree of*

M.D. (MICROBIOLOGY)

BRANCH – IV



**GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
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CERTIFICATE

This is to certify that this dissertation entitled “**CLINICO-INVESTIGATIVE PROFILE OF CERVICO-VAGINAL DISCHARGE IN HIGHRISK WOMEN ATTENDING SEXUALLY TRANSMITTED INFECTION CLINIC IN TERTIARY CARE HOSPITAL**” is the bonafide original work done by **Dr. M. SWAPNA**, Post graduate in Microbiology, under my overall supervision and guidance in the department of Microbiology, Stanley Medical College, Chennai, in partial fulfilment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV)**.

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DECLARATION

I solemnly declare that this dissertation “**CLINICO-INVESTIGATIVE PROFILE OF CERVICO-VAGINAL DISCHARGE IN HIGHRISK WOMEN ATTENDING SEXUALLY TRANSMITTED INFECTION CLINIC IN TERTIARY CARE HOSPITAL**” is the bonafide work done by me at the Department of Microbiology, Government Stanley Medical College Hospital, Chennai, under the guidance and supervision of **Prof. Dr.R.SELVI, M.D.**, Professor and head of department of Microbiology, Government Stanley Medical College, Chennai-600 001.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of degree of M.D. Branch IV Microbiology examinations to be held in April 2013.

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CONTENTS

SL.NO.	TITLE	PAGE NO.
1.	<i>INTRODUCTION</i>	1
2.	AIM OF THE STUDY	4
3.	REVIEW OF LITERATURE	5
4.	MATERIALS AND METHODS	26
5.	OBSERVATION & RESULTS	52
6.	DISCUSSION	66
7.	SUMMARY	75
8.	CONCLUSION	77
10.	ANNEXURES	
	I) APPENDIX	
	II) BIBLIOGRAPHY	
	III) MASTER CHART	
	IV) KEY TO MASTER CHART	
	V) PROFORMA	

INTRODUCTION

Sexually transmitted disease (STD) refers to a variety of clinical syndromes caused by organisms that can be acquired or transmitted sexually ¹⁵. Even though STI is a broader entity than STD, the term STD and STI are now used synonymously.

STDs rank one among the five leading health problems in the developing nations (WHO). Women are more susceptible than men for acquiring STI from infected partners due to anatomical, physiological, immunological, hormonal and social factors. Also, social stigma in health care seeking behaviour, specimen collection method, diagnostic problems, management difficulties and adverse outcomes further complicates the magnitude of the problem in women. ³⁷

STDs in women are classified into Ulcerative and Non-ulcerative diseases and vaginal discharge is the commonest presentation of Non-ulcerative disease. The problem of vaginal discharge is the most frequently narrated complaint of woman in the reproductive age group ²⁹. The prevalence of vaginal discharge in India was found to be 30% in general population ⁵⁷ and 50% among commercial sex workers⁷⁴.

Vaginal discharge can be either physiological or pathological. A pathological discharge may be ignored by some females as normal discharge, whereas normal physiological discharge may be considered as abnormal by some meticulous women.⁶⁴

Infectious causes of abnormal vaginal discharge can be of bacterial, viral, fungal or parasitic etiology and successful management of vaginal discharge depends on the diagnostic approach. The traditional method of diagnosis is through laboratory tests but most of the times, this is not possible due to unavailability and expensive nature of the tests⁷³. In such situations, a presumptive diagnosis is made and syndromic management is given, which is often inaccurate. As a result, the patient may remain infective and continue transmitting the disease to their contacts.

Complications of vaginal discharge syndrome includes adverse pregnancy outcomes, pelvic inflammatory disease, infertility, malignancy, easy transmission of HIV and others.^(37,63) The complications and long-term consequences of untreated STDs not only affect the productive population of the society but also the newborn babies, who suffer from STDs acquired from infected mothers and face potentially serious consequences.

In contrast to the previous assumption of only Ulcerative STDs favours the acquisition of HIV, Non-ulcerative STDs also increases HIV acquisition by two fold⁴¹ thus increasing the HIV burden of the Nation in addition to the increased morbidity and mortality. The prevalence and incidence of STIs can be used as a proxy marker for assessing the behavioural changes in the target population and thus indirectly measures HIV incidence.

Since Cervico-vaginal discharge is a common problem which is manageable, the list of adverse outcomes that occur due to vaginal discharge syndrome are preventable and female sex workers form one of the core group for the spread of STIs to the general population, this study on Cervico-vaginal discharge in high risk women is important for the patient management as well as for preventing transmission of the infection to the general population through their clients who acts as bridge group connecting the High risk group and the general population. Therefore this study was done to analyse the various aspects of Cervico-vaginal discharge with the aim of establishing etiological diagnosis and achieving complete cure.

AIMS & OBJECTIVES

1. To study the prevalence of various etiological agents of Cervico-vaginal discharge
2. Comparison of different methods of diagnosis for various etiologies of Cervico-vaginal discharge.
3. Identification of co-infections
4. Determination of antimicrobial susceptibility pattern of the bacterial and fungal isolates
5. Evaluation and comparison of syndromic , clinical and etiological diagnosis.

REVIEW OF LITERATURE

History:

“Venereal diseases are like fine arts –it is pointless to ask who invented them”- Voltaire (French philosopher).¹¹

The history of sexually transmitted disease in India has been dated to mid sixteenth century in an ancient book called Bhavaprakasa. It has been mentioned in Bible that syphilis and gonorrhoea were ancient as well as contemporary burdens. Gonorrhoea and syphilis were called "the great imitator" by the eminent physician William Osler. The late nineteenth and early twentieth centuries saw major breakthroughs in the understanding of syphilis and gonorrhoea when, the causative agent of gonorrhoea, *Neisseria gonorrhoeae* was discovered by Albert Neisser and the causative agent of syphilis, *Treponema pallidum* was identified by Fritz Richard Schaudinn and Paul Erich Hoffmann.

The sexually transmitted diseases were major problems during World Wars I and II. The caption of a poster from world war II read "V.D. Worst of the Three," suggesting that venereal disease could destroy American troops faster than either of America's two declared enemies (Germany and Japan).

Prostitution as a profession has been there since long time in India. The devadasi (handmaiden of god) system also called as sacred prostitution has been there since 300 AD. In the modern era, many women enter this profession due to life circumstances and its consequences. 1. Women having no support either widowed or abused by their husbands and in-laws, entering this profession as a means to support themselves 2. Women choosing this profession for fulfilling their sexual urge or were curious¹⁸. Female sex worker (FSW) by definition is a woman who provides sexual service for immediate cash or for in kind returns²². These sex workers and their clients form the “core group” and “bridge group” for transmission of STDs and HIV to the general population.

Sexually transmitted infection (STI) rates are occurring in higher percentage among population subgroups such as commercial sex workers, since they tend to have high rates of partner change and unprotected sex. Therefore, maximum preference is given to this group in targeted intervention for prevention of HIV/AIDS.⁶⁷ The entry of deadly HIV into India in 1986 also contributed to the increased awareness and fear on STD and its prevention.

Anatomy of vagina⁶⁵:

The female external genitalia comprises of vulva, urethra, vagina and uterus. Vagina is a fibro muscular tube extending from vestibule to cervix. Length of vagina is about 9 cm anteriorly and 11.5 cm posteriorly. It forms a cuff around the lower two thirds of the cervix and thus forming an anterior, posterior and two lateral fornices. Functions of vagina are, it plays role in sexual activity, child birth and acts as a passage for the uterine secretions to be passed out.

Vaginal discharge:

Vaginal discharge constitute a major problem for many women causing discomfort and anxiety affecting their quality of life. Normal vaginal discharge has the following characteristics⁴. It is whitish, non-offensive, floccular in consistency and the pH varies for different age groups.

Age group	New born to Neonate	Neonate to puberty	Puberty to Menopause	Pregnancy	Post menopause
pH	4	7	3.8-4.5	4-4.2	6.0-7.5

The constituents of normal vaginal discharge are sloughed columnar epithelial cells of cervix and squamous epithelial cells of vagina, serous transudate of vagina and mucous secretion of cervix, organic compounds like carbohydrates, fatty acids, proteins and normal flora which includes *Lactobacillus crispatus*, *L.jensenii*, *L.gasseri* and *L.iners*. The uterus and fallopian tube contribute very less to the vaginal discharge.

Vaginal discharge should be considered abnormal when any one of the following feature is present.³²

1. Increased amount of discharge not associated with menstruation
2. Abnormal odour
3. Yellow coloured discharge

The causes of vaginal discharge can be physiological or pathological.⁴

I. Physiological causes

1. Age dependent

Neonate, Pre puberty and Child bearing

2. Excessive secretion

Sexual arousal and Pregnancy

II. Pathological:

1. Infective:

- a. *Gardnerella vaginalis* and list of Anaerobes
- b. *Trichomonas vaginalis*
- c. *Candida species*
- d. *Neisseria gonorrhoeae*
- e. *Chlamydia trachomatis D-K*
- f. Aerobic bacteria like *Staphylococcus aureus*, *Streptococcus species*, *Escherichia coli* and others

2. Non-infective:

- a. Chemical irritants:
Antiseptics, Detergent spermicide, Douches, Deodorants
- b. Gynaecological conditions:
Endo cervical polyp, Fistula, Radiation effects, Post operative and tumors
- c. Foreign bodies:
Intrauterine contraceptive device (IUCD)
- d. Medication and nutrition.

Epidemiology⁷⁰:

The estimated global occurrence of curable STI among adults was about 400 million in 1995. Out of this total disease burden, south and south-east Asia holds about 150 million, for which India's contribution is about 50 million. In 1990, WHO estimated that over 250 million new cases of STIs had occurred in that year globally. In 1995, the number of new cases of STIs had shot up to 333 million and in 1999, it became 340 million.

The STI trend in countries of Southeast Asian region shows wide differences. In Bangladesh, about 2.5 million persons are infected with STIs. In Bhutan, STIs are a major burden with gonorrhoea being the most common STI. In Myanmar the prevalence of major STIs per 100 000 population for the year 2000 are: syphilis -12.6; gonorrhoea - 4.2; non-gonococcal urethritis - 5.4; and chancroid -1.8. Genital herpes is the commonest reported STI and bacterial STIs are showing a decline in Srilanka. STIs are of low prevalence in Maldives. In Thailand the prevalence of major bacterial STI has decreased since 1989 with a slight increase in gonorrhoea cases in 2000.

In India, Tamil Nadu showed the prevalence of gonorrhoea to be 3.7% and chlamydia to be 3.9%, syphilis 0.3% and trichomoniasis 5.1%. A study carried out amongst sex workers revealed that 75% had at least one STI and also multiple infections occurred commonly. The prevalence of commonly encountered STIs include hepatitis B (5.3%), trichomoniasis (5.1%), chlamydial infection (3.9%), gonorrhoea (3.7%), HIV infection (1.8%), syphilis (0.3%), and chancroid (0.1%). Amongst high-risk groups, syphilis was the commonly found STI in Tamil Nadu, while trichomoniasis was the commonest STI in Kerala⁷³.

The data from southeast region shows that wide variations exist between regions and within countries in the same region, between urban and rural population, and even in similar population groups. There is a need to have this information for instituting preventive measures and providing good quality STI care to the population. According to WHO, effective control of STI needs research activities like STI prevalence study, Gonococcal antimicrobial surveillance and studies on cervicitis syndrome.

Vaginal discharge syndrome and HIV:

Women with bacterial vaginosis has two fold increased risk of acquiring HIV infection. Trichomoniasis was associated with 1.7 fold⁴¹ and 1.5 fold⁵² increased risk for acquiring HIV infection. Untreated vulvovaginal candidiasis is a possible risk for acquisition of HIV⁷². Gonorrhoea increases the risk of acquisition of HIV by 2-5 fold and Chlamydia trachomatis infection have 3-5 fold increased risk for acquiring HIV infection.

BACTERIAL VAGINOSIS¹⁷:

Introduction:

Bacterial vaginosis is also called as Anaerobic vaginitis /Vaginal bacteriosis. It is called vaginosis due to absence of inflammation. Bacterial vaginosis is described as a polymicrobial syndrome characterised by replacement of normal vaginal lactobacilli by anaerobic bacteria and mycoplasmas. Gardner and Dukes were the first to describe this syndrome as Hemophilus vaginalis vaginitis in 1955. Bacterial vaginosis was the most common infection among women visiting reproductive health clinic. In rural population of south India, the prevalence was found to be 17 % among sexually active age group.

Etiology^(1,17,55)

List of organisms responsible for this syndrome are *Gardnerella vaginalis*, *Mobiluncus species*, *Prevotella*, *Mycoplasma hominis*, *Bacteroides ureolyticus*, *Porphyromonas*, *Peptostreptococcus*, *Corynebacterium*, *Streptococcus viridians*, *Coagulase negative staphylococcus*, *Enterococcus faecalis*, *Clostridium species* and *Fusobacterium*.

Pathogenesis:

Normal vaginal flora is replaced by profuse mixed flora and there is also a quantitative increase in bacteria to 100-1000 times the normal. Production of amines by decarboxylation of amino acids and fatty acids by the mixed flora results in rise of vaginal pH and promotes growth of anaerobic organisms.

Hypothesis in pathogenesis are

- Gvh toxin is a haemolytic toxin produced by *Gardnerella vaginalis* and women who manifest bacterial vaginosis are those who lack an adequate IgA response against this toxin.
- Anaerobic organisms produce hydrolytic enzymes like sialidase and dipeptidase which lyse the mucin and helps in adherence of bacteria to the vagina.

Clinical features:

Homogenous, white discharge that smoothly coats the vaginal walls, with characteristic amine odour, pruritis, dyspareunia and lower abdominal pain.

Diagnosis:

Amsel's criteria is used to clinically evaluate the discharge based on simple tests. **Nugent scoring** of gram stained smear based on the number of certain bacterial morphotypes. **Hays/Ison system** is based on gram stain observation of ratio of different morphotypes rather than the exact number of bacteria present. **Schimdt's scoring system** is done on wet mount of vaginal smears, similar to nugent scoring system but does not recognise *Mobiluncus species*. **Anaerobic culture** methods are needed since most organisms responsible for bacterial vaginosis are obligate anaerobes. **Gas liquid chromatography** measures the ratio of succinate to lactate peaks. A value of greater than 0.4 is used as a predictor for bacterial vaginosis. **Sialidase activity and DNA probes** for *Gardnerella vaginalis* are also available.

Complications:

Miscarriage, pre term delivery, low birth weight baby, premature rupture of membranes, chorioamnionitis, postpartum endometritis, vaginal cuff cellulitis, pelvic inflammatory disease.

Treatment:

Metronidazole / Tinidazole 2g single dose should be given orally or Metronidazole 400 mg should be given twice daily for 7 days.

TRICHOMONIASIS⁶³:**Introduction:**

Trichomoniasis is a protozoal parasitic sexually transmitted disease. Donne first described *Trichomonas vaginalis* in the vaginal discharge in 1936. Annual worldwide incidence of Trichomoniasis is more than 170 million and this infection accounts for almost half of all curable sexually transmitted infections(WHO).The prevalence of Trichomoniasis was found to be 0.4- 27.4 % in women.

Etiology:

This is an obligate parasite that lacks the ability to synthesise the macromolecules de novo. It also lacks mitochondria and possesses hydrogenosomes to accomplish fermentative carbohydrate metabolism with hydrogen as electron acceptor.

Pathogenesis:

The three mechanisms involved in pathogenesis are

1. Contact dependent: *Trichomonas vaginalis* attaches to the epithelial cells by means of adhesion proteins AP 65, AP 33, AP 23. The laminin protein of the host can act as target for adhesion of the parasite. The haemolytic activity of the parasite also has a role in virulence.
2. Contact independent: The parasitic product called cell detaching factors (CDF) have trypsin like activity, which is supposed to play role in pathogenesis.
3. Immune response: *Trichomonas vaginalis* evades the immune system by complement mediated destruction, molecular mimicry and by its ability to coat itself with host proteins.

Clinical features:

About 50% are asymptomatic and others can present with symptoms like vaginal discharge which is frothy, foul smelling, green or yellow in colour, pruritis, dysuria, dysparenuia and lower abdominal pain

Diagnosis:

Clinical evaluation: Characteristic frothy discharge will be present in only 10% female and strawberry cervix will be present in only 2% female. The typical features are present only in fewer percentage of individuals and if clinical diagnosis was made, about 88% of infected women will not be diagnosed and 29% of uninfected female will be diagnosed as having Trichomoniasis.

Wet mount: The presence of *Trichomonas vaginalis* was determined by the morphology (10X7 μm size, pear shaped) and characteristic twitching motility. It needs a minimal concentration of 10^4 organisms /ml of vaginal secretion to be picked up by this method. Since the parasite lose its motility on cooling at room temperature and can survive in saline only for 20 min⁴⁹, the microscopy should be done immediately. Sensitivity depends on the expertise of the examiner and is about 55%.

Staining methods: Various stains that can be used are Giemsa stain, Acridine orange, Leishman stain, Periodic acid –Schiff and Fontana stain.

Culture method: Culture is the gold standard for diagnosis of Trichomoniasis. It can pick up positivity if 300-500 organisms/ml were present in the specimen. The positivity of culture is as twice as wet mount. It needs 2-7 days to show positivity. The media widely available for culture are Diamond's/ modified diamond's medium, CPLM (Cysteine peptone liver infusion maltose) /modified CPLM medium, Feinberg Whittington medium, Lash medium, Kupferberg medium, Agar culture and In Pouch TV culture.

Cell culture method: This method was reported to be superior to broth culture and microscopy since it can detect parasite even as low as 3 organisms/ml present in the vaginal fluid. It is not used routinely due to cost and time factors.

Rapid tests: Immunochromatographic based tests and Polymerase chain reaction (PCR) based methods are also available.

Complications:

Pre term labour, pre mature rupture of membranes, low birth weight infant, infertility, malignancy like Cervical cancer (Seema sood et al),risk of developing prostate cancer that was diagnosed at an

advanced stage (Jennifer et al ³³), risk factor for acquisition of HIV, other STI s and vice versa.

Treatment:

Metronidazole / Tinidazole 2g single dose should be given orally or Metronidazole 500 mg orally should be given twice daily for 7 days.

Resistance: Trichomoniasis is said to be clinically resistant if there is failure to cure the infection after atleast 2 consecutive courses of metronidazole. Prevalence of metronidazole resistance was estimated to be 2.5-5% .

VULVOVAGINAL CANDIDIASIS⁴

Introduction:

Vulvovaginal candidiasis is a fungal disease which is not only sexually transmitted but can be acquired due to variety of other reasons. Lagenbeck first demonstrated the fungus in thrush in 1839. Candidiasis accounts for 15-30% of etiological agent of vaginal discharge.

Etiology:

Candidiasis is caused mostly by *Candida albicans* and the rest by non –albicans, among which *Candida glabarata* is the commonest species.

Pathogenesis:

The important defense mechanism against candidiasis is the normal natural bacterial flora. The organism should adhere to the epithelial cells, proliferate and germinate for establishing colonisation and symptomatic inflammation. Candida can gain access to the vaginal epithelium from adjacent perianal area. Spontaneous phenotypic switching occurs and this variant phenotype has the capacity to form mycelia, express virulence factors, adhere and invade to cause disease. This spontaneous phenotypic switching can convert asymptomatic colonization to symptomatic vaginitis.

Risk factors:

Pregnancy, uncontrolled diabetes mellitus, corticosteroid therapy, tight fitting synthetic underclothing, antimicrobial therapy, oestrogen therapy, HIV infection and contraceptives like IUCD, sponge, nonoxynol-9 and diaphragm.

Clinical features:

Thick curdy white discharge-cottage cheese like in character, pruritis, soreness or irritation, burning/ dysuria and dyspareunia.

Diagnosis:

KOH mount: Direct examination of the discharge done with 10% KOH will show yeast cells of size 4-6 μ m which will be smaller than pus cells ($\geq 7\mu$ m). It has a greater significance if pseudohyphal forms are seen. **Gram stain:** Gram positive yeast cells with budding and pseudohyphae will be seen. If direct microscopy is positive, Culture is usually not needed to establish diagnosis. **Culture:** Culture should be done in microscopy negative clinically suspicious cases and also in resistant and recurrent cases. **Rapid tests:** Latex agglutination test are available with reported sensitivity and specificity of 81% and 98%.

Complications:

Recurrence and psychological stress

Treatment:

Fluconazole 150 mg single dose should be given orally.

GONOCOCCAL CERVICITIS⁷¹:**Introduction:**

Gonorrhoea is derived from two words- gono meaning 'seed' and rhoea meaning 'flow'. Albert Neisser identified the organism *Neisseria gonorrhoeae* in 1879.

Etiology:

Neisseria gonorrhoeae.

Pathogenesis:

N.gonorrhoeae affects the columnar epithelium. Infection is established in three steps.1.Adherence 2.Invasion and 3.Tissue damage.

Clinical features:

About 50% are asymptomatic or can have mucopurulent discharge, dysuria, menorrhagia and other menstrual irregularities.

Diagnosis:

Microscopy: Specificity is 40-60% in endocervical secretion and is negative in asymptomatic individuals while the specificity is 95-97% in male urethral samples. **Culture:** Various culture media used are Modified Thayer Martin medium, Chacko Nayar medium , Martin Lewis medium and New York City medium. **Polymerase chain reaction and Ligase chain reaction and serological methods like** Latex agglutination, anti surface pili assays and ELISA are available.

Complications:

Acute pelvic inflammatory disease, infertility, ectopic pregnancy, neonatal complications like ophthalmia neonatorum, Gonococcal arthritis, meningitis, endocarditis and disseminated gonococcal infection, the prevalence of which is about 0.1 to 0.3 of the total infection.

Treatment:

Cefixime 400mg single dose or ciprofloxacin 500 mg single dose should be given orally or ceftriaxone 125mg single dose should be given intramuscularly.

CHLAMYDIAL CERVICITIS⁶⁶:**Introduction:**

Chlamydia is derived from word 'cloak'. Neisser (who also discovered *N.gonorrhoeae*) along with Ludwig Halberstaedter and Stanislaus von Prowazek discovered *Chlamydia*. Ludwig Waelsch, famous German microbiologist was the first to describe *Chlamydia*. It is the most commonly reported STI and the commonest notifiable infectious disease in developed countries. The prevalence was 36-41% in northern states of India.

Etiology:

Chlamydia genus involves 4 species. *Chlamydia trachomatis* is one among them and is divided into many serovars. Serovars D-K are associated with chlamydial cervicitis.

Pathogenesis:

Chlamydia is an obligate intracellular pathogen and exhibits a biphasic cycle altering between two morphologically distinct forms, elementary body (infectious) and reticulate body.

Clinical features:

87% of women are asymptomatic and others can have mucopurulent discharge or hypertrophic ectopy.

Diagnosis:

Isolation in cell culture: McCoy, HeLa are the cell lines that support the growth of *Chlamydia*. Cell culture is highly specific (100%) and this method should be adopted to establish the diagnosis in medico legal cases. **Antigen detection:** Direct fluorescent antibody (DFA) test, Enzyme linked immune sorbent assay (ELISA), Nucleic acid hybridisation technique and Polymerase chain reaction can be used for antigen detection. **Serological methods** like Microimmuno fluorescence and ELISA are also available

Complications:

Pelvic inflammatory diseases, tubal occlusion leading to ectopic pregnancy and infertility, Fitz-hugh-curtis syndrome, spontaneous abortion, low birth weight baby and neonatal complications like conjunctivitis and pneumonia.

Treatment

Doxycycline 100 mg orally should be given twice daily for 7 days or Azithromycin 1g single dose should be given orally.

MATERIALS AND METHODS

TYPE OF STUDY: Cross sectional comparative study.

PLACE OF STUDY:

This study has been conducted in the Department of Microbiology, Stanley Medical College in association with Department of Sexually Transmitted Diseases, Government Stanley hospital, Chennai.

PERIOD OF STUDY: May 2011 to April 2012.

STUDY POPULATION:

Female outpatients attending Sexually Transmitted Infection clinic, were enrolled into the study.

INCLUSION CRITERIA

1. Women who presented with C/O vaginal discharge (symptomatic/subjective vaginal discharge).
2. Women who presented with complaints other than vaginal discharge, but had discharge on examination (asymptomatic/objective vaginal discharge).
3. Sexually active age group (15-49 years)

4. High risk group - Female sex workers

Housewife with multiple partners

Female with high risk partner

Female with other venereal diseases

Female with H/O diabetes and steroid intake

5. Low risk group - Absence of the above mentioned risk factors

EXCLUSION CRITERIA

1. Extremes of age.
2. Women in Menstruation or with Genital prolapse
3. Females with H/O treatment for discharge syndrome within 2 weeks prior to the visit.

ETHICAL CONSIDERATION

Ethical and research clearance was obtained from the Ethical Committee, Stanley Medical College. Informed consent was obtained from the patients before enrolment into the study.

STATISTICAL ANALYSIS

Statistical analysis was done using IBM-SPSS Statistics -20 statistical package. Variables were analysed using student 't' test, Chi square test and Z test of proportions wherever necessary. The P value less than 0.05 ($P < 0.05$) were treated as significant in two tail test.

SPECIMEN COLLECTION:

Consent was obtained from the patient and after wearing sterile gloves, four High vaginal swabs, two cervical swabs and 5 ml of blood were collected from each patient.

Speculum examination: Patient was asked to lie in dorsal position and draw the heels towards her bottom and then widely separate the knees. The speculum was inserted into the vagina and all the aspects of vagina and cervix are examined for presence or absence of erythema, ulceration, Mucopurulent cervicitis and vesicular lesions of vulva or vagina. The nature, colour, amount, consistency and location of discharge in the vagina were noticed.

High vaginal swab⁵⁵: A sterile cotton swab was inserted into the vagina and the material collected from the posterior and lateral fornices.

Cervical swab⁵⁵: Ectocervix was cleaned using sterile cotton swab and using another sterile cotton swab, the sample was collected from the endocervical canal.

Serum: About 5 ml of blood was collected and serum was separated by centrifuging at 1500 rpm for 5-10 min at room temperature. Serum was then stored in sterile container at -20°C.

PROCESSING OF SPECIMEN:

Discharge from speculum was subjected to Saline wet mount, KOH mount and Amsel's criteria

High vaginal Swab 1: Gram stain for Nugent score, Modified Donder's score, Giemsa stain for *Trichomonas vaginalis*

High vaginal Swab 2: Inoculated into Blood agar and Human bilayer tween Blood agar for culture of Aerobic and Anaerobic organisms

High vaginal Swab 3: Inoculated into Modified CPLM (Cysteine, Peptone, Liver infusion, Maltose) medium for isolation of *Trichomonas vaginalis*

High vaginal Swab 4: Inoculated into Sabouraud's dextrose agar for culture of *Candida species*

Cervical Swab 1: Gram stain to look for pus cells with or without organisms especially intracellular diplococci

Cervical Swab 2: Inoculated into Modified Thayer martin medium for isolation of *Neisseria gonorrhoeae*

Serum was subjected to test for HIV, Rapid Plasma Reagin test for Syphilis and ELISA for detection of Chlamydia IgM, IgG antibodies

Tzanck smear-Giemsa stain was done to look for multinucleated giant cells of Herpes simplex virus

Saline wet mount: A drop of vaginal discharge was put in a clean grease free slide, mixed with a drop of normal saline and examined first under 10X, then under 40X magnification to look for normal vaginal epithelial cells, Clue cells, Pus cells and motile *Trichomonas vaginalis*.

KOH mount: A drop of 10% KOH was added to the discharge placed on a clean slide and examined for budding yeast cells and pseudohyphae.

Amsel criteria⁵⁰:

1. Homogenous discharge
2. PH \geq 4.5
3. Presence of amine odour which is demonstrated by adding 10% KOH to the discharge and this occurs due to the production of amines like cadaverine, trimethyl amines and putrescene.
4. Presence of clue cells (20%) in the wet mount. Clue cells are defined as squamous epithelial cells studded with coccobacilli thus obscuring the nucleus and giving a granular appearance.

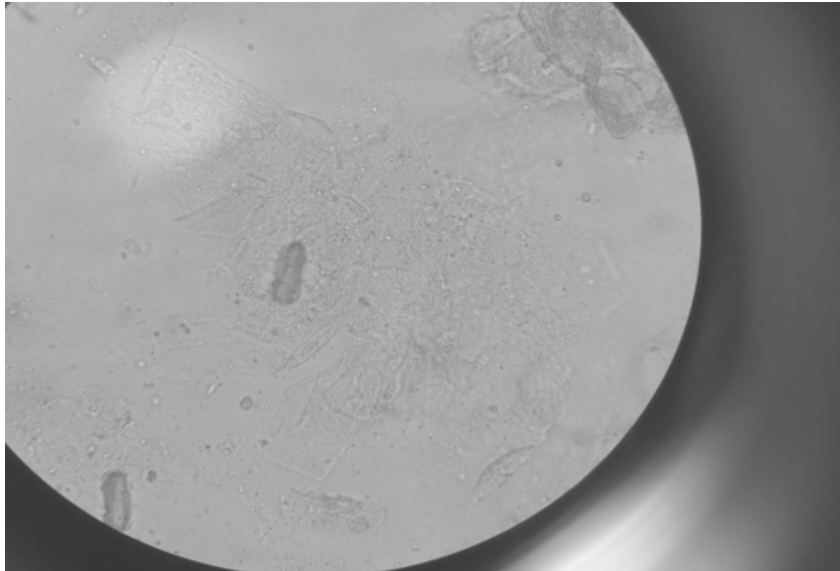
Presence of 3 out of 4 is significant for diagnosis of bacterial vaginosis

5. Absence of lactobacilli is an additional criteria.

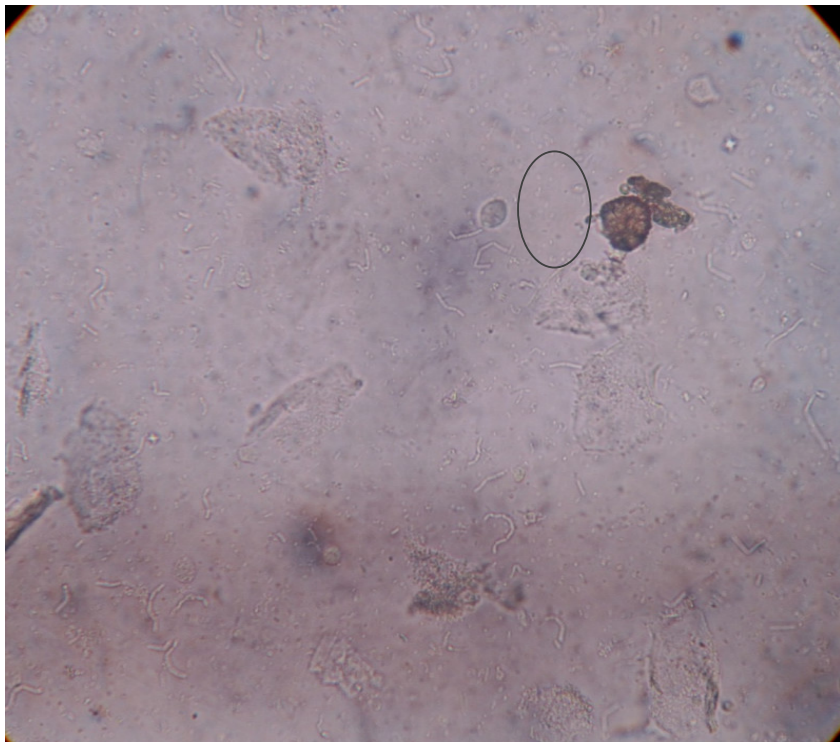
Vaginal Swab 1: was used for Gram stain and Giemsa stain

Preparation of smear: Clean grease free slide was taken and labelled. Smear was prepared by rolling the swab on the slide in one direction. Unidirectional smearing minimizes distortion and breakage of polymorphonuclear leukocytes (PMNL) and thereby preserves the

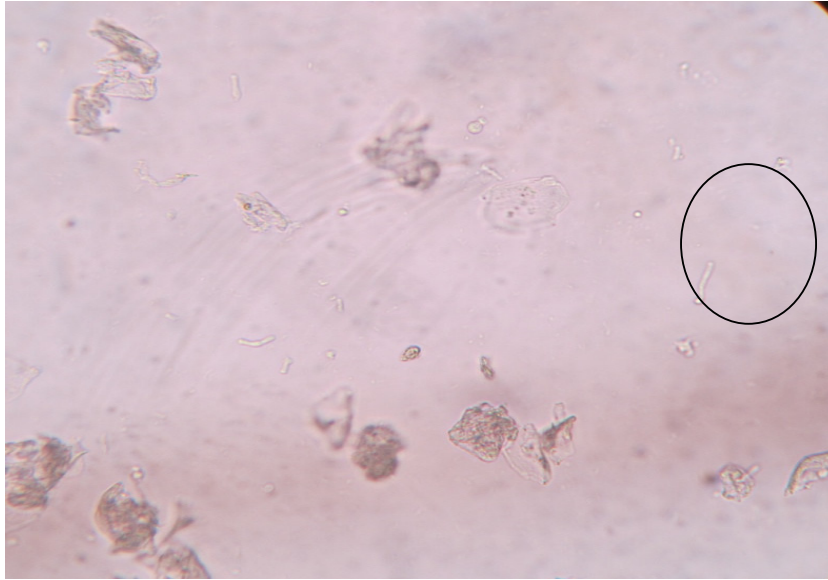
Wet mount under 40x showing clue cells



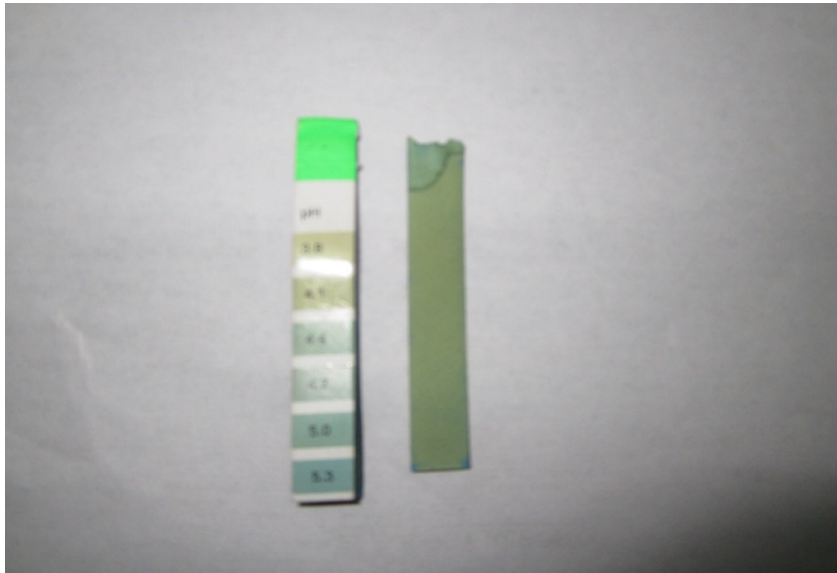
Wet mount under 40x showing pear shaped *Trichomonas vaginalis*



KOH mount under 40x showing Yeast cells & Pseudohyphae



pH measurement for Amsel criteria



morphology of the cells and location of microorganisms. The smear was air dried and gram stain was performed.

Nugent scoring of Gram stained smear for Bacterial vaginosis.⁹

ORGANISM MORPHOTYPE	NUMBER/OIL IMMERSION FIELD	SCORE
<i>Lactobacillus</i> - like(parallel sided, gram positive rods)	>30	0
	5-30	1
	1-4	2
	<1	3
	0	4
<i>Mobiluncus</i> – like(curved,gram negative rods)	>5	2
	<1-4	1
	0	0
<i>Gardnerella bacteroides</i> – like(tiny,gram variable coccobacilli and pleomorphic rods with vacuoles)	>30	4
	5-30	3
	1-4	2
	<1	1
	0	0

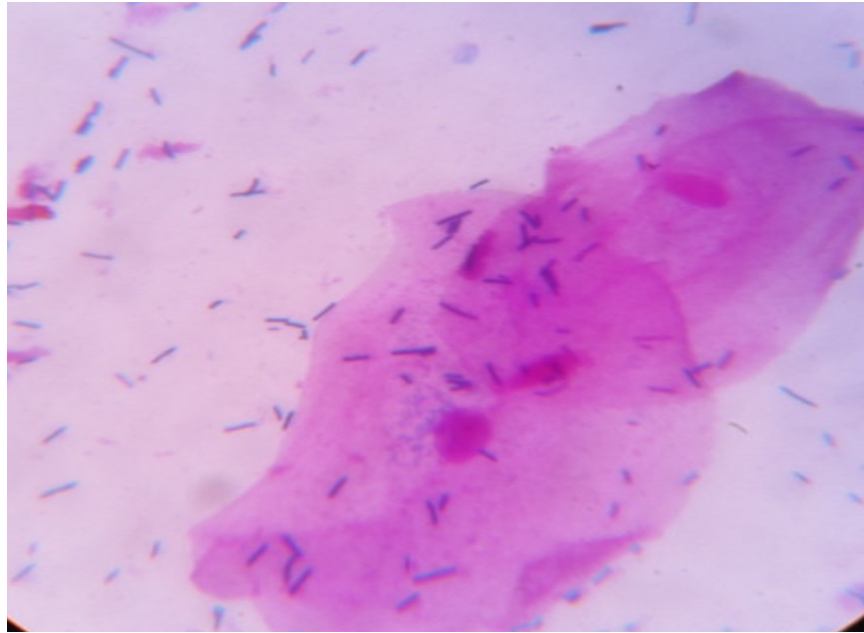
Total score:-

0-3 Normal

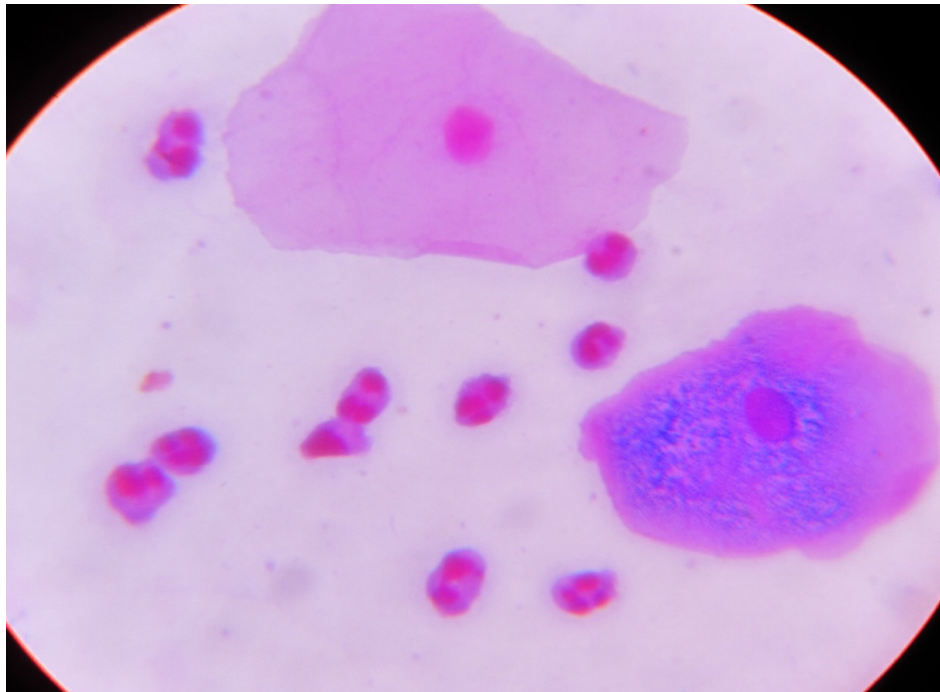
4-6 Intermediate, repeat test later

7-10 Bacterial vaginosis

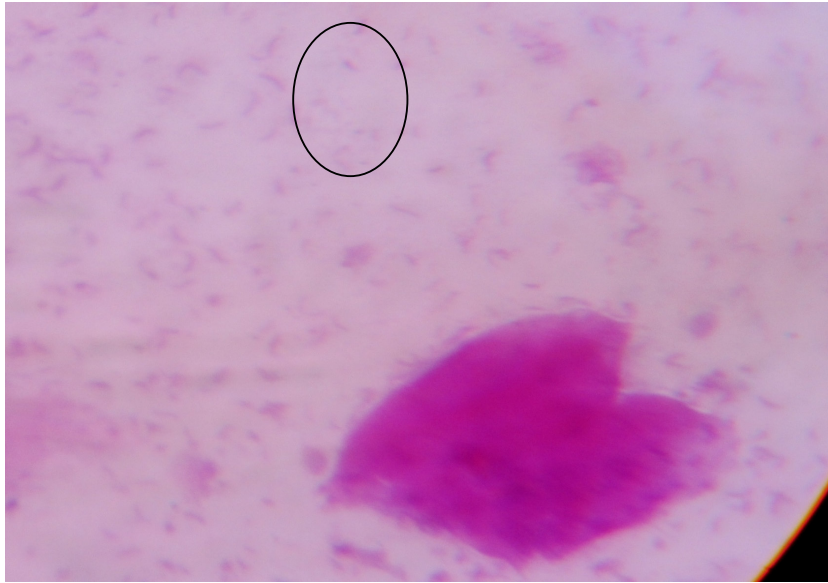
Gram stain under oil immersion showing Normal vaginal epithelial cells with *Lactobacilli*



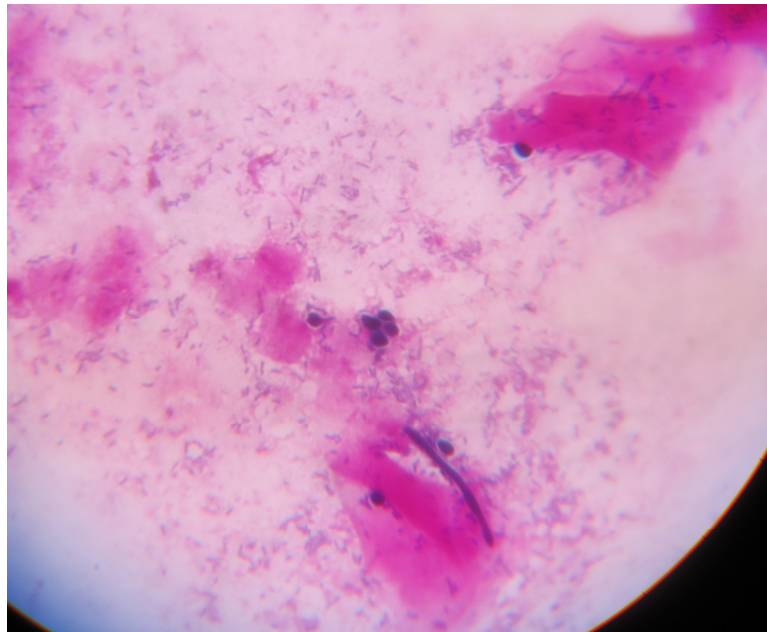
Gram stain under oil immersion showing Cluecells with absence of *Lactobacilli*



Gram stain under oil immersion showing *Mobiluncus species*
(Curved Gram negative rods)



Gram stain vaginal smear showing Gram positive yeast cell with
pseudohyphae



Modified Donder's grading of Gram stained smear for Aerobic
vaginosis²⁵

Score	Lacto bacillary grade	No.of leucocytes	Proportion of toxic leucocytes	Background flora	Proportion of parabasal cells
0	I,II A	≤10/hpf	None or sporadic	Unremarkable/ cytolysis	None or <1%
1	II B	>10/hpf or ≤10/epi cell	≤50%of leucocytes	Small coliforms	≤10%
2	III	>10/epi cell	>50% leucocytes	Cocci in chains	>10%

Total score:-

<3 - Normal

3-4- Slight aerobic vaginosis

5-6- Moderate aerobic vaginosis

6-10- Severe aerobic vaginosis

Giemsa stain was done to look for pear shaped *Trichomonas vaginalis*.

1. The smear was fixed in methanol for 5 min
2. The slide was stained with Giemsa stain diluted to 1 in 10 with buffered distilled water
3. The stain was allowed to stand for 30 min
4. The slide is then washed with distilled water.
5. The findings were observed under oil immersion.

Vaginal Swab 2: was inoculated in Blood agar and Human blood bilayer tween agar. The blood agar was incubated aerobically and Human blood bilayer tween agar was incubated in anaerobic atmosphere using McIntosh Fildes jar at 37°C for 48 hrs.

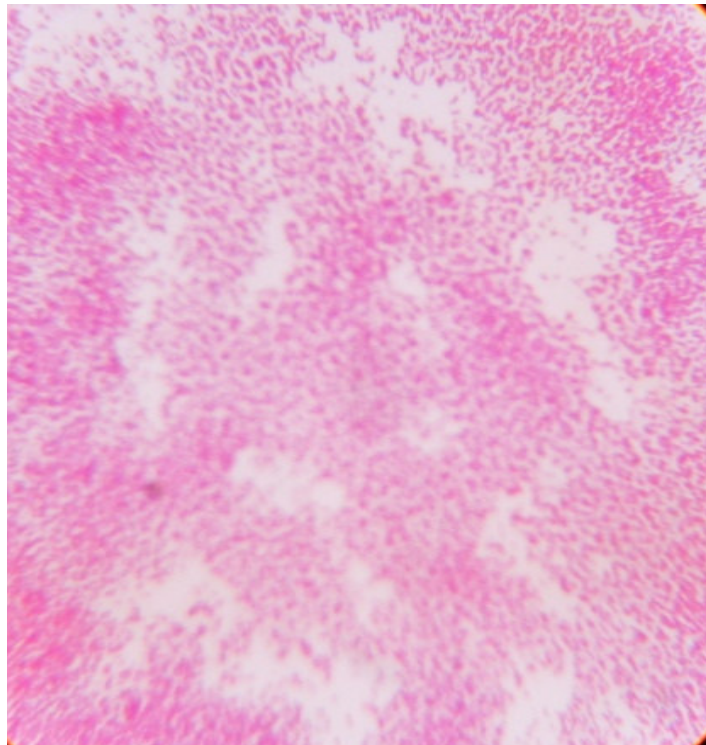
Aerobic organisms were identified based on the colony morphology, Gram stain and biochemical reactions. Antibiotic susceptibility test performed by Kirby-bauer disc diffusion method as per Central Laboratory Standards Institute (CLSI) guidelines.

*Gardnerella vaginalis*³⁹ were identified by observing for tiny translucent beta haemolytic colonies which were catalase and oxidase negative and Gram stain showing gram negative coccobacilli.

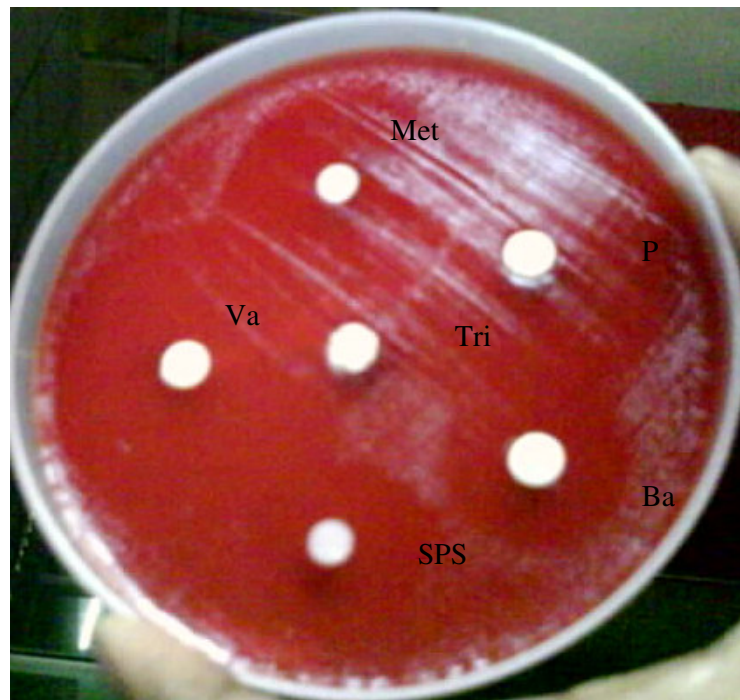
Giemsa stain under oil immersion showing pear shaped
Trichomonas vaginalis



Gram stain of culture smear showing Gram negative cocobacilli
(*Gardnerella vaginalis*)



Antibiotic sensitivity test for *Gardnerella vaginalis*



Identification discs like Metronidazole 50µg, Bacitracin 5 IU and SPS (Sodium Polyanethol Sulphonate) disc 100µg prepared by adding 20µl of 5% solution of SPS to sterile Whatman No.1 filter paper disc were used⁴³.

Obligate anaerobes were identified based on colony morphology, beta hemolysis, pigment production, swarming, aerotolerance test and Gram stain findings. Differential discs used were Vancomycin 5µg, Colistin 10µg and Kanamycin 1 mg.

Identification of anaerobes using differential discs⁸

Organism	Kanamycin 1mg	Vancomycin 5µg	Colistin 10µg
<i>Bacteroides ureolyticus</i>	S	R	S
<i>Prevotella</i>	R	R	S
<i>Porphyromonas</i>	R	S	R
<i>Fusobacterium</i>	S	R	S
<i>Peptostreptococcus</i>	R	S	R
<i>Clostridium</i>	S	S	R
<i>Eubacterium</i>	S	S	R

S (sensitive) >10 mm

R (resistant) <10 mm

Vaginal Swab 3: was inoculated in Modified CPLM (Cysteine, Peptone, Liver infusion, Maltose) medium and incubated in Co₂ atmosphere for 7 days. Wet mount was prepared from the medium everyday to look for motile *Trichomonas vaginalis*.

Vaginal Swab 4: was inoculated into Sabouraud's dextrose agar and incubated at 25-27°C.

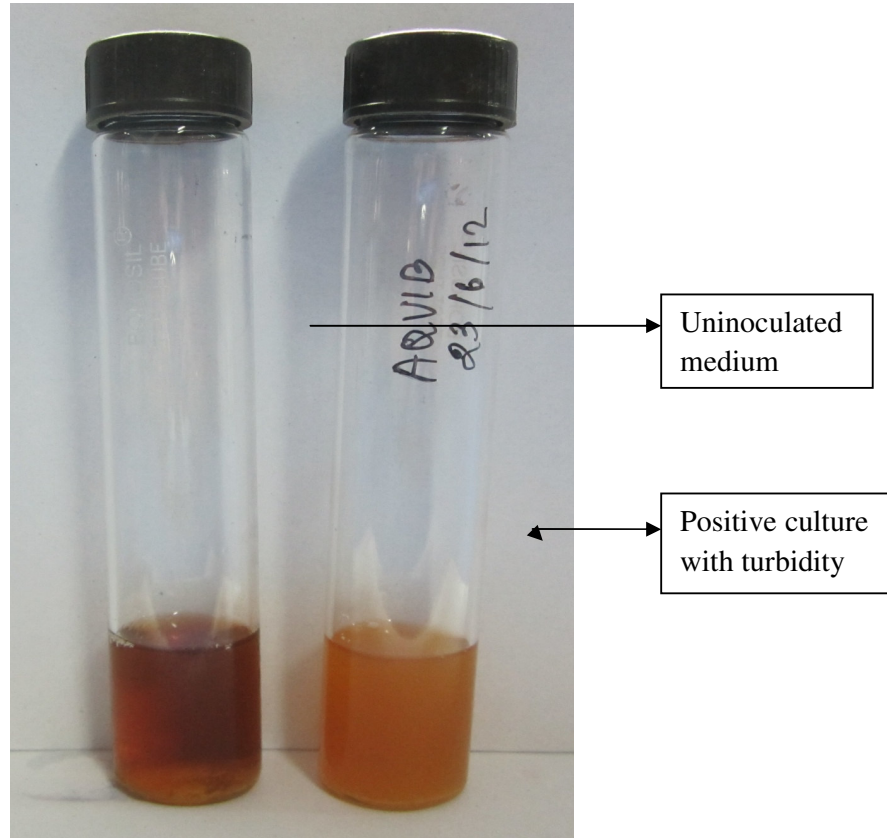
Colony morphology- Pasty, opaque, cream coloured circular colonies grow in 24-48 hours.

Gram stain –Gram positive budding yeast cells.

Germ tube test⁴⁰:

Few colonies were inoculated into 0.5 ml of human serum and incubated at 37°C for 2 hours. After 2 hours, wet mount was prepared and observed under 40 X objective. A germ tube is a short, lateral hyphal extension (filament) of the yeast cell without constriction at the base. Observation of germ tube suggests the presence of *Candida albicans*. Absence of germ tube indicates that the species belongs to non-albicans candida.

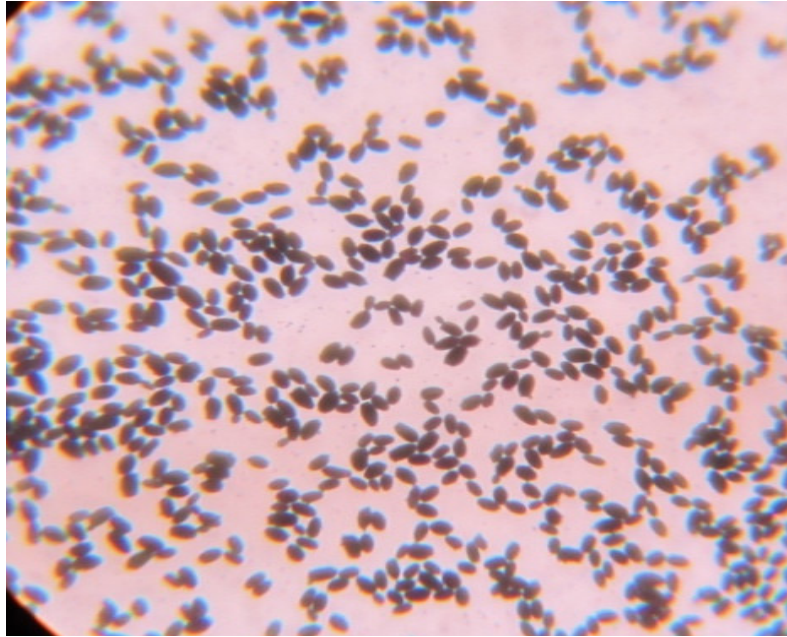
Modified CPLM medium for *Trichomonas vaginalis*



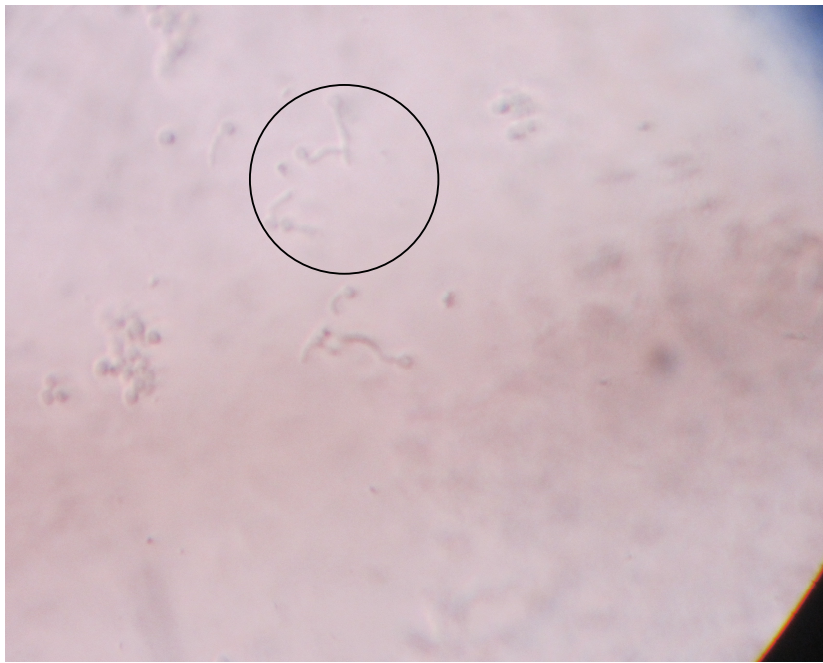
Cultures of various species of *Candida* in SDA



Gram stain of Candida culture showing Gram positive yeast cells



Germ tube test positivity of *Candida albicans*



CHROM agar

The colony was inoculated on CHROM agar medium and incubated at 30°C for 48 hrs and species were identified based on the colour.

Speciation of *Candida* by CHROM agar³¹

Species	Colour
<i>C.albicans</i>	Light green
<i>C.glabarata</i>	Pink to purple
<i>C.tropicalis</i>	Blue with pink halo
<i>C.parapsilosis</i>	Cream to pale pink
<i>C.krusei</i>	Pink
<i>C.dublinensis</i>	Dark green

Corn meal agar⁷

The tween incorporated in Corn meal agar medium and the subsurface inoculation technique provides a low surface tension to allow for the development of pseudohyphal and hyphal forms, blastoconidia and chlamydoconidia. Isolated colony from pure culture was streaked in cornmeal agar medium as two parallel streak lines ½ inch apart at 45 degree angle to the medium. A sterile cover slip was

placed on the streaked area and incubated at 27°C for 48 hrs and observed under 10 X and 40 X.

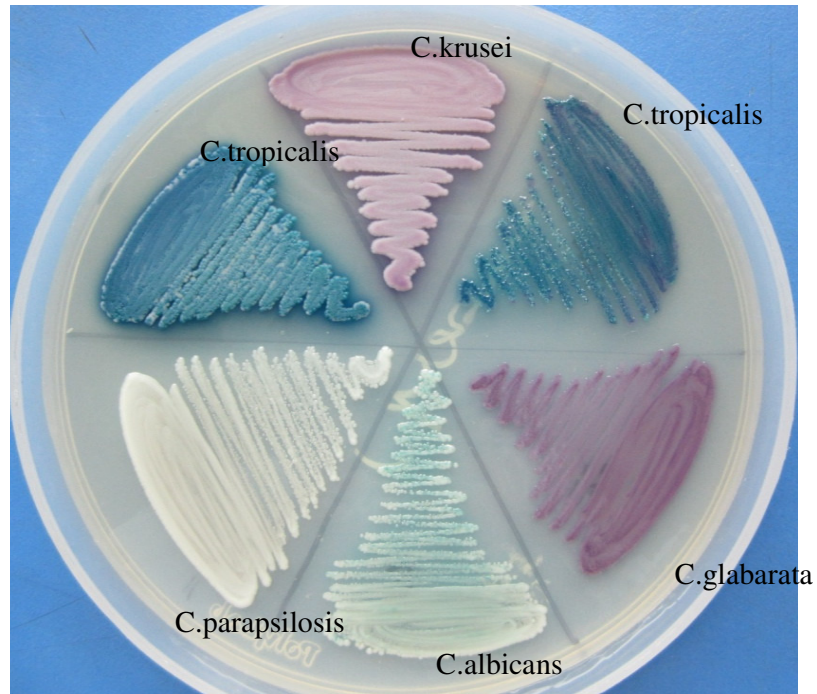
Speciation of *Candida* by cornmeal agar⁵⁸

Species	Morphology
<i>C.albicans</i>	Terminal and intercalary chlamydo spores
<i>C.glabarata</i>	No pseudohyphae, only blastoconidia
<i>C.tropicalis</i>	Branching pseudohyphae and blastoconidia
<i>C.parapsilosis</i>	Curved pseudohyphae and blastconidia
<i>C.krusei</i>	Pseudohyphae and blastoconidia resembles crossed match stick
<i>C.dublinensis</i>	Terminal and intercalary chlamydo spores

Carbohydrate assimilation test ⁷

Carbohydrate free medium (Yeast nitrogen base agar) containing bromocresol purple was inoculated with suspension of yeast corresponding to McFarland No.4 turbidity standard. Carbohydrate discs were placed on the inoculated medium and incubated at 30°C for 48 hrs. Change in colour of the medium or

CHROM agar showing various species of Candida



Corn meal agar under 40X showing blastospores and terminal chlamydospores of *Candida albicans*



growth around the disc were considered positive for utilisation of carbohydrate.

Speciation of *Candida* by carbohydrate assimilation³⁰

Species	Xyl	Lac	Suc	Mal	Mel	Cel	Tre
<i>C.albicans</i>	+	+	+	-	+	+	+
<i>C.tropicalis</i>	+	-	+	+	-	+	+
<i>C.glabarata</i>	+	-	-	-	-	-	+
<i>C.parapsilosis</i>	+	-	+	+	-	-	+
<i>C.krusei</i>	+	-	-	-	-	-	-
<i>C.dublinensis</i>	+	+	+	-	+	+	+

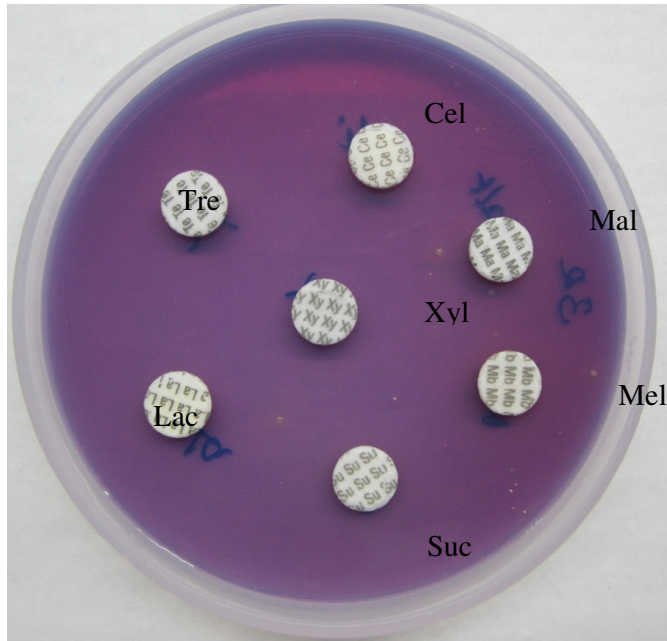
Xyl-xylose, Lac-lactose, Suc-sucrose, Mal-maltose, Mel-melibiose, Cel-celibiose, Tre-trehalose

Antifungal susceptibility test:

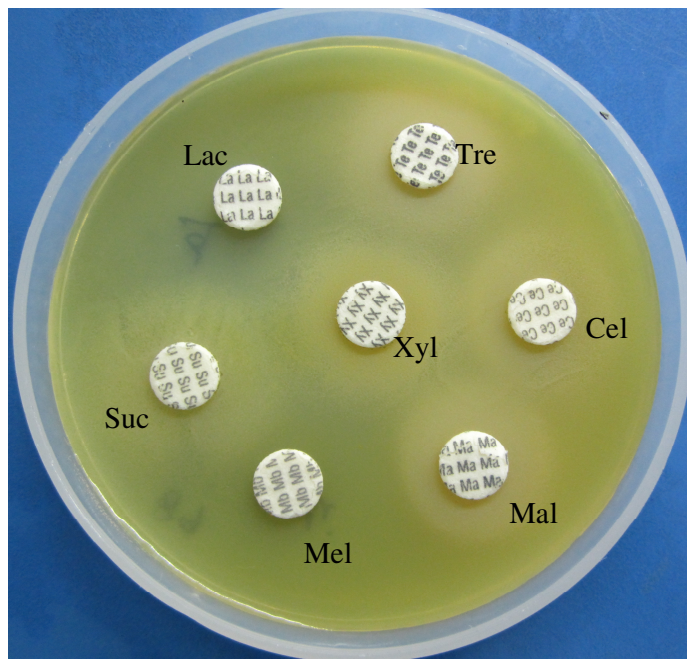
Disc diffusion (M44- A2):

Mueller Hinton agar with 2% glucose, 0.5µg/ml methylene blue added to the medium for easy identification of zone size was used. Inoculum standardised to 0.5 McFarland standard and after placing the discs, plates were incubated at 35⁰C for 24 hrs. For azoles the zones

Carbohydrate assimilation test for Candida (before incubation)



Carbohydrate assimilation test for Candida tropicalis (after incubation)



Candida tropicalis –Assimilation positive for Xylose, Sucrose, Maltose, Celibiose and Trehalose

were measured up to colonies of normal size and for Amphotericin B, the clear zone with no visible growth was measured

Interpretive zone diameter

Drug	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Fluconazole 25µg	≥19	15-18(SDD)*	<14
Itraconazole 10 µg	≥23	14-22(SDD)	<13
Amphotericin B 20µg	≥15	10-14	<10

SDD*-Susceptible dose dependent

Minimum Inhibitory Concentration by Microbroth dilution (M 27 -A3):

Procedure was done using RPMI-1640 medium (with glutamine without bicarbonate) supplemented with 0.2% glucose and buffered to pH of 7.0 with 0.165 mol/L MOPS(3-[N-morpholino] propane sulfonic acid). Standard inoculum of 0.5 McFarland was used and incubated at 35⁰C. Interpretation was done at 24 hrs using a reading mirror and the growth in each well was compared with that of the growth control.

The MIC for Amphotericin B is the lowest concentration with a score of 0 (optically clear). The MICs for the azoles are the lowest concentrations with a score of 2 (prominent decrease in turbidity).

Interpretive MIC in µg/ml

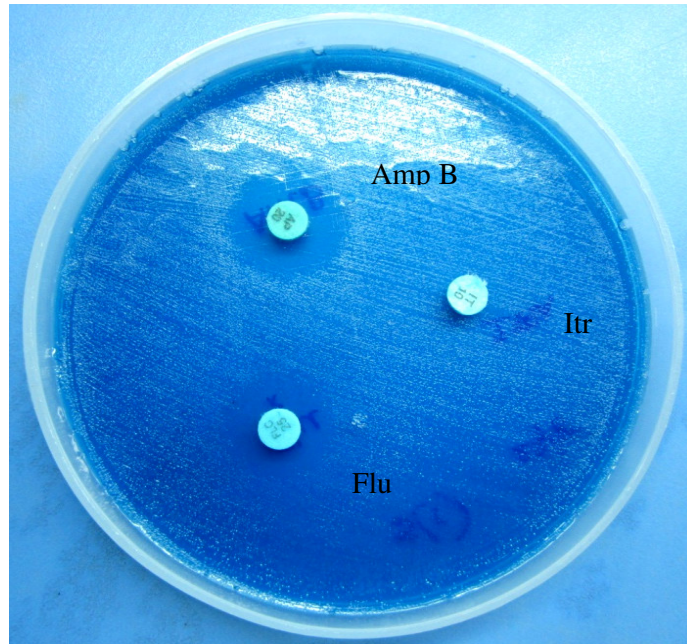
Drug	Susceptible	SDD*	Intermediate	Resistant
Fluconazole	<8	16-32	-	>64
Itraconazole	<0.125	0.25-0.5	-	>1
Amphotericin B	≤0.5	-	-	≥2

SDD*-Susceptible dose dependent

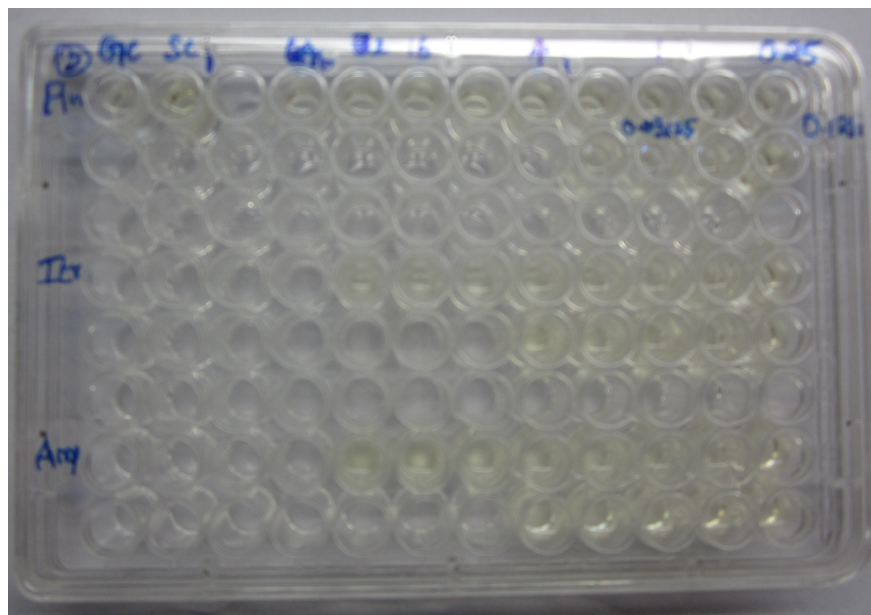
Minimum Inhibitory Concentration by Hi comb strips:

Hi comb MIC is based on the principle of disc diffusion. Hi comb strip consists of a predefined and continuous concentration gradient of antifungal agent and therefore directly measures the discrete MIC values similar to that of broth dilution results. Advantage is that Hi comb MICs are unaffected by molecular weight, aqueous solubility, diffusion and other properties of the drug or by varying growth rates of yeast. The procedure was performed similar to that of disc diffusion except that Hi comb strips were placed instead of discs.

Antibiotic susceptibility test for Candida

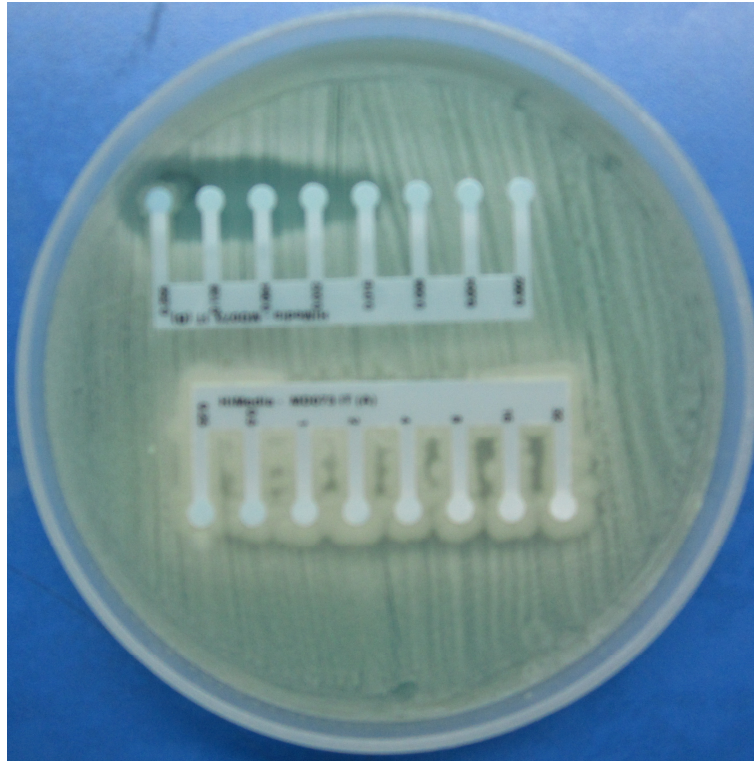


Microbroth dilution for Candida albicans



MIC for Flu-8 µg/ml, Itr-0.25 µg/ml and Amp B-0.25 µg/ml

Hi comb MIC test for Candida albicans



MIC for Fluconazole- 0.64 µg/ml

For Amphotericin B, reading was taken at complete inhibition of all growth. For Azoles, reading was taken at first point of significant inhibition i.e.so-called 80% inhibition.

Cervical Swab 1: was subjected to Gram stain.

Neisseria gonorrhoeae: The smear was examined for epithelial cells, polymorphonuclear leucocytes(PMNs), organisms and their location whether extracellular or intracellular. The gonococci are intracellular; bean shaped and are usually arranged in pairs, $0.8\mu\text{m} \times 0.6\mu\text{m}$ in size. They are Gram negative in reaction and are stained pink along with the pus cells. Intracellular diplococci atleast 3 pairs or more is strongly suggestive of *Neisseria gonorrhoeae* and the slide was examined for at least 2 minutes before declaring as negative.

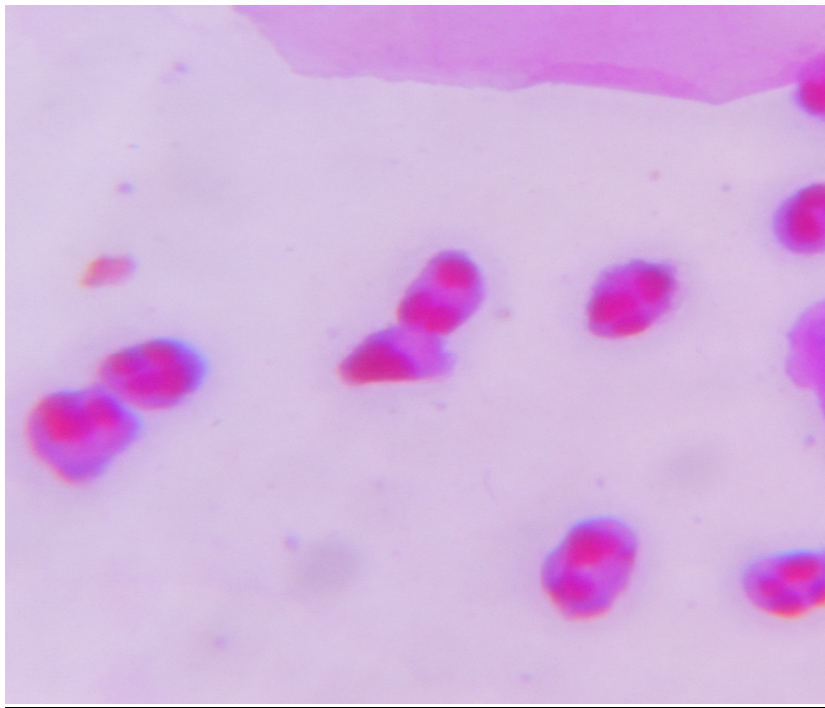
Chlamydial cervicitis: Presence of >30 PMNL/ High power field with no gram negative diplococci and occasional bacteria is an indirect clue for *Chlamydial cervicitis*.⁶⁶

Cervical Swab 2: was inoculated in “Z” pattern, and then cross streaked with a sterile platinum/ nichrome loop on Chocolate agar and Modified Thayer Martin medium with VCNT (Vancomycin to inhibit gram positive bacteria, Colistin to inhibit gram negative bacteria, Nystatin to inhibit yeasts and moulds, Trimethoprim to inhibit

Gram stain of Endocervical smear showing Intracellular Gram negative diplococci



Cervical gram stain showing pus cells under oil immersion field



swarming of Proteus) and incubated at 37⁰C in 5-10% CO₂ atmosphere. A moist cotton ball/ wet paper towel was placed inside the jar to provide >70% humidity and a lighted candle to provide 5% CO₂. The plates were examined after 18-24 hours and incubated for another 24 hours if there was no growth and re examined after 48 hours. Small pin point, 0.5 to 1 mm in diameter, grey to white in colour, smooth, translucent, raised convex colonies suggests *Neisseria*.

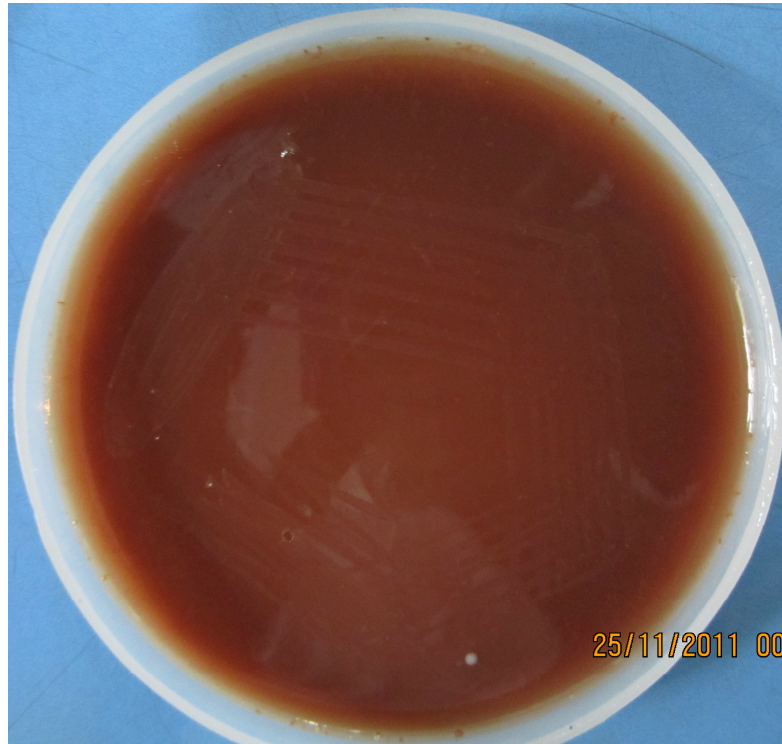
Presumptive identification of *Neisseria gonorrhoeae*:

1. **Gram staining:** Gram stain was done from smear prepared from suspected colony and it showed typical Gram negative diplococci.
2. **Oxidase test:**

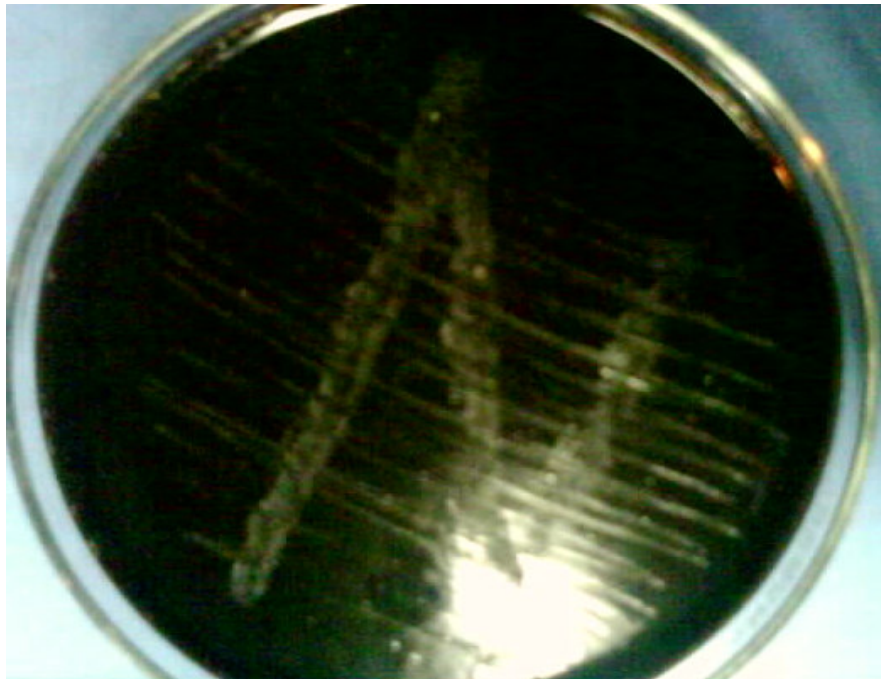
Method I (Direct colony method):

1% fresh solution of oxidase reagent (N,N-Tetra methyl paraphenylenediamine dihydrochloride) was prepared by dissolving 10 mg of reagent in 1 ml of distilled water. One drop of the reagent was added on the colony to be tested. Development of purple colour within 10 seconds indicates that the isolate is probably *Neisseria gonorrhoeae*.

Culture of *N.gonorrhoeae* on chocolate agar



'Z' Streaking of Endocervical swab on Modified Thayer Martin medium



Method II (Wet filter paper method):

A filter paper strip was moistened with 2-3 drops of oxidase reagent. A single colony was picked with the wooden applicator stick, tip of glass slide, glass rod or platinum loop and rubbed on filter paper. Production of purple colour means positive result.

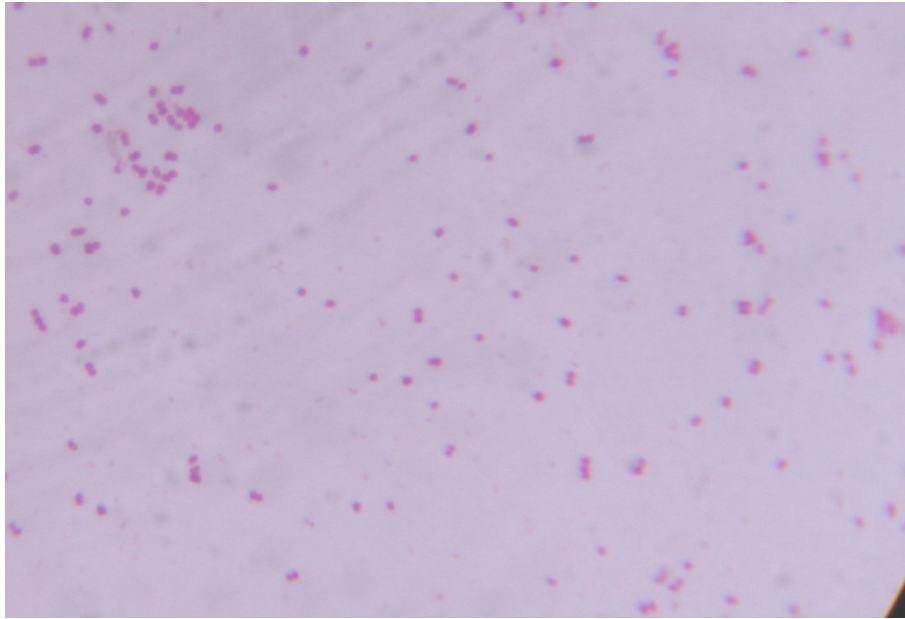
Method III (Dry filter paper method):

Whatman filter paper was cut into small strips and 1% fresh solution of oxidase reagent was poured on to the strips placing it in a petri dish and dried in hot air oven. The strips were stored in a brown bottle in refrigerator and tested with the suspected colony.

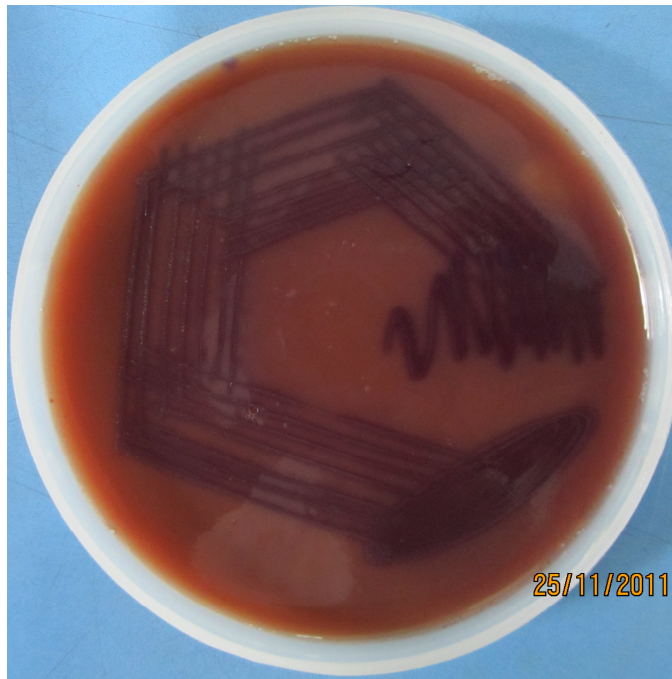
- i. Dark deep purple colour in 5 – 10 sec – positive test
- ii. Deep purple colour in 10 – 60 sec – Delayed positive test
- iii. No colour or colour development after 60 sec – negative test

The test was performed with positive control (*Pseudomonas aeruginosa* ATCC 27853) and negative control (*Escherichia coli* ATCC 25922)

Culture smear of *N. gonorrhoeae* showing Gram negative diplococci



Oxidase test positive -Direct colony method



3) Superoxol test:

Method I(Slide method):

A drop of 30% w/v hydrogen peroxide was placed in the centre of a clean glass slide. Few colonies of the culture to be tested were picked with a glass rod and emulsified directly in the drop of hydrogen peroxide.

Method II(Plate method):

One drop of 30% w/v hydrogen peroxide solution was put on the suspected colony on chocolate agar. Immediate production of bubbles (within 1 to 2 seconds) was defined as a positive result. A negative reaction was defined by weak or delayed bubbling after 3 seconds. A negative test means that the isolate is not *Neisseria gonorrhoeae*.

Confirmatory test for *Neisseria gonorrhoeae*:

Rapid carbohydrate utilization test (RCUT):

This is the most frequently used method for confirmation of *Neisseria gonorrhoeae*. This method is non-growth dependent and combines the use of carbohydrate utilisation and acidometric test for detection of beta-lactamase. Ampicillin is more stable and more sensitive to TEM β -lactamase, was used instead of penicillin G in this test.

Procedure: Pure culture of suspected *Neisseria gonorrhoeae* was obtained by sub culturing a single colony into Chocolate agar. Two full 3 mm loop ful of the isolate of 24 hr pure culture was emulsified into a tube containing 0.3 to 0.4 ml buffered balanced salt solution (BSS). Six wells of a microtiter plate were required for each test. First well serves as control. 100 micro litre of BSS was added to all the wells, followed by 50 micro litre of 10% sterile glucose, maltose, sucrose, lactose and ampicillin solution to the respective wells. Finally, 50 micro litre of bacterial suspension was added to each of the five wells except the control. The wells were incubated aerobically at 37°C and read after 2 to 4 hrs.

Results: Control tube/well = Red
Positive reaction = Yellow colour
Negative reaction = Orange Red colour

Only the tube containing glucose showed colour change, since *Neisseria gonorrhoeae* utilizes glucose only.

Sugar utilization reactions of *Neisseria* species

Species	Glucose	Maltose	Lactose	Sucrose
<i>N.cinera</i>	-	-	-	-
<i>N.gonorrhoeae</i>	A	-	-	-
<i>N.meningitidis</i>	A	A	-	-
<i>N.lactamica</i>	A	A	A	-

c) Antimicrobial susceptibility testing methods:

The following antibiotics recommended by WHO were used for sensitivity testing.

Penicillin, Ciprofloxacin, Tetracycline, Spectinomycin, Azithromycin, Ceftriaxone or other cephalosporins and test performed following CLSI guidelines.

Neisseria gonorrhoeae colonies were picked up and emulsified in 5ml of phosphate buffered saline (PBS) to attain 0.5 Mc Farland's standard which corresponds to 150 million organisms/ml. 2 ml of colony suspension was poured on the plate with a sterile pasteur pipette. Then the plate was tilted in all directions to spread the inoculum over the entire surface of plate and the excess fluid was aspirated using a pasteur pipette. The plate was inverted and kept in the incubator at 37° C for 30 min for the inoculum to dry. After placing the

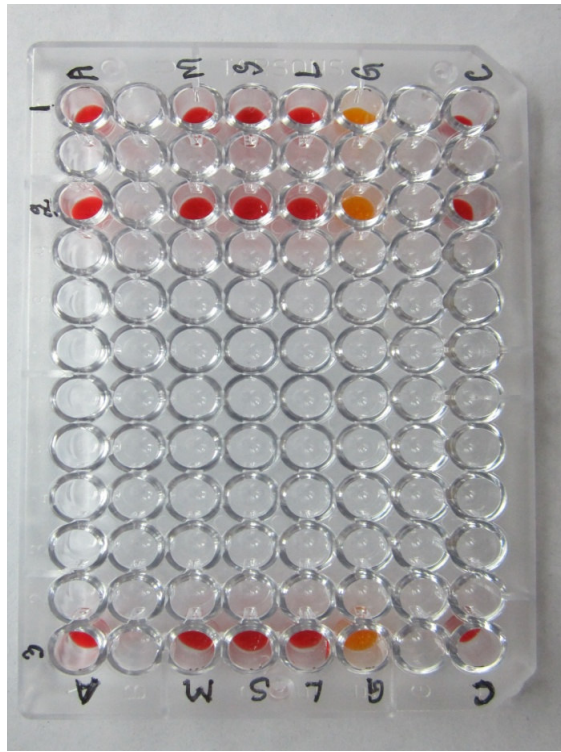
discs, the plate was incubated at 37° C in Co₂ atmosphere for 18-24 hours. The diameter of the zones of complete inhibition was measured and were interpreted by referring to the CLSI standards and reported as susceptible, intermediate, or resistant to the antibiotics that have been tested.

The three isolates of *Neisseria gonorrhoeae* were sent to the Apex Regional STD Teaching, Training and Research centre, Vardhaman Mahavir Medical College and Safdarjung Hospital and confirmed.

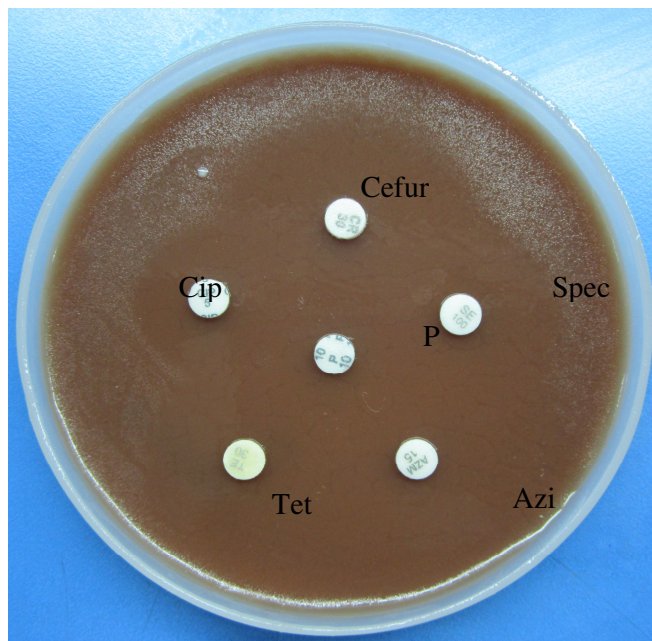
Zone size interpretative chart (CLSI)

Drug	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Penicillin 10 U	≤26	27-46	≥47
Tetracycline 30ug	≤30	31-37	≥38
Spectinomycin 100ug	≤14	15-17	≥17
Ceftriaxone 30ug	≤25	26-30	≥31
Ciprofloxacin 5ug	≤27	28-40	≥41
Azithromycin 15ug	≤25	-	-

Rapid Carbohydrate Utilisation Test(RCUT)-Only Glucose was utilised



Antibiotic sensitivity test for N.gonorrhoeae



The sensitivity test results of Reference centre done by Calibrated Dichotomous sensitivity method using Low potency discs were compared with that of CLSI method of our College.

CLSI method (Stanley medical college) and CDS method (Regional reference centre) for 3 isolates of *N.gonorrhoeae*

DRUG	CLSI		CDS	
	Sensitive (mm)	Resistant (mm)	Sensitive (mm)	Resistant (mm)
Penicillin	1	2	2	1
Tetracycline	2	1	3	0
Spectinomycin	3	0	3	0
Ceftriaxone	2	1	3	0
Ciprofloxacin	2	1	2	1
Azithromycin	3	0	3	0

CLSI-Central Laboratory Standards Institute, CDS-Calibrated Dichotomous sensitivity test

Serum for Indirect ELISA for IgM, IgG antibodies to Chlamydia trachomatis (Novalisa-Germany)

Procedure:

Dilute the samples by adding 1mL sample diluent to 10 μ L of sample.



Dispense 100 μ L controls & diluted samples into their respective wells.
Well A1 was left for Blank



Cover microtitre plate with foil and incubate at 37°C for one hour.



Wash the wells three times, each with 300 μ L of washing solution.



Dispense 100 μ L Chlamydia trachomatis anti Ig conjugate into all wells except the blank.



Incubate at room temperature for 30 min. Do not expose to direct sunlight.



Repeat the wash step three times.



Dispense 100 μ L TMB substrate solution into all wells including blank.



Incubate at room temperature for 15 min in dark.

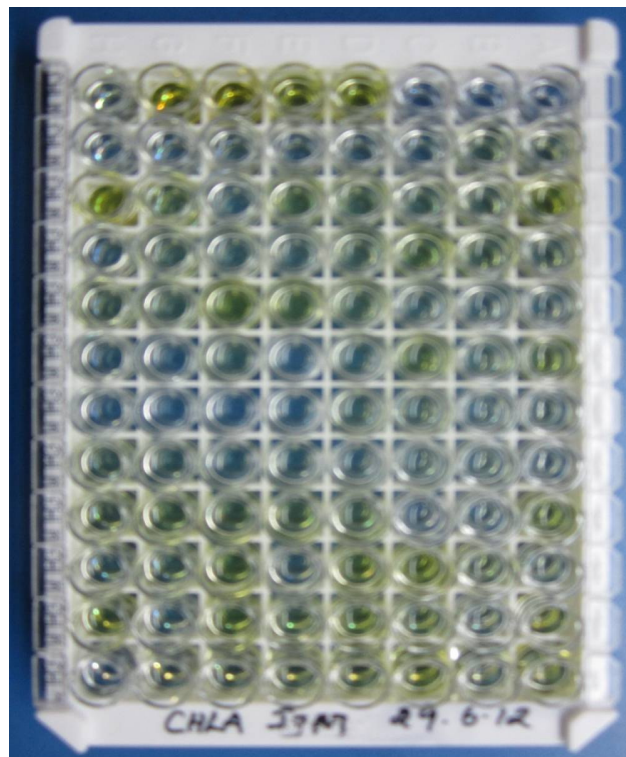


Dispense 100 μ L stop solution into all wells including blank.

IgM ELISA Kit for Chlamydia trachomatis



IgM ELISA for Chlamydia trachomatis

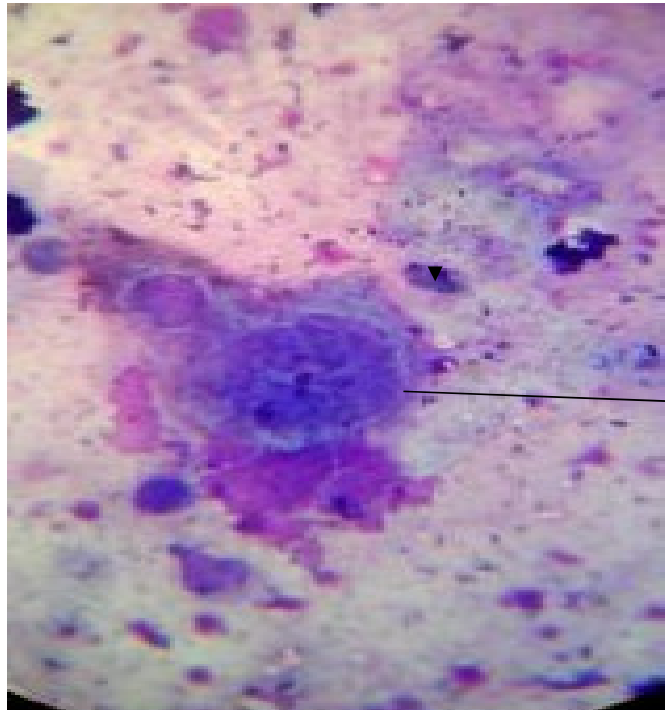


The absorbance value was read immediately at 450/620 nm after addition of stop solution. The values above and below cut off were considered positive and negative respectively and for subjects whose values fall in grey zone, the result needs to be repeated after 1 month.

Consent was obtained from each patient for HIV testing and pre test counselling was given. Tests for HIV was done according to National AIDS Control Organisation (NACO) guidelines and Rapid Plasma Reagin test for syphilis was also performed.

Tzanck smear: The vesicle is unroofed and the base of the lesion is scraped with the edge of scalpel blade. The material is then touched to a glass slide, air dried is then stained with Giemsa stain

Tzanck smear under oil immersion showing multinucleated giant cells



Multi
nucleated
giant cell

OBSERVATION AND RESULTS

Total number of patients studied was 130, which included 65 High risk females with subjective vaginal discharge (Symptomatics), 20 High risk females with objective vaginal discharge (asymptomatics), 35 Low risk females with subjective vaginal discharge (Symptomatics) and 10 Low risk females with objective vaginal discharge (asymptomatics). The results were analysed and tabulated as follows.

Table 1:Distribution of females into groups based on risk and symptoms

Symptom	High risk	Low risk	Total	%
Subjective vaginal discharge	65(50.0%)	35(26.92%)	100	76.92
Objective vaginal discharge	20(15.38%)	10(7.69%)	30	23.07
Total	85	45	130	34.61
%	65.38	34.61	100	100

Table 2:Age distribution of High risk and Low risk groups

Age(years)	High risk		Low risk	
	Number	%	Number	%
15-19	0	0	0	0
20-29	23	27.05	13	28.88
30-39	48	56.47	26	57.77
40-49	14	16.47	6	13.33
Total	85		45	
Mean±SD	34.0±7.5		31.4±6.7	
Significance	<i>t</i> value=1.932 degree of freedom=128 P value >0.05			

Commonest age group in both high risk and low risk groups was 30-39 years of age. The two groups were similar in age group distribution and did not show significant difference with respect to age ($P>0.05$) and therefore the two groups were comparable.

Figure 1: Distribution of females into groups based on risk and symptoms

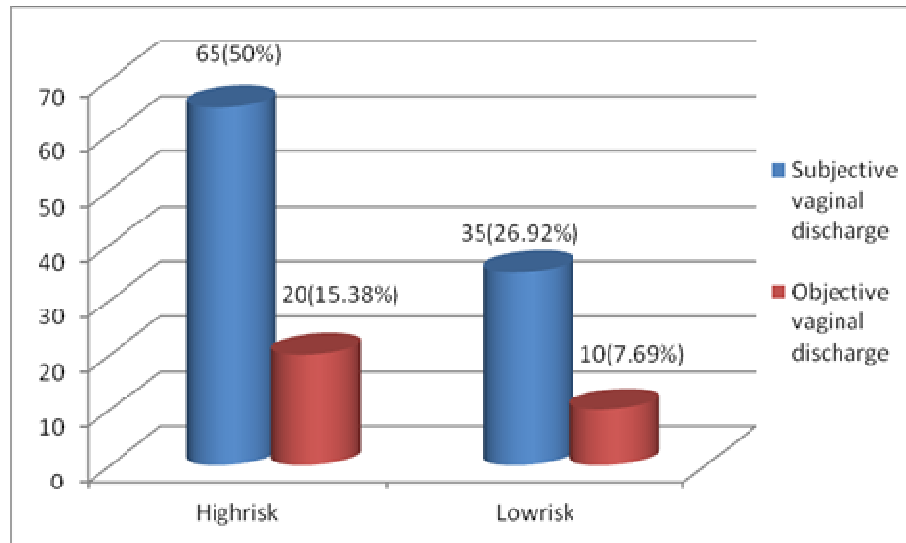


Figure 2: Age distribution of High risk and Low risk groups

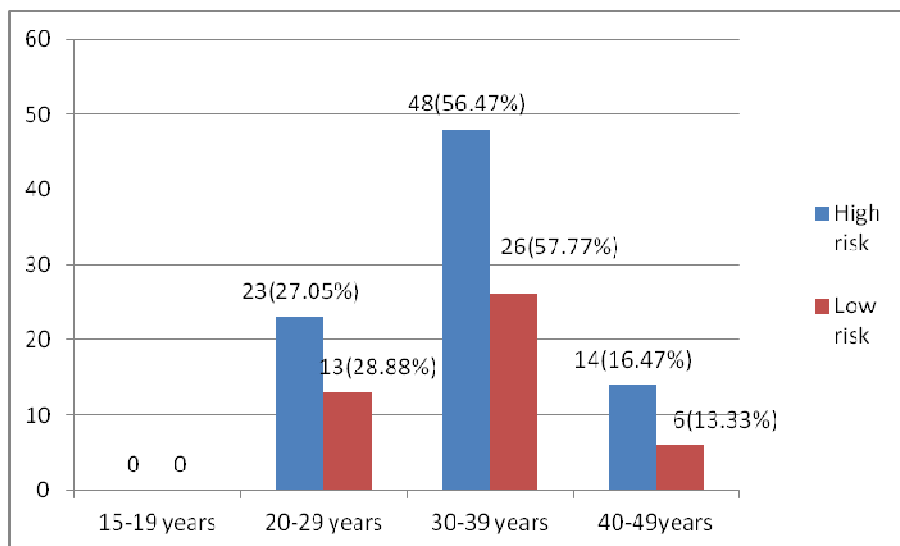


Table 3:Distribution of subjects based on Educational status

Education	High risk		Low risk	
	Number	%	Number	%
Nil	32	37.64	13	28.88
Primary school	21	24.70	10	22.22
Middle school	23	27.05	16	35.55
High school	7	8.23	5	11.11
Higher secondary	2	2.35	0	0
Degree	0	0	1	2.22
Total	85	100	45	100
Significance	$\chi^2 = 3.156$ degree of freedom = 5 P>0.05			

Commonest educational level in high risk group was nil education and low risk group was middle school level. But statistically the two groups did not show significant difference with respect to Educational status (P>0.05) and therefore both groups were comparable in education level.

Table 4: Distribution of subjects based on Marital status

Marital status	High risk	%	Low risk	%
Unmarried	1	0.11	0	0
Married	74	87.05	43	95.55
Widow	5	5.88	1	2.22
Separated	5	5.88	1	2.22
Total	85	100	45	100
Significance	$\chi^2 = 4.353$ degree of freedom = 4 P>0.05			

Married women the commonest of about 87.05% in high risk and 95.5% in low risk group. The two groups did not show significant difference with respect to Marital status (P>0.05).

Figure 3: Distribution of subjects based on Educational status

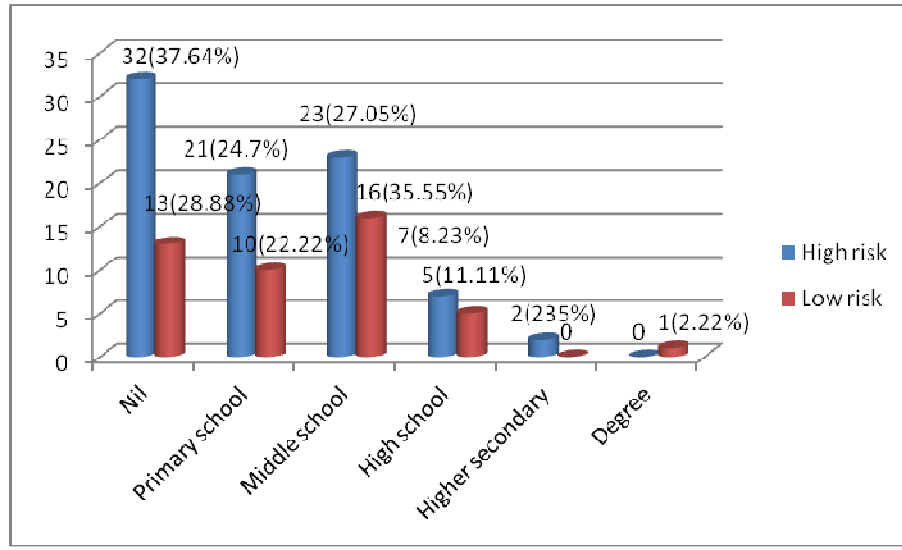


Figure 4: Distribution of subjects based on Marital status

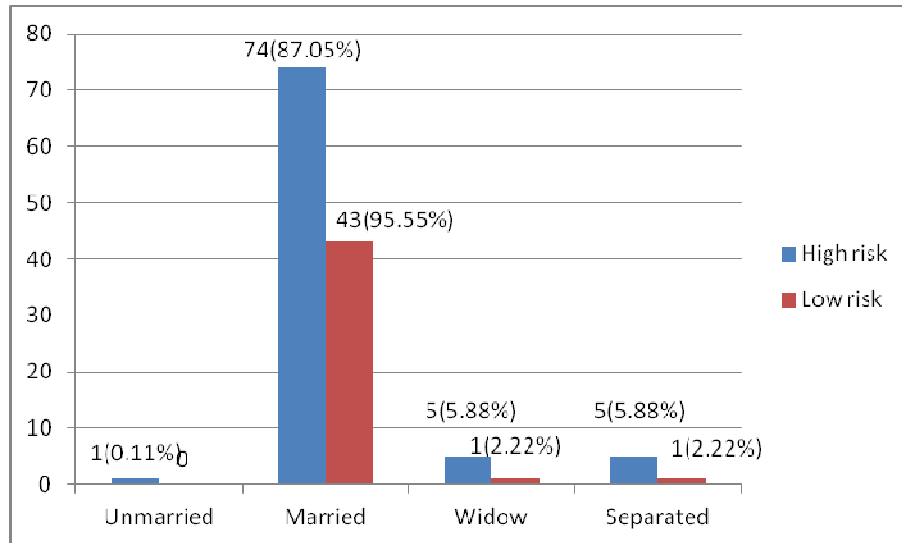


Table 5: Distribution of Protected/ Unprotected sex in high risk group

Sexual Contact	Number	%
Protected	46	54.11
Unprotected	37	43.52
No recent contact	2	2.35
Total	85	100

About 43.52% females in high risk group followed unprotected sex.

Table 6: Distribution of risk factors

Risk factor (n=130)	Number	%
Female sex worker	23	17.69
House wife with extramarital contact	28	21.53
Diabetes and steroid	8	6.15
Past VD	14	10.76
Partner HR	18	13.84
Low risk	45	34.61

The most common risk factor was housewives with extramarital contact (21.53%)

Figure 5: Distribution of sex among high risk group based on protection

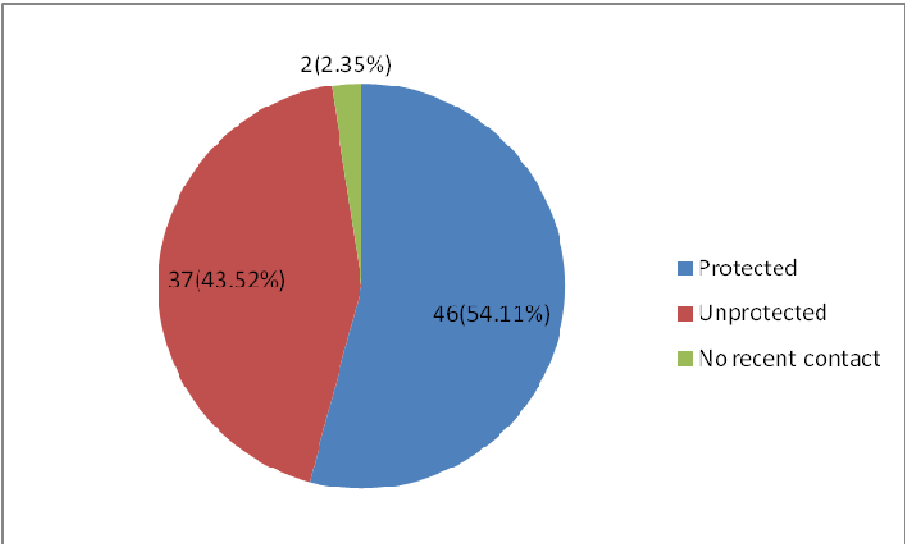
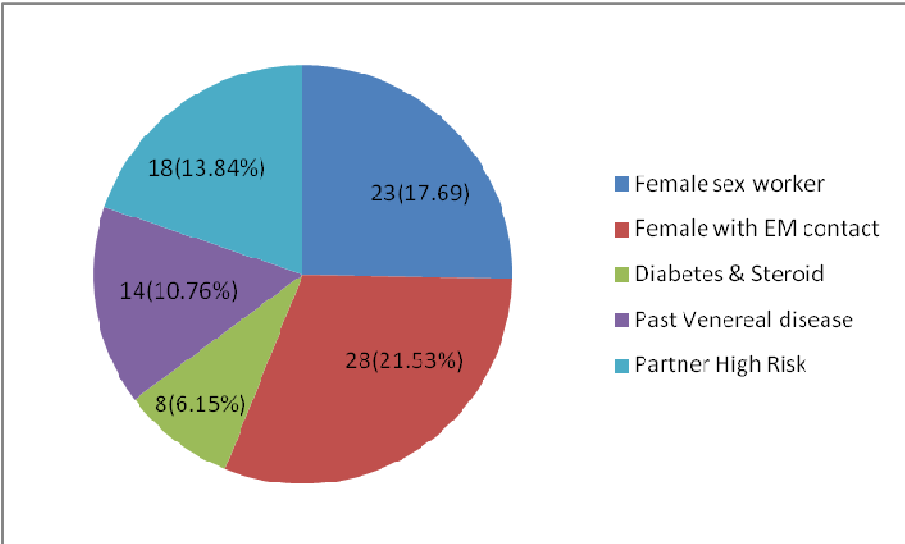


Figure 6: Distribution of risk factors in high risk group



BACTERIAL VAGINOSIS:

Table 7: Amsel criteria

Amsel	Number	%
≥ 3	46	35.38
< 3	84	62.22
Total	130	100

Amsel was significant in 35.38% of females studied.

Table 8: Nugent scoring:

Nugent score	Number	%
< 4	68	52.30
4-6	43	33.07
≥ 7	19	14.61
Total	130	100

Nugent score was suggestive of Bacterial vaginosis in 14.61% of females studied.

Table 9: Culture positivity of Gardnerella and Anaerobes :

Culture	Number	%
Positive	21	16.15
Negative	109	83.89
Total	130	100

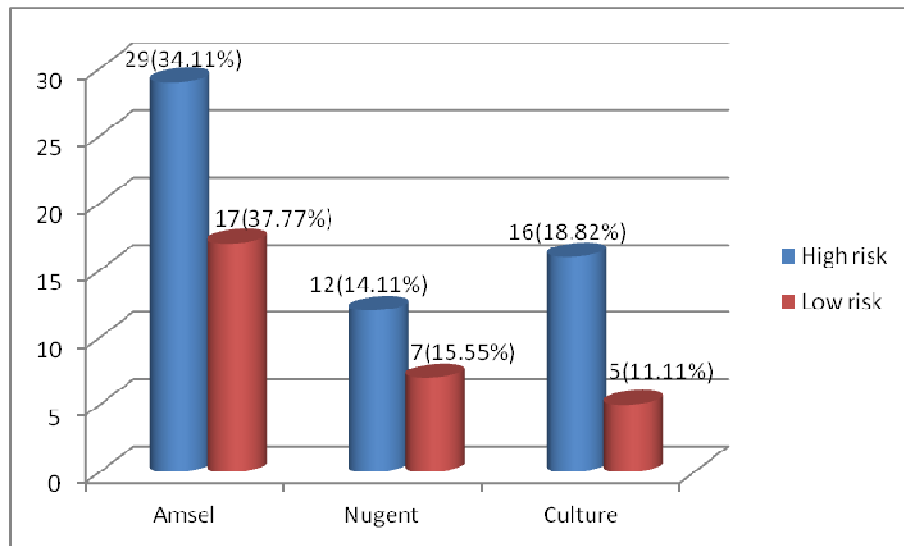
Culture was positive in 16.15% of females

Table 10: Comparison of positivity by Amsel, Nugent and Culture

n=130	Amsel	Nugent	Culture	χ^2	Value
High risk(85)	29	12	16	0.803	p>0.05
Low risk(45)	17	7	5		
Total	46	19	21		
n=130					
%	35.38	14.61	16.15		

All the three methods detected positives more in high risk group. Among the three methods Amsel identified more positives than other two methods.

Figure 7: Comparison of positivity by Amsel, Nugent and Culture
for Bacterial vaginosis



TRICHOMONIASIS

Table 11: Comparison of positivity by Wet mount, Giemsa and Culture

n=130	Wet	Giemsa	Culture	χ^2	Value
High risk (85)	5	4	6	1.125	P>0.05
Low risk (45)	2	1	2		
Total	7	5	8		
%	5.38	3.84	6.15		

The detection of positivity by Culture was more compared to Wet mount and Giemsa stain.

VULVOVAGINAL CANDIDIASIS

Table 12: Comparison of positivity by KOH, Gram stain, Culture

n=130	KOH	Gram	Culture	χ^2	Value
High risk (85)	24	26	36	11.021	P<0.05
Low risk (45)	12	15	15		
Total (n=130)	36	41	51		
%	27.69	31.53	39.23		

The detection of positivity by Culture was more and statistically significant than KOH mount (P<0.001).

Figure 8: Comparison of positivity by Wet mount, Giemsa and

Culture for Trichomoniasis

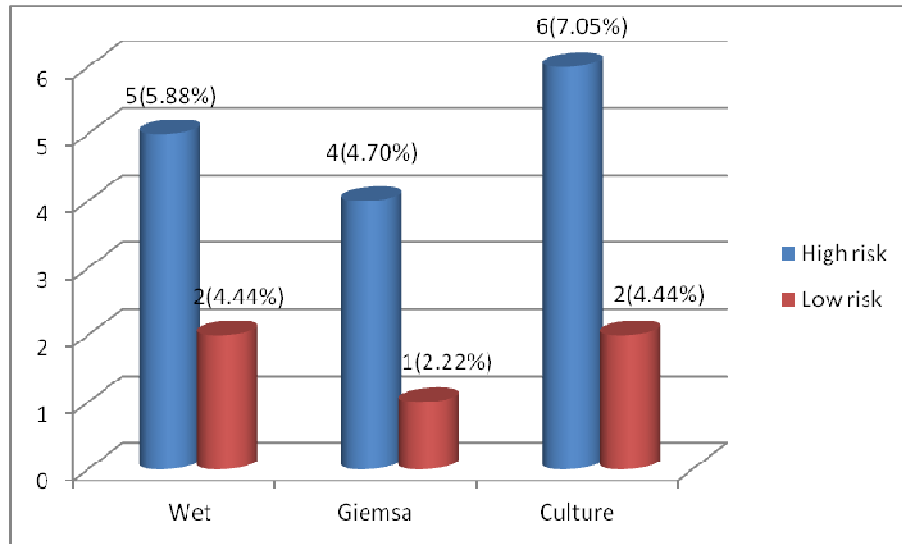
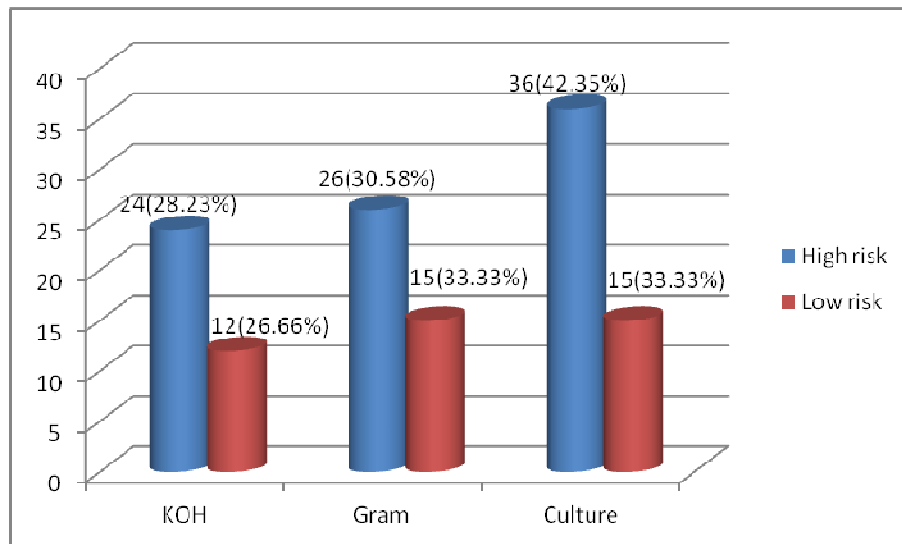


Figure 9: Comparison of positivity by KOH, Gram stain &

Culture for Candidiasis



N.GONORRHOEAE

Table 13: Comparison of positivity by Gram stain and Culture

n=130	Gram	Culture	Z	Value
High risk (85)	3	3	0.0	P=1.00
Low risk (45)	0	0		
Total (n=130)	3	3		
%	2.30	2.30		

Positives were seen only in high risk group and both the methods detected equal number of positives in our study.

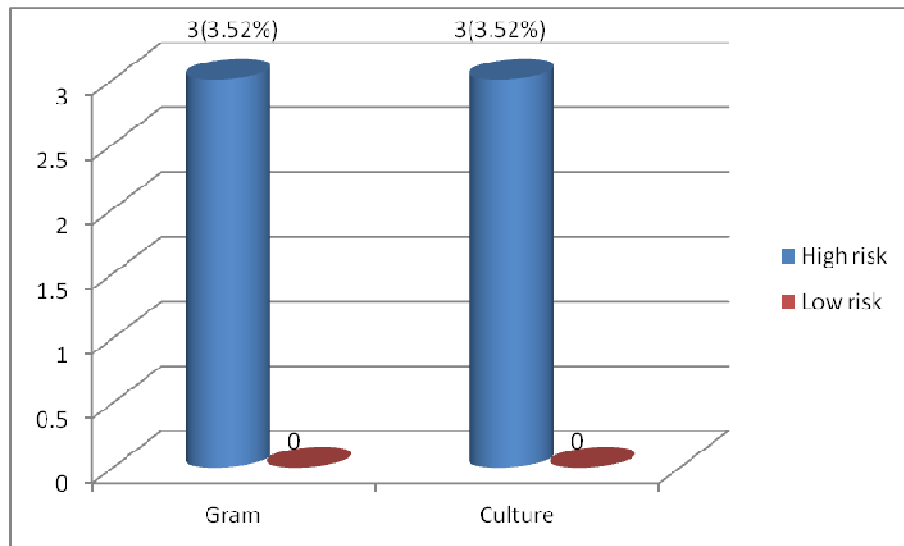
CHLAMYDIA TRACHOMATIS

Table 14: Comparison of positivity by Gram stain (number of pus cells) and Serology (ELISA)

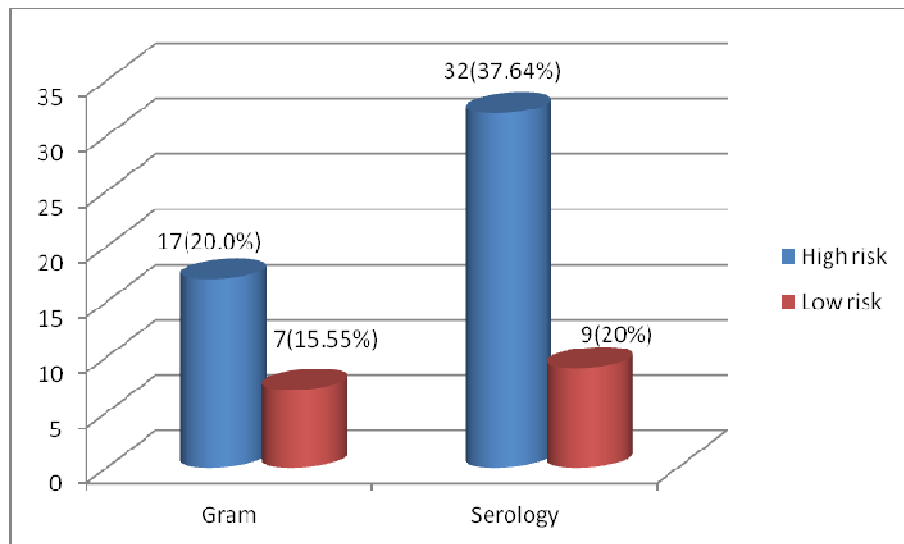
n=130	Gram	Serology	Z	Value
High risk (85)	17	32	2.368	p <0.05
Low risk (45)	7	9		
Total (n=130)	24	41		
%	15.38	31.53		

Serology identified more positives and was statistically significant in the low risk group (P<0.05).

**Figure 10: Comparison of positivity by Gram stain and Culture for
Gonococcal cervicitis**



**Figure 11: Comparison of positivity by Gram stain and Serology
for Chlamydial cervicitis**



AEROBIC BACTERIA

Table 15: Comparison of positivity by Gram stain and Culture:

n=130	Gram	Culture	Z	Value
High risk (85)	8	9	0.57	p>0.05
Low risk (45)	2	2		
Total (n=130)	10	11		
%	7.69	8.46		

Aerobic bacteria isolated were Staphylococcus aureus 3, Group A Streptococcus 2, Group B Streptococcus 2, Escherichia coli 3, Enterococci 1.

Table 16: Comparison of total result among high risk and low risk group

Results	High risk (85)	Low risk (45)	Total (130)	%	χ^2	Df	Significance
Positives	74	26	100	76.92	16.08	1	P<0.001
Negatives	11	19	30	23.07			
Total	85	45	130	100			

Out of 130 females studied, 100 (76.92%) of them showed positive for pathogenic isolates. Total positives were more in high risk than low risk group.

Figure 12: Comparison of positivity by Gram stain and Culture for

Aerobic vaginosis

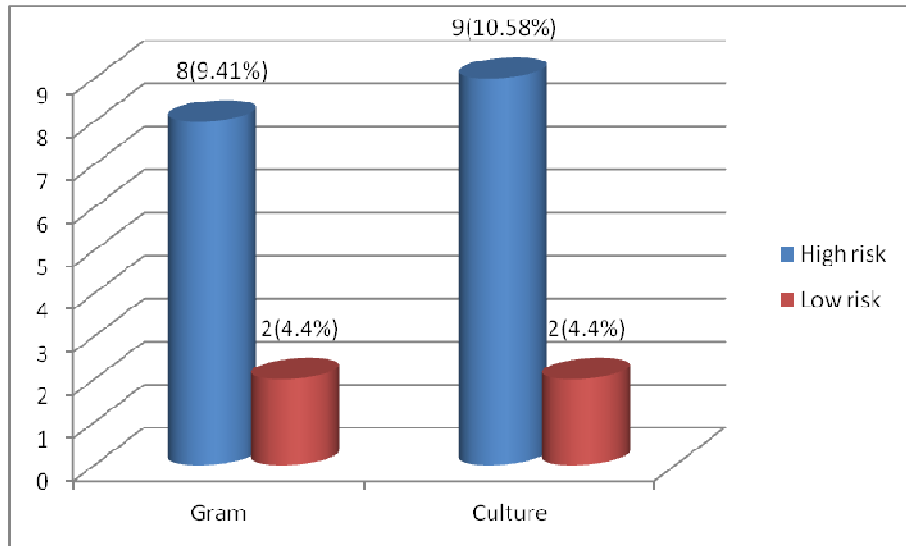


Table 17: Multiple isolates positivity among highrisk and lowrisk group

Results	High risk(85)	Low risk(45)	Total (130)	χ^2	Df	Significance
Mixed infection	30	10	40	2.985	1	P>0.05
Single infection	44	16	60			
Total positive	74	26	100			

Mixed infections were seen in 40 (40%) of total positives, more in high risk than low risk group.

Table 18: HIV, RPR reactivity & Tzanck smear positivity:

Total(n=130)	Reactive	Non reactive	Not tested
HIV	7(5.38%)	120(92.30%)	3(2.30%)
RPR	3(2.30%)	124(96.92%)	3(2.30%)
Tzanck	4(3.07%)	1(0.7%)	125(96.15%)

HIV, RPR reactivity and Tzanck smear positivity were seen exclusively in high risk group.

Figure 13: HIV reactivity

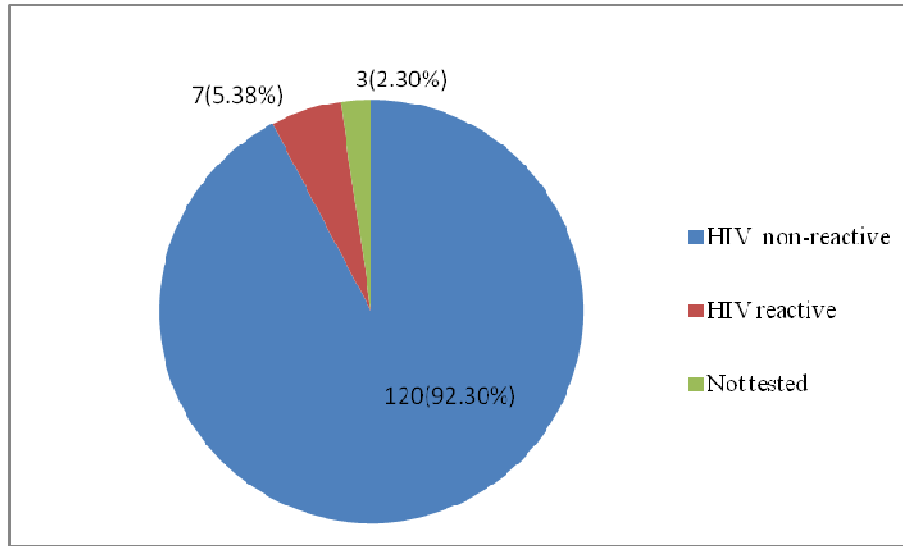


Figure 14: RPR reactivity

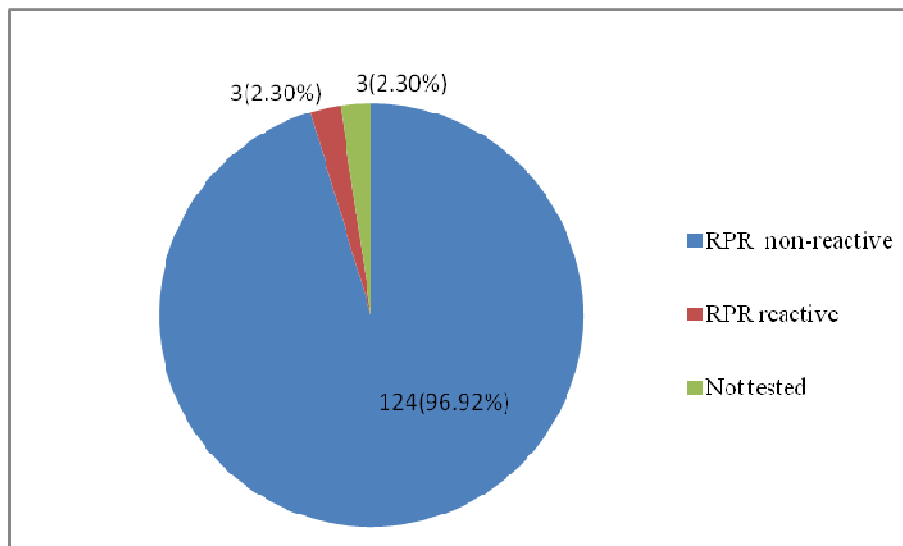


Figure 15: Tzanck smear positivity

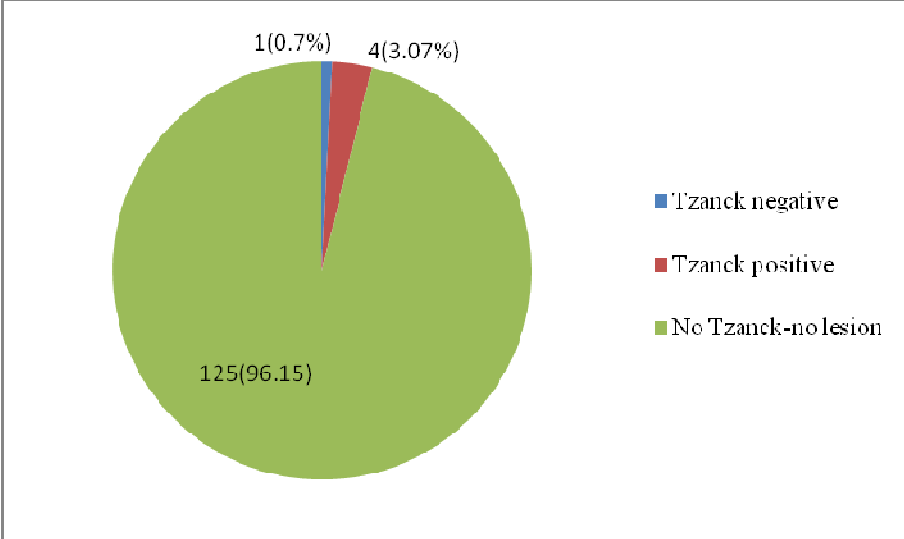


Table 19: Total number isolates among high risk and low risk groups

Isolates	High risk	Low risk	Total	%
<i>Gardnerella & Anaerobes</i>	16	5	21	14.38
<i>Trichomonas vaginalis</i>	6	2	8	5.47
<i>Candida</i>	42	17	59 ^a	40.47
<i>N.gonorrhoeae</i>	3	0	3	2.05
<i>Chlamydia</i>	32	9	41	28.08
<i>Aerobic bacteria</i>	9	2	11	7.53
<i>Herpes Simplex Virus</i>	4	0	4	2.73
Total	112*(76.71%)	34*(23.77%)	146*	100%

*Total number of isolates grown in Culture was more than the total number of patients studied, since multiple isolates were obtained from 30 high risk and 10 low risk individuals. ^aIn 8 patients with Candidiasis, more than one species of *Candida* was grown in Culture.

The Commonest etiology was *Candida* followed by *Chlamydia trachomatis*, *Gardnerella vaginalis* and *anaerobes*, *Aerobic bacteria*, *Trichomonas vaginalis* and *Neisseria gonorrhoeae* in order. Isolation of pathogens were more in high risk compared to low risk group.

Table 20: Follow up after treatment

Syndromic management(n=120)	N	%
Responded	105	87.5
Not responded	15	12.5

Out of 120 patients treated on the day of visit, 87.5% showed relief of symptoms and 12.5% continued to have symptoms.

Table 21: Co infection

Multiple Etiology(n=40)	Number	%
Vaginal+ cervical	15	37.5
Multiple vaginal	11	27.5
Multiple cervical	0	0
Discharge etiology+HIV	7	17.5
Discharge etiology+RPR	3	7.5
Discharge etiology+Tzanck	4	10
Total	40	100

Combined vaginal and cervical etiologies were identified in 37.5% of those who showed multiple etiologies.

Figure 16: Total isolates among high risk and low risk groups

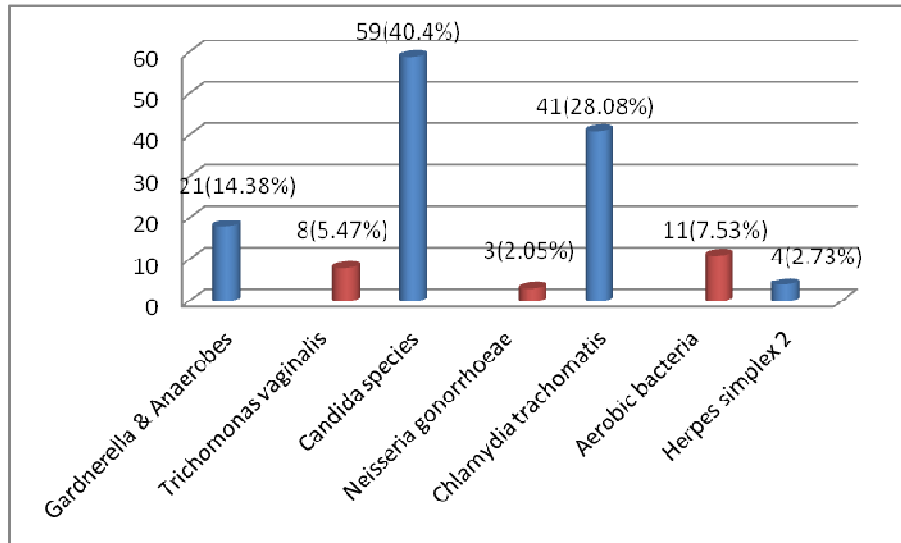


Figure 17: Follow up after treatment

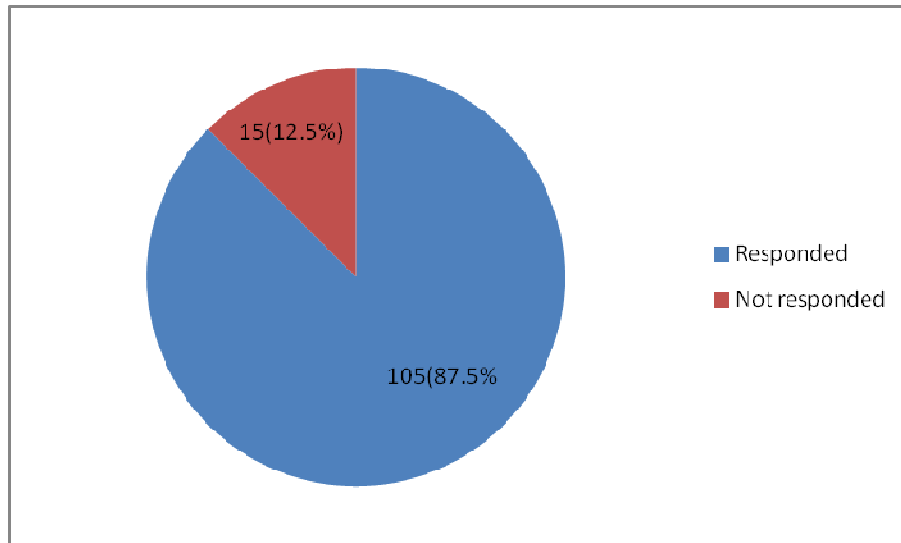


Table 22: Sensitivity pattern of isolates

Isolate (n)	Sensitive (%)	Resistant (%)
Gardnerella vaginalis (11)	Penicillin (19.0%) Metronidazole (100%) Vancomycin (100%)	Penicillin (81.81%) Metronidazole (0%) Vancomycin (0%)
Anaerobic bacteria (10)	Penicillin (30%) Vancomycin (80%) Metronidazole (100%)	Penicillin (70%) Vancomycin (20%) Metronidazole (0%)
Aerobic bacteria (11)	Co-trimoxazole (19.0%) Penicillin (28.28%) Gentamicin (28.28%) Cefataxime (0%) Amikacin (0%)	Co-trimoxazole (81.81%) Penicillin (72.72%) Gentamicin (72.72%) Cefataxime (0%) Amikacin (0%)
Candida (59)	Itraconazole (83.06%) Fluconazole (93.3%) Amphotericin B (100)	Itraconazole (16.94%) Fluconazole (6.77%) Amphotericin B (0%)
Neisseria gonorrhoeae (3)	Penicillin (66.67%) Ciprofloxacin (66.67%) Azithromycin (100%) Spectinomycin (100%) Ceftriaxone (100%)	Penicillin (33.33%) Ciprofloxacin (33.33%) Azithromycin (0%) Spectinomycin (0%) Ceftriaxone (0%)

Anaerobic isolates showed resistance to penicillin to a greater percentage, but metronidazole remained sensitive. Aerobic isolates showed resistance to co-trimoxazole in higher percentage. Among *candida* isolates resistance to Itraconazole (16.94%) was more than Fluconazole and Amphotericin B and 33.33% *N.gonorrhoeae* were resistant to Penicillin and Ciprofloxacin.

DISCUSSION

STIs rank the fourth common health problem in the global scenario, with an annual incidence exceeded only by diarrheal diseases, malaria and lower respiratory tract infections. In the developing world, STIs are one among the important causes of disability adjusted life years (DALYs) lost for women of sexually active age group, next to maternal causes and HIV.

In the present study, out of 130 female patients studied, 85(65.38%) were found to be highrisk individuals and 45(34.61%) were low risk individuals. Among the total patients studied, 100 (76.92%) of them had subjective vaginal discharge and 30(23.07%) of them had objective vaginal discharge (Table 1). This is similar to the observation in a study done by A Das et al ³ in which 68% were symptomatics and 32 % were asymptomatics.

The females in our study belonged to 15-49 years (sexually active) age group since this is the age group that is vulnerable for STIs. The age group of the population in our study was categorised based on decades and was found that, about 48(56.47 %) females in the highrisk group and 26(57.77%) females in the low risk group belonged to 30-39

years of age group. In our study 30-39 years was the commonest age group affected. A study done by Madeline Sutton et al ⁴⁸ showed that eventhough 15-20 years was the most common age group affected in their study (32%), positives were detected more in 30-39 years age group which comprised to about 20% of their study population. The mean age was 34.0 ± 7.5 years in high risk group and 31.4 ± 6.7 years in lowrisk group (Table 2). This is similar to the age group finding of 31.1 ± 6.9 years in study done by Ros anarlu ⁵⁹ and is different from the mean age of 28 ± 0.2 years in another study ²⁸.

Analysis of educational status of the females in our study showed that illiterates were more common in highrisk group (37.64%) compared to lowrisk group (28.88%). About 35.55% of females in lowrisk group had middle school level of education (Table 3). The percentage of illiterates in our study was higher than 26% seen in a study conducted in Mysore district of Karnataka by P Madhivanan et al ⁵⁴. The reason for difference in percentage of illiterates of our study and the other study may be due to the difference in literacy rates ⁷⁴ of the two states and the difference in study population. Madhivanan et al study was focussed on females of general population, our study was focussed on high risk females which included sex workers. Sexworkers

of our study gave lack of education and income as one of the reasons for entering sex work, which is supported by the report of study done by Z Fiona scorgie et al.⁷⁶

Married women were common in both the groups but widows and separated females were more in high risk group 5.88% each compared to low risk group (2.22%)(Table 4). Similar percentage of widows and separated females (8.45%) was seen in study conducted by Madeline Sutton et al.⁴⁸

In the present study, unprotected sex was carried out by 43.52 % of females (Table5). In the study done by A Das et al, the percentage of unprotected sex was found to be 70%, which is better than our observation. This emphasis the need for creating awareness regarding protective measures through health education campaigns among population subgroups especially commercial sex workers.

The most common high risk factor seen was housewife with extramarital contact followed by professional sex worker, female with highrisk partners, past venereal disease, diabetes and steroid intake in order (Table 6).

In the present study, Amsel criteria identified 35.38% of positives (Table 7) which is similar to a study by Aggarwal et al¹ where Amsel positivity was 40%.¹ In our study, Nugent score detected 52.30% as having normal flora, 33.07% as having intermediate flora and 14.61% are diagnosed as bacterial vaginosis (Table 8). A study conducted by P Madhivanan et al⁵⁴ showed 65.4% had normal flora, 15.4% had intermediate flora and 19.1% had bacterial vaginosis which supports the observation of our study. Culture detected 16.15% in our study (Table 9) which correlates well with the study by PS Rao et al⁵⁵ where culture positivity was 17.42%.

For diagnosis of bacterial vaginosis, positivity by Amsel's criteria (35.38%) was more than Culture (16.15%) followed by Nugent scoring (14.61%) (Table 10). A study by Marijane et al⁵⁰ showed Culture detected more positives followed by Amsel and Nugent in order. Initial difficulties faced in isolation of Anaerobes could be the reason for the lower percentage of culture positivity in our study.

For Trichomoniasis, Culture (6.15%) identified more cases than Wet mount (5.38%) than Giemsa stain (3.84%) (Table 11). The order of sensitivity of three methods of our study correlates well with the findings of the study done by Akujobi et al⁵ where the culture

(9.04%) was more sensitive than wet mount (8.01%) than Giemsa stain (6.71%) even though the positive percentage by all the three methods were slightly lower in our study. The reason could be due to variation in prevalence in various geographical areas.

For vulvovaginal candidiasis, culture (39.23%) identified more cases than gram stain (31.53%) than KOH mount (27.69%) (Table 12). This finding is supported by a study by Mirela et al⁵³ where culture detected 25.4% cases which is more than microscopy positivity (23.8%).

For Gonococcal cervicitis, both gram stain (2.30%) and culture (2.30%) detected equal number of cases (Table 13). Our study showed gram stain detected 100% of cases identified by culture which is different from the study done by Knud Hansen et al³⁸ showed endocervical gram stain had 47% sensitivity to diagnose gonococcal cervicitis.

For Chlamydia, Serology (31.53%) identified more cases than Gram stain (15.38%) (based on number of pus cells in endocervical smear) (Table 14). The reason could be due to the low sensitivity of Microscopy (17%) shown in a study done by Linda Myziuk⁴⁵ and non-

specificity of ELISA, since the antigen used was LPS (Lipopolysaccharide), non-specific antigen which may give false positive result with any LPS containing organism.

For Aerobic vaginosis, Culture (8.46%) detected more cases than Gram stain (7.69%) (Table 15). Though the aerobic bacteria were not sexually transmitted, they have been implicated as causative agents of vaginal discharge. A study conducted by Gilbert et al ²⁵ showed Culture positivity was 17% compared to the smear positivity of 7.9% which correlates with the order of sensitivity of the two methods in our study.

Out of 130 females studied, etiological agents of cervicovaginal discharge was identified in 100 (76.92%) females. Females in high risk group yielded more positives than females in low risk group. Multiple isolates was seen in 40 (40 %) of positives, which is more in high risk group than low risk group (Table 16 & 17).

In our study, HIV reactivity was found to be 5.38% , RPR (Rapid Plasma Reagin) reactivity was 2.30%, Tzanck smear positivity suggestive of Herpes simplex was 3.07% (Table 18). Genital herpes was diagnosed based on Tzanck smear only due to non availability of Herpes serological tests during the test period The detection of HIV in

patients with STI is important since they are at increased risk of getting HIV because of common mode of transmission for STI and HIV and also due to the reason that any STI increases the acquisition and transmission of HIV and the chances of acquiring STI in presence of HIV is also increased.

The total number of isolates obtained was 146, of which 112 (76.71%) isolates were in high risk group and 34 (23.77%) isolates were in the low risk group. The percentage of various isolates in our study were *candidia* 40.47%, *Chlamydia trachomatis* 28.08%, *Gardnerella vaginalis* and *anaerobes* 14.38%, *Aerobic bacteria* 7.53%, *Trichomonias vaginalis* 5.47 % and *N.gonorrhoeae* 2.05% (Table19). In a study by PS Rao⁵⁵ among reproductive age group women in rural areas ,the prevalence of vulvovaginal candidiasis was 10.69%, Bacterial vaginosis was 17.42%, Trichomoniasis was 1.18%, Gonococcal cervicitis was 0%. In a study conducted among female sex workers in Mumbai and Hyderabad ³, the prevalence of bacterial vaginosis was found to be 67.38%, *Trichomoniasis* 29.73%, *Vulvovaginal candidiasis* was 20.38%, Gonococcl cervicitis was 14.14% and Chlamydial cervicitis was 16.06%. The higher prevalence of positivity seen in Mumbai and Hyderabad could be due the reason

that their study population were professional sex workers of redlight area.

The follow up of patients showed that 87.5% of females showed complete relief of symptoms after treatment and 12.5% of females continued to have symptoms inspite of initial treatment given on the day of visit(Table 20)

The results of microbiological techniques showed that the reasons for treatment failure were existence of more than one infection in many of those females and resistant isolates in a few. Complete cure was achieved on providing specific treatment. Coinfection with cervical and vaginal etiologies were identified in 37.5% females (Table 21) suggesting that a combined treatment approach of vaginal discharge and cervicitis would be more useful for management of discharge syndrome in females. A study conducted by A Das et³ al had stated that female sex workers having vaginal discharge should be treated for both vaginal and cervical infectious etiology.

In the present study, about 70% of *Gardnerella vaginalis* and anaerobes were resistant to penicillin, aerobic isolates were resistant to cotrimoxazole, penicillin and gentamicin, 16.94% of *Candida* isolates were resistant to Itraconazole and 33.33% of *N.gonorrhoeae* were resistant to penicillin and ciprofloxacin (Table 22).

Establishing an etiological diagnosis helps to identify all etiological agents in coinfections thus helping to achieve complete cure. Also isolation of pathogen is essential for susceptibility testing in resistant and recurrent cases. Therefore the possibility of co infections and resistant pathogen should always be considered, atleast in treatment failure cases, which emphasises the need for isolation of the pathogen and susceptibility testing.

SUMMARY

This study was conducted to find out the prevalence of various etiological agents responsible for cervico vaginal discharge and to evaluate syndromic, clinical and etiological diagnosis. Total of 130 female patients were studied which included 85 high risk and 45 low risk females.

The various etiological agents of cervico vaginal discharge found were Vulvovaginal candidiasis 40.47%, Chlamydial cervicitis 28.08%, Bacterial vaginosis 14.38%, Aerobic vaginosis 7.53%, Trichomoniasis 5.47% and Gonococcal cervicitis 2.05%. The prevalence of all the etiological agents were found to be higher in high risk group compared to the low risk group.

For diagnosis of Bacterial vaginosis, Amsel's criteria detected more positives than Culture and Nugent score. For Trichomoniasis, culture identified more cases than wet mount and Giemsa stain. For Vulvovaginal candidiasis, Culture was better than Gram stain and KOH mount. For Gonococcal cervicitis, both Gram stain and Culture detected equal number of cases in our study. For Chlamydial cervicitis, Serology identified more cases than Gram stain. For Aerobic vaginosis, Culture was better than Gram stain.

Coinfections were seen in 40% of positives and combined cervical and vaginal infections were seen in 37.5 % of Coinfections. So this study suggests a combined approach of treating female patients for both vaginal discharge and cervicitis syndrome. HIV test reactivity was found to be 5.38% among the total study population

Anaerobic bacterial isolates showed resistance to penicillin, but were sensitive to Metronidazole. Aerobic isolates were resistant to Cotrimoxazole. Candida isolates showed resistance to Itraconazole more than Fluconazole and Amphotericin B. *Neisseria gonorrhoeae* showed resistance to penicillin and ciprofloxacin but third generation cephalosporins remained sensitive.

Syndromic management was successful in 87.5% of treated female patients and thus it can be considered as the treatment method in resource poor settings. Etiological diagnosis was considered as a better diagnostic approach than syndromic or clinical diagnosis which was evident from the outcome of 12.5% treatment failure cases, who showed complete relief of symptoms only after specific treatment was given, in addition to the initial syndromic management.

CONCLUSION

This work was done to study the various aspects of Cervico vaginal discharge and found that among the various etiologies of cervico vaginal discharge, Candidiasis was the commonest followed by Chlamydial cervicitis, Bacterial vaginosis, Aerobic vaginosis, Trichomoniasis and Gonococcal cervicitis in order.

Amsel criteria for Bacterial vaginosis, Culture for Trichomoniasis, Vulvovaginal candidiasis and Aerobic vaginosis, Serology for Chlamydial cervicitis were the methods which showed greater sensitivity compared to other methods. For Gonococcal cervicitis, both Gram stain and Culture were equally sensitive.

Coinfections were found in more than one third of total positive females suggesting a combined treatment method for vaginal and cervical etiologies.

Resistance to penicillin and co-trimoxazole was seen among the bacterial isolates in higher percentage with *Neisseria gonorrhoeae* showing resistance to penicillin and ciprofloxacin. Resistance to Itraconazole was seen among candida isolates.

Syndromic management still remains as the method of choice for treatment in resource poor settings. Etiological diagnosis should be achieved wherever laboratory facilities are available in order to

minimise treatment failure to prevent complications and disease spread. Thus Etiological diagnosis and appropriate management helps to achieve complete cure and prevent complications including infertility, malignancy and easy acquisition of HIV. Since high risk females form one of the core groups, effective treatment of these females, contact tracing, partner treatment and Health education to the high risk population can reduce the disease spread from core group to the general population significantly.

APPENDIX

1.BLOOD AGAR

Ingredients:

Nutrient agar 100ml

Sterile sheep blood 5 ml

Procedure:

Autoclave the nutrient agar base at 121°C for 15 minutes.

Cool to 45-50 °C and add blood with sterile precautions and distribute in petri dishes.

2.HUMAN BLOOD BILAYER TWEEN AGAR:

Columbia agar base

1% Proteose peptone

Tween 80 0.00075%

Amphotericin B 2.0 µg/ml

Prepare Basal medium , add the supplements ,pour in petri plates and overlay with 5% human blood.

3.MODIFIED CYSTEINE PETONE LIVERINFUSION MALTOSE MEDIUM

Ingredients:

Peptone 32 gms

Maltose 1.6 gms

L-Cysteine hydrochloride 2.4 gms

Liver digest 20 gms

Ringer's solution ,1/4 strength 1 lit

NaOH 1mol/lit 0.9 ml

Distilled water 900 ml

1. Mix the contents in distilled water.
2. Steam the medium at 100°C for 30 minutes.
3. Adjust pH to 6.0

Penicillin streptomycin solution:

Penicillin 0.06 gms

Streptomycin 0.1 gms

Sterile water 10 ml

Nystatin solution:

Nystatin 5×10^4 units

Sterile water 10 ml

Procedure:

Basal medium 90 ml

Sterile horse serum 10 ml

Penicillin streptomycin solution 1 ml

Nystatin solution 1 ml

Complete medium is prepared and distributed in sterile screw capped

4.SABOURAUD'S DEXTROSE AGAR

Ingredients:

Neopeptone 10.0 gm

Agar 15.0 gm

Dextrose 40.0 gm

Distilled water 1000 ml

Procedure:

1. Mix the ingredients in distilled water by boiling. Adjust pH to 5.6.
2. Sterilize by autoclaving at 115° C for 15 min.
3. Allow to cool to 50° C.
4. Pour into 15 ml test tubes.
5. Allow the test tubes to rest at an angle so that agar slopes (slants) are obtained.
6. After the medium solidifies, cotton wool stopped dextrose agar slants in the refrigerator.

5.CHOCOLATE AGAR

Ingredients:

Nutrient agar base 30 gm.

Sheep blood 100 ml.

Distilled water 1000 ml.

Procedure:

1.Dissolve 30 g of Columbia agar base to 1 litre of distilled water in a flask.

Heat in a stem sterilizer to dissolve it completely.

2.Adjust the pH to 7.5 – 7.6.

3.Sterilize by autoclaving at 121° C for 15 minutes.

4.Cool to 70° C in a water bath.

5.Aseptically add 100 ml of sheep blood to it and leave at 70° C for 30

minutes.

6. Mix the blood and agar by gentle agitation from time to time till blood

becomes chocolate brown in colour. This will take about 10 minutes.

7. Pour as slopes or plates in sterile tubes or sterile Petri dishes.

6. MODIFIED THAYER MARTIN MEDIUM

A selective medium for the isolation of *N. gonorrhoeae*.

Ingredients:

1. Modified Thayer Martin agar base 21 gm
2. 2% Hemoglobin solution 250 ml
3. Distilled water 450 ml.
4. VCNT inhibitor 10ml
 - i). Vancomycin 2.0 to 4.0 mg or lincomycin 1 mg/litre
 - ii. Colistin 300,000 units to 7.5 mg/litre
 - iii. Nystain 12.5 IU/ml
 - iv. Trimethoprim 2mg/litre.

Procedure:

1. Mix 21gms of MTM agar in 450 ml of distilled water by boiling.
2. Sterilize by autoclaving at 121° C for 15 minutes.
3. Cool to 50-55° C in a waterbath.
4. Aseptically add freshly prepared 250 ml of 2% hemoglobin solution and VCNT inhibitors and mix well.
5. Pour 20 ml of medium in a sterile Petri dish of 90 mm diameter under strict aseptic precautions.

6. Allow the medium to cool and store in refrigerator.

7. BUFFERED BALANCED SALT INDICATOR SOLUTION (BSS)

Ingredients:

Dipotassium hydrogen phosphate 0.4 gms

Potassium dihydrogen phosphate 0.1 gm

Potassium Chloride 8.0 gms

Phenol red indicator 0.6 gms

Distilled water 1000 ml

Procedure:

1. Mix the salts and indicator in distilled water.
2. Adjust the pH to 7.1 to 7.2.
3. Sterilise by Seitz filter.
4. Store in the refrigerator at 4°C.

8. PHOSPHATE BUFFERED SALINE:

Ingredients:

NaCl 9.0 gm

Potassium chloride 0.2 gms

Disodium hydrogen phosphate 1.15 gms

Potassium dihydrogen phosphate 0.2 gms

Distilled water 1000 ml

Mix the salts in distilled water.

Adjust the pH to 7.3.

9. MUELLER HINTON Agar

Ingredients:

Beef Infusion 300.00 gms

Casein acid hydrolysate 17.50 gms

Starch 1.50 gms

Agar 17.00 gms

Distilled water 1000 ml

Final pH at 25° C 7.3 ± 0.2 .

Procedure: Suspend 38 gms in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Mix well before pouring.

Pour 20-25ml of it into petridishes of 9cm diameter to give a thickness of 4 mm.

BIBLIOGRAPHY

1. A Aggarwal,P Devi,R Jain-Anaerobes in Bacterial vaginosis-IJMM,2003,21(2):124-126 (BRIEF)
2. A C S Machado,E M B Guimaraes,E Sakurai FCR Floravante ,W N Amarai , F C A Ives-High titres of chlamydia trachomatis antibodies in Brazilian women with tubal occlusion or previous ectopic pregnancy-Infectious diseases in obstetrics and gynaecology-vol 2007;24816: 6.
3. A Das,P Prabhakar,P Narayanan,G Neilsen, T Wi,S Kumta, G Rao,R Gangakhedkar ,A Risbud-Prevalence and assessment of clinical management of sexually transmitted infections among female sex workers in two cities of India-Infectious diseases in obstetrics and gynaecology, vol 2011;494769:pages 8
4. A J Kanwar-Clinical approach to vaginal /urethral discharge-IASSTD 2 nd edition,pg 777-789
5. Akujobi.C.N,Ojukwu.C.L-Comparision of Saline wet preparation ,Giemsa staining and culture methods for the

detection of *Trichomonas vaginalis*-African Journal of Clinical and Experimental Microbiology, 2006;Vol 7(3):208-211

6. Alice Reyn-Antibiotic sensitivity of Gonococcal strains isolated in the south –east Asia and Western pacific regions in 1961-68- Bull Wld Hlth Org 1969;40:257-262
7. Bailey and scott- Laboratory methods in basic mycology- Diagnostic Microbiology-12 th edition; pg 629-713,procedure 50-13
8. Bailey and scott -Laboratory considerations- Diagnostic Microbiology-12 th edition;chapter 44,pg 463-477.
9. Bailey and scott-Genital tract infections- Diagnostic Microbiology-12 th edition;chapter 58,pg 843-871
10. Barbara Van Der Pol,Cynthia Kwok,Bosny Pierre –Louis,Anne Rinaldi, Robert A.Salata,Pai-Lien Chen,Janneke Van de Wijgert, Fran is Mmiro,Roy Mugerwa,Tsungai Chipato and Charles S Morrison,*Trichomonas vaginalis* infection and Human Immunodeficiency Virus Acquisition in African women-The Journal of Infectious diseases 2008:548-54

11. Bingham JS, Historical aspects of sexually transmitted infections
–Sexually transmitted infections ,1 st edition pg 5-17
12. C S Jones, P A C Maple N J Andrews I D Paul, E O Caul-
Measurement of IgG antibodies to chlamydia trachomatis by
commercial enzyme immunoassays and immunofluorescence in
sera from pregnant women and patients with infertility ,Pelvic
inflammatory disease ,ectopic pregnancy and laboratory
diagnosed chlamydia psittaci/chlamydia pneumonia infection-J
Clin Pathol 2003;56:225-230
13. Carol A. Spiegel, Richard Amsel , King K. Holmes –Diagnosis of
Bacterial vaginosis by Direct gram stain of vaginal fluid-
JCM, July 1983;170-177
14. Celine Costello Daly, Irving Hoffman, Marcia Hobbs , Martin
Maida , Dick Zimba, Rachael Davis , G. Mughogho, Myron
S. Cohen –Development of an Antimicrobial susceptibility
surveillance system for Neisseria gonorrhoeae in
Malawi: Comparison of Methods-JCM , Nov 1997; 2985-2988
15. Centres for Disease Control and Prevention-Sexually
Transmitted Diseases Surveillance-2009-Atlanta: US department
of health and human services:2010

16. D Heather watts,Gayle springer,Howard Minkoff,Sharon Hillier,Lisa Jacobson,Michael moxley,Jessica justman,Helen cejtin, Casey O Connell ,Ruth M Greenblatt-The occurrence of vaginal infections among HIV-infected and High risk – uninfected women-Longitudinal findings of women’s interagency study HIV study-J Acquir Immune Defic Syndr – 2006;43(2)
17. Devinder M Thappa,Balaji Adityan-Bacterial vaginosis- IASTD- 2nd edition pg 398-404
18. Devinder M Thappa-Historical aspects of sexually transmitted diseases and AIDS-IASTD 2nd edition pg 70-83
19. Donald P Orr,Krystal Johnson,Edward brizendine,Barry katz,Dennis fortentberry-Subsequent sexually transmitted infection in urban adolescents and young adults-Arch pediatr adolesc med vol 155,aug 2001
20. Elizabeth Taylor,I.Phillips-The identification of Gardnerella vaginalis-J.Med.Microbiol,1983;16:83-92
21. Erik kihlstrom, Richard lindgren ,Gunnar ryden –Antibodies to chlamydia trachomatis in women with infertility ,Pelvic

inflammatory disease and ectopic pregnancy-European journal of obstetrics and gynaecology and reproductive biology,1990;35:199-204

22. Francis A Plumer,Roel A Countino,Elizabeth N Ngugi,Stephen Moses-Sexworkers and their clients in the epidemiology and control of sexually transmitted diseases –King holmes sexually transmitted diseases 3 rd edition Chapter 10,pg 143-150
23. Frank sorvillo,Andrea kovacs,Peter kerndt,Alice stek,Laila muderspach and Lorriane sanchez-keeland,Risk factors for Trichomoiasis among women with Human Immunodeficiency Virus(HIV) infection at a public clinic in Los Angeles county,California:Implications for HIV Prevention-Am J Trop Med Hyg,1998,58(4):495-500
24. George Schmid ,Lauri Markowitz,Riduan Joesoef,Emily Koumans,Bacterial vaginosis and HIV infection-
25. Gilbert G.G.Donders,Annie vereecken,Eugene Bosmans, Alfons Dekeersmaecker,Geer salembier,Bernard spitz –Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis :aerobic vaginitis-BJOG,Jan 2002;109: 34-43

26. Gregory T Spear, Elizabeth St John and M Reza Zariffard, Bacterial vaginosis and Human Immunodeficiency Virus infection –AIDS Research and Therapy 2007;4:25 .1-5
27. Henn EW, Kruger TF, Siebert TI- Vaginal discharge reviewed: the adult pre-menopausal female - SA Fam Pract 2005;47(2): 30-38
28. Inayat magasi, Shahida sheikh, Saleem akhter sheikh Vaginal discharge syndromic management among internally displaced women living in camps at Larkana-Professional Med J Oct-Dec 2011;18(4): 598-603
29. Jabeen N, Soomro U- Bacterial vaginosis. Gynaecologist, 2001;5:56-7.
30. Jagdish chander- Candidiasis-Textbook of medical mycology- Third edition; Chapter 20:pg 267-290
31. Jagdish chander- Fungal culture media-Textbook of medical mycology-Third edition; appendix:pg 508-513
32. Jeanne M Marrazzo, King H Holmes-Sexually transmitted infections:overview and clinical approach Harrison's textbook of medicine,chapter 130,pg 1095-1109

33. Jennifer R.Stark,Gregory Judson,John F Alderete,Vasantha Krishna Mundodi,Ashwin S Kucknoor, et al.Prospective study of *Trichomonas vaginalis* infection and prostate cancer incidence and mortality-Physician's health study- JNCI 2009;Vol. 101, Issue 20 ,October 21
34. Jill S.Huppert,Bryon E Batteiger ,Phillip Braslins,James A Feldman,Marcia M. Hobbs,Heather Z.Sankey, Arlene C.Sena,Karen A Wendel-Use of an Immunochromotographic Assay for rapid detection of *Trichomonas vaginalis* in vaginal specimens-JCM,2005:684-687
35. JLS Jolly –Minimal criteria for the identification of *Gardnerella vaginalis* isolated from the vagina-J Clin Pathol 1983;36:476-478
36. Jose A ,Simoes,Farhad B.Hashemi,Alla A.Aroutcheva,Ira Heimler,Gregory T Spear,Susan Shott and Sebastian Faro,Human Immunodeficiency Virus type 1 stimulatory activity by *Gardnerella vaginalis* :Relationship to biotypes and other pathogenic charecteristics- The Journal of Infectious diseases 2001;184:22-7

37. King K Holmes ,Walter E Stamm-Lower genital tract infection syndromes in women King holmes sexually transmitted diseases 3rd edition ,pg 761-779
38. Knud Hansen CR, Dallabetta GA,Reichart C, Pabst KM et al- Surrogate markers to diagnose gonococcal and chlamydial cervicitis-comparision of leukocyte esterase dipstick,Endocervical gram stain and culture-Sex Trans Dis 1991,oct-dec:18(4);211-6
39. Koneman- Gardnerella vaginalis-Colour atlas and Textbook of Diagnostic Microbiology-6th edition-pg 834-838
40. Koneman- The laboratory identification of yeasts-Colour atlas and Textbook of Diagnostic Microbiology-6th edition-pg 1216-1237
41. Laga M,Alary M,Nzila N et al,Condom promotion,Sexually transmitted diseases treatment and declining incidence of HIV-1 infection in female zairian sex workers.Lancet 1994;344:246-8
42. Landon Myerl,Lynette Denny,Robin Telerant,Michelle de Souza,Thomas C.Wright Jr,Louise Kuhn,Bacterial vaginosis and

susceptibility to Hiv infection in south African women:A nested case control study-

43. Larry G .Reimer,Barth Reller-Use of a sodium polyanetholsulfonate disc for the identification of Gardnerella vaginalis -JCM,1985; 146-149
44. Laura H.Bachmann,Marcia M. Hobbs,Arlene C.Sena,Jack D.Sobel,Jane R Schwebke ,John N K reiger,R Scott McClelland,Kimberly A.Workowski-Trichomonas vaginalis Genital infections:Progress and challenges-Clinical Infectious Diseases 2011;53(S3):S160-72
45. Linda Myziuk,Barbara Romonawski,Myra brown-Endocervical gram stain smears and their usefulness in the diagnosis of Chlamydia trachomatis –Sex Transm Inf 2001;77:103-106
46. Lucio Pattullo,Sarah Griffeth,Lili Ding,Joel Mortensen,Jennifer Reed,Jessica Kahn, Jill Huppert-Stepwise diagnosis of Trichomonas vaginalis in adolescent women-JCM,Jan 2009; 59-63
47. Mackie & Mc Cartney Practical Medical Microbiology-14 th edition

48. Madeline Sutton, Maya Stenberg, Emilia H Koumans, Geraldine Mc Quillan, Stuart Berman- The Prevalence of *Trichomonas vaginalis* infection among reproductive –age women in the United states, 2001-2004-*Clinical Infectious Diseases* 2007;45:1319-26
49. Marcia M Hobbs, Arlene C Sena- Methods for detection of *Trichomonas vaginalis*- *JCM*, June 1993, p 266-271
50. Marijane A. Krohn, Sharon L. Hiller, David A. Eschenbach- Comparison of Methods for Diagnosing Bacterial vaginosis among pregnant women-*JCM*, June 1989, p 1266-1271
51. Maysaa El Sayed Zaki, Douaa Raafat, Wafaa El Emshaty, Manar Sobh Azab, Hossam Goda, Correlation of *Trichomonas vaginalis* to bacterial vaginosis : a laboratory-based study-*J Infect Dev Ctries* 2010;4(3):156-163
52. Mc Clelland RS, Sangare L, Hassan WM, et al. Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. *J infect Dis* 2007;195:698-702.
53. Mirela Babic, Mirsada Hukic –*Candida albicans* and Non-*albicans* species as etiological agent of vaginitis in pregnant and

Non-pregnant women-Bosnian Journal of Basic medical sciences 2010;10(1):89-97

54. P Madhivanan,K Krupp,V Chandrasekaran,C Karat,A Arun,CR Cohen,AL Reingold,JD Klausner-Prevalence and Correlates of bacterial vaginosis among young women of reproductive age in Mysore,India-IJMM,2008;26(2):132-7
55. PS Rao,S Devi, A Shriyan, M Rajaram, K Jagdishchandra-Diagnosis of Bacterial vaginosis in rural setup: Comparision of clinical algorithm,smear scoring and culture by semiquantitative technique- IJMM,2004;22(1):47-50
56. R Scott McClelland,Laura Sangare,Wisal M Hassan,Ludo Lavreys,Kisorchandra Mandaliya,James Kirarie,Jeckoniah Ndinya-Achola,Walter Jaoka and Jared M .Baeten,Infection with Trichomonas vaginalis increases the risk of HIV-1 acquisition - The Journal of Infectious diseases 2007;195:000-000
57. Rekha, S. Jyothi -Comparison of visual, clinical and microbiological diagnosis of symptomatic vaginal discharge in the reproductive age group-Int J Pharm Biomed Res 2010; 1(4): 144-148

58. Rippon JW et al Medical mycology –Th pathogenic fungi and the pathogenic actinomycetes.3 rd edition WB Saunders,1988
59. Ros Anorlu,Donald Imosemi,NKIRU Odunukwe et al- Prevalence of HIV among women with vaginal discharge in gynaecological clinic- Journal of the national medical association 2004 ;vol 96,No 3,Mar 367
60. S.Cu-vin ,Joseph W .Hogan,A.M.Caliendo,J.Harwell,K.H.Mayer and C.C.J.Carpenter,Association between bacterial vaginosis and Expression of HumanImmunodeficiency Virus type 1 RNA in the female genital tract-Clinical infectious diseases,33(6):894-896.
61. Sandra Aparecida Marinho,Alice Becker Teixeira,Otavio Silveira Santos,Ricardo Flores Cazanova,Carlos Alexandre Sanchez Ferreira,Karen Cherubini,Silvia Dias de Oliveira- Identification of candida spp by phenotypic tests and PCR- Brazialian Journal of Microbiology 2010;41:286-294
62. Sandra S.Richter ,Rudolph P.Galask,Shawn A.Messer ,Richard J.Hollis,Daniel J.Diekema,Michael A.Pfaller-Antifungal susceptibilities of candida species causing vulvovaginitis and Epidemiology of recurrent cases- JCM May 2005; 2155-2162

63. Seema Sood,Arti Kapil-An update on Trichomonas vaginalis-
Indian J Sex Transm Dis 2008 ;Vol 29,No .1
64. Shazia A Khan,Fauzia Amir,Shagufta Atlaf,Raazia Tanveer-
evaluation of common organisms causing vaginal discharge-J
Ayub Med Coll Abbottabad 2009;21(2).
65. Thami P Gurvindar-Applied anatomy of male and female
reproductive tract-IASTD 2 nd edition ,pg240-258
66. Usha Gupta ,D K Gupta –Non gonococcal urethritis and
chlamydial infections- IASTD- 2nd edition pg 368-383
67. V K Desai, J K Kosambiya, H G Thakor, D D Umrigar, B R
Khandwala, K K Bhuyan-Prevalence of sexually transmitted
infections and performance of STI syndromes against
aetiological diagnosis, in female sex workers of red light area in
Surat, India-Sex Transm Infect 2003;79:111–115
68. Veena Manjunath,Vidya GS,Archana sharma,Mridula Raj
Prakash,Murugesh-Speciation of candida by Hichrome agar and
sugar assimilation test in both HIV infected and non infected
patients-Int J Biol Med Res 2012;(2):1778-1782

69. Vikram Singh, Manju Bala, Monika Kakran, V Ramesh- Comparative assessment of CDS ,CLSI disc diffusion and Etest techniques for Antimicrobial susceptibility testing of neisseria gonorrhoeae:6 year study-BMJ open 2012
70. Vinod K Sharma, Jyoti Dhawan , Trilokraj Tejasvi-Trichomoniasis and other protozoal diseases-IASTD- 2nd edition pg 488-496
71. VS Dorairaj, K Venkateswaran-Gonorrhoea-IASTD -2nd edition.Pg-358-366
72. Wadicho¹, D. Z. Egah², O. O. Alao³ and E. Iheanacho- Risk factors for vaginal candidiasis among women attending primary health care centers of Jos, Nigeria- Journal of Clinical Medicine and Research Vol. 2(7), pp. 110-113, July 2010
73. World health organisation. World health report 1998. Geneva :WHO 1998.
74. www.mapsofindia.com, Census 2011, Literacy rate in India
75. Zachariah R, Spielman MP, Harries et al –Sexually transmitted infections and sexual behaviour among commercial sex workers in rural district of Malawi-International Journal of STD & AIDS 2003; 14: 185–188

76. Z fiona scorgie,Matthew F-Socio demographic characteristics and behavioural risk factors of female sex workers in sub-Saharan Africa-Systematic review-AIDS Behav 2011:Substative review

PROFORMA

Name:

Age:

Address:

STD OP number.:

Micro ID no:

Presenting complaints:

Menstrual history:

Sexual history:

Personal history:

H/O previous STD:

H/O diabetes / Steroid intake:

Treatment history:

For Past & present STD

EXAMINATION

General examination :

Genital examination:

Other system examination :

INVESTIGATIONS:

Discharge from speculum

- AMSEL criteria

Character of the discharge

pH

amine odour on addition of 10%

KOH

- Saline mount
- KOH mount

High vaginal swab 1: Gram stain: Nugent score

Modified Donder's score

High vaginal swab 2: culture for *Gardnerella vaginalis* &
Anaerobes

High vaginal swab 3: culture for *Trichomonas vaginalis*

High vaginal swab 4: culture for *Candida*

Endocervical swab 1: Gram stain for cervicitis and
intracellular diplococci

Endocervical swab 2: culture for *Neisseria gonorrhoeae*

Geimsa stain – Tzanck smear for Herpes simplex virus

Serum for HIV testing

ELISA – Chlamydia IgG &IgM antibodies

Rapid plasma reagin test for syphilis

Diagnosis:

Drug kit given:

Follow up:

KEY TO MASTER CHART

Sub dis-Subjective vaginal discharge

Obj dis-Objective vaginal discharge

FSW-Female sex worker

Mul con-Multiple contact

Last MC-last Marital contact

Last EMC-last Extramarital contact

Dia & ste-Diabetes and steroid intake

Past VD-Past venereal disease

Partn HR-Partner High risk

HIV-Human Immunodeficiency virus infection

RPR-Rapid plasma reagin test

Wet-Wet mount

KOH-KOH mount

Ams-Amsel criteria

Nug-Nugent score

Giem-Giemsa stain

GS- v,c smear-Gram stain of vaginal &cervical smear

Ae.ba-Aerobic bacteria

An.ba-Anaerobic bacteria

P/A-Penicillin/Ampicillin

E/G-Erythromycin/Gentamicin

Co-Cotrimoxazole

Ak-Amikacin

M-Metronidazole

V-Vancomycin

Gono-Neisseria gonorrhoeae

C-Ciprofloxacin

T-Tetracycline

Az-Azithromycin

S-Spectinomycin

Ce-Ceftriaxone

TV-Trichomonas vaginalis

F-Fluconazole

I-Itraconazole

Am-Amphotericin B

GPC-Gram positive cocci

GNC-Gram negative cocci

GNB-Gram negative bacilli

Yea-Yeast cells

PSE-Pseudohyphae

GV-Gardnerella vaginalis

MOB-Mobiluncus

Peptost- Pepto streptococcus

E.coli-Escherichia coli

S.aureus-Staphylococcus aureus

NG-No growth

S-Sensitive

R-Resistant

C.al-C.albicans

C.pa-Candida parapsilosis

C.kr-Candida krusei

C.tr-Candida tropicalis

C.gl-Candida glabarata

Tzan-Tzanck smear

Chla-Chlamydia trachomatis IgM,IgG




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