MICROBIOLOGICAL PROFILE OF LOWER GENITAL TRACT INFECTIONS IN WOMEN OF REPRODUCTIVE AGE GROUP WITH SPECIAL REFERENCE TO CHLAMYDIA TRACHOMATIS BY REALTIME PCR ASSAY IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

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for the award of the degree of

M.D. (MICROBIOLOGY)

BRANCH – IV

CHENGALPATTU MEDICAL COLLEGE

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CERTIFICATE

This is to certify that this dissertation titled **"MICROBIOLOGICAL PROFILE OF LOWER GENITAL TRACT INFECTIONS IN WOMEN OF REPRODUCTIVE AGE GROUP WITH SPECIAL REFERENCE TO CHLAMYDIA TRACHOMATIS BY REALTIME PCR ASSAY IN A TERTIARY CARE HOSPITAL''** is a bonafide record of work done by **DR.M.MALATHI.,** during the period of her Post graduate study from 2013 to 2016 under guidance and supervision in the Department of Microbiology, Chengalpattu Medical College and Hospital, Chengalpattu – 603 301 in partial fulfillment of the requirement for **M.D. MICROBIOLOGY** degree Examination of The Tamil Nadu Dr. M.G.R. Medical University to be held in April 2016.

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DECLARATION

I declare that the dissertation entitled **"MICROBIOLOGICAL PROFILE OF LOWER GENITAL TRACT INFECTIONS IN WOMEN OF REPRODUCTIVE AGE GROUP WITH SPECIAL REFERENCE TO CHLAMYDIA TRACHOMATIS BY REALTIME PCR ASSAY IN A TERTIARY CARE HOSPITAL"** submitted by me for the degree of M.D. is the record work carried out by me during the period of **June 2014 to June 2015** under the guidance of Professor **DR.A.VIJAYALAKSHMI ,M.D.,M.B.A.,(HM)** Professor & HOD, Department of Microbiology, Chengalpattu Medical College, Chengalpattu. This dissertation is submitted to The TamilnaduDr.M.G.R. Medical University, Chennai,in partial fulfillment of theUniversity regulations for the award of degree of M.D.,Microbiology (Branch IV) examinations to be held in April 2016.

Place: Chengalpattu **Signature of the Candidate**

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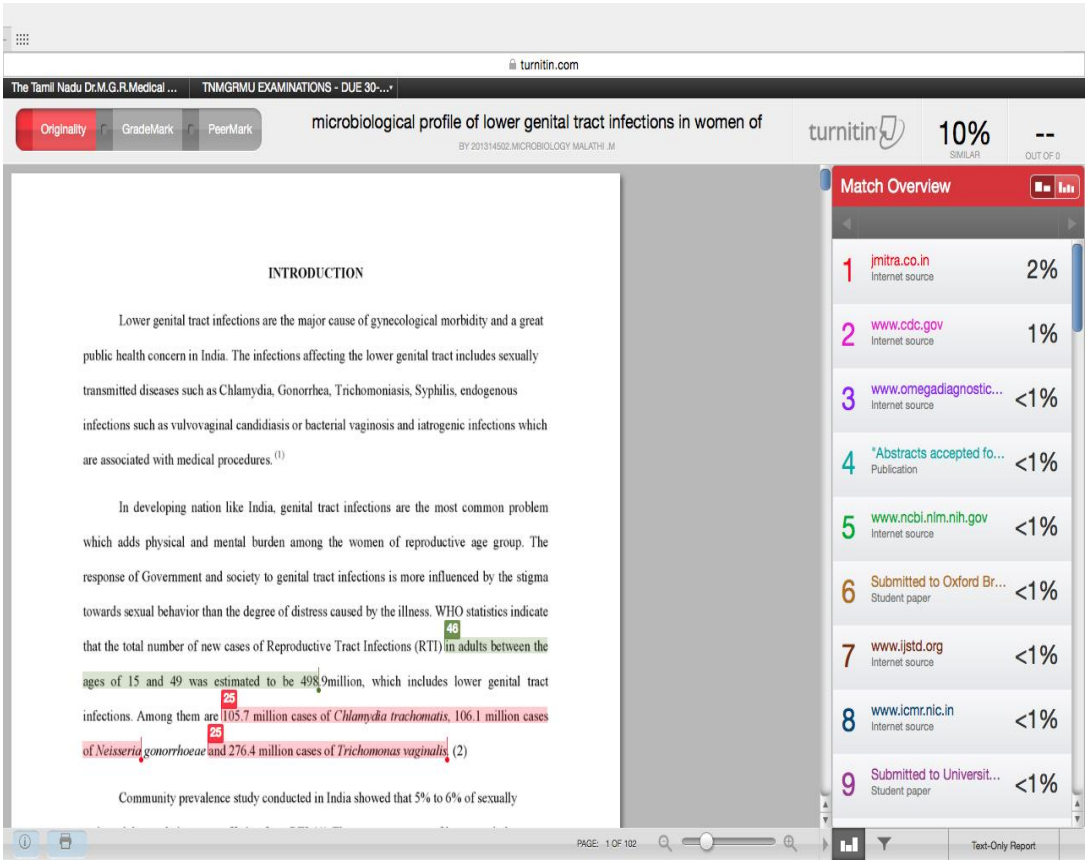
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INTRODUCTION
Lower genital tract infections are the major cause of gynecological morbidity and a great public health concern in India. The infections affecting the lower genital tract includes sexually transmitted diseases such as Chlamydia, Gonorrhea, Trichomoniasis, Syphilis, endogenous infections such as vulvovaginal candidiasis or bacterial vaginosis and iatrogenic infections which are associated with medical procedures. (1)
In developing nation like India, genital tract infections are the most common problem which adds physical and mental burden among the women of reproductive age group. The response of Government and society to genital tract infections is more influenced by the stigma towards sexual behavior than the degree of distress caused by the illness. WHO statistics indicate that the total number of new cases of Reproductive Tract Infections (RTI) in adults between the ages of 15 and 49 was estimated to be 498.9million, which includes lower genital tract infections. Among them are 105.7 million cases of Chlamydia trachomatis, 106.1 million cases of Neisseria gonorrhoeae and 276.4 million cases of Trichomonas vaginalis (2)
Community prevalence study conducted in India showed that 5% to 6% of sexually
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ABSTRACT

MICROBIOLOGICAL PROFILE OF LOWER GENITAL TRACT INFECTIONS IN WOMEN OF REPRODUCTIVE AGE GROUP WITH SPECIAL REFERENCE TO CHLAMYDIA TRACHOMATIS BY REALTIME PCR ASSAYIN A TERTIARY CARE HOSPITAL

Background and Aims:

Lower genital tract infections are the major cause of gynecological morbidity and a great public health concern in India. Inadequate laboratory diagnostic facilities in all the levels of health care, stigma and discrimination associated with RTI services are some of the reasons of lack of exact incidence/prevalence rate of RTI in India. Hence this study is conducted to assess the microbiological profile of lower genital tract infections in women of reproductive age group with special reference to *Chlamydia trachomatis* by Real Time PCR assay in a tertiary care hospital

Methodology:

Women with symptoms suggestive of lower genital tract infections were selected and vaginal swab, endocervical swab and 5ml blood sample were collected after obtaining detailed history and informed consent from the patient. Vaginal swab was used for evaluation by Gram staining and culture for bacterial and fungal pathogens. Endocervical swab was subjected to culture for Neisseria gonorrhea and Real time PCR analysis of Chlamydia trachomatis. The serum sample was subjected to analysis for HBV, HCV, HIV, HSV and syphilis..

Results:

Out of 110 subjects, 43 subjects (39.09%) were identified with laboratory findings for lower genital tract infections. 18.75% of *Chlamydia trachomatis* was identified by Real

time PCR assay. Factors such as women with age ≤ 30 years, parity belonging to L2 group and IUCD users were seem to have significant influence in the outcome.

Conclusion:

Laboratory diagnosis for women with symptoms and signs is suggested when compared to syndromic approach. Screening for sexually transmitted infections by serological methods are mandatory in women with symptoms and signs of genital tract infections.

Keywords:

Lower genital tract infections, *Chlamydia trachomatis*, Real time PCR, Reproductive tract infections (RTI)

TABLE OF CONTENTS

INTRODUCTION

Lower genital tract infectionsare the major cause of gynecological morbidity and a great public health concern in India. The infections affecting the lower genital tract includes sexually transmitted diseases such as Chlamydia, Gonorrhea, Trichomoniasis, Syphilis, endogenous infections such as vulvovaginal candidiasis or bacterial vaginosis and iatrogenic infections which are associated with medical procedures. [(1)]

In developing nation like India, genital tract infections are the most common problem which adds physical and mental burden among the women of reproductive age group. The response of Government and society to genital tract infections is more influenced by the stigma towards sexual behavior than the degree of distress caused by the illness. WHO statistics indicate that the total number of new cases ofReproductive Tract Infections (RTI) in adults between the ages of 15 and 49 was estimated to be 498.9million including lower genital tract infections. Among them are 105.7 million cases of *Chlamydia trachomatis*, 106.1 million cases of *Neisseria gonorrhoeae* and 276.4 million cases of *Trichomonasvaginalis.* [(2)]

Community prevalence study conductedin India showed that 5% to 6% of sexually active adult populations are suffering from RTI.[(3)] The common causes of lower genital tract infections are*Chlamydia trachomatis,* Candidiasis, Trichomoniasis, Bacterial vaginosis, Gonorrhea, Syphilis, Human papilloma virus(HPV) infection, Herpes simplex virus(HSV) infectionand other bacterial infections.[(4)]

A study conducted at Regional STD teaching and training centre, New Delhi figures out that 19.9% of the study group were infected with *Chlamydia trachomatis*.[(5)] In females, the asymptomatic Chlamydial infection, if left untreated leads to Pelvic Inflammatory Disease (PID), infertility and its complications. Henceforth, screening

women for*Chlamydia trachomatis* helps in prevention of PID, ectopic pregnancy and infertility. Nucleic acid amplification technique like Polymerase chain reaction increased the chance of diagnosing and reporting *Chlamydia trachomatis* infection over those of culture and other serological tests.

Likewise, a similar study conducted at New Delhi estimated that 9.4% of the study group was infected with candidiasis, 7.8% with bacterial vaginosis, 1.8% with syphilis, 1% with Trichomoniasis, 0.7% with gonorrhea and 0.3% with chancroid.[5] This implies that screening for lower genital tract infections by laboratory methods is advisory before giving empirical treatment.

Individuals suffering from RTI have significantly increased chance of acquiring and transmitting Human Immunodeficieny Virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV). Hence screening for HIV, HbsAg and HCV is mandatory for all cases with complaints suggestive of RTI.[6]

The low level of awareness among women is because of stigma and it adds up a major contribution factor for the increase in the incidence of genital tract infections. Inadequate laboratory diagnostic facilities in all the levels of health care, limited resources in material and manpower, stigma and discrimination associated with RTI services are some of the reasons of lack of exact incidence/prevalence rate of RTI in India, which forms an important data tool for determining intervention and treatment strategies.[7]Moreover, epidemiological studies in RTI are not abundant in south India.[8]Hence this study is conducted to provide a reliable laboratory based data on the microbiological profile of lower genital tract infections.

AIMS AND OBJECTIVES

1. To study the various socio demographic profiles associated with lower genital tract infections in women of reproductive age group.

2. To isolate and to study the microbiological profile of lower genital tract infection in women of reproductive age group.

3. To determine the proportion of *Chlamydia trachomatis* among the study group by Real-time PCR assay.

4. To study the co infections caused by HBV, HCV, HSV, HIV and Syphilis by various serological methods.

REVIEW OF LITERATURE

Genital tract infections are most commonly caused by various organisms which include bacteria, viruses, parasites and fungi. They are transmitted by vaginal, anal and oral sexual contact. Some organisms also spreads through other modes like blood transfusion, skin abrasions that add on more threat. E.g. HBV, HCV, HIV, HSV and Syphilis. Lower genital tract infections are the leading cause of morbidity in both developing and developed countries, which increases the psychological stress in the young sexually active females. WHO estimates that more than 1 million people acquire RTI everyday.[9]

Background:

The normal vaginal epithelium is an important immune lining which helps in preventing many infections. Vaginal cells contain glycogen and it is continually shed in the lumen of the vagina. The normal microbial flora of vagina has predominantly *Lactobacillus crispatus* and *Lactobacillus jensenii*.[10] The glycogen is depolymerized to glucose and utilized by the lactobacilli which on metabolism results in the production of lactic acid. This is responsible for the acidic pH of the normal vagina (3.5 to 4.5) providing immunity against pathogens (Eg:gonorrhea).[11] In addition, Lactobacilli produce hydrogen peroxide which is highly bactericidal. The normal vaginal flora is altered because of exposure to sexually transmitted pathogens or due to overgrowth of endogenous organisms.[12] The differential diagnosis of a patient presenting with discharge per vaginum includes trichomoniasis, vulvo-vaginal candidiasis, bacterial vaginosis, desquamative inflammatory vaginitis, cervicitis and vulvovaginitis associated with oestrogendeficiency.

Epidemiology of Genital tract infections:

Epidemiological data based on previous studies show that the incidence and prevalence of RTI vary greatly between countries, which are influenced by the differences in the characteristics of each pathogen as well as other biological, behavioral, medical, social, and economic factors.[13] It was estimated that in developing countries, gonorrhea rates are 10–15 times higher, the rates of Chlamydia are 2–3 times higher, and syphilis rates are 10–100 times higher than the rates among women in industrialized countries.[14] However, because of dissimilarities in study design, specimen collection, and laboratory methods across the world, it is difficult to compare and interpret the data on prevalence of RTIs. Still, the data that are available suggest that RTI are common in almost all of the developing nations, with high prevalence rates among commercial sex workers and clients of STD clinics.[15]

According to WHO, an estimated 499 million new cases of curable infections like Gonorrheae, Chlamydia, Syphilis and Trichomoniasis occur each year, 536 million people have incurable HSV-2 infection and 291 million women have HPV infection.[16]NACO estimates that 3 crore episodes of RTI occurs per year.[17]Several studies have been conducted in India to estimate the prevalence of RTI, but the stigma and culture of silence among women in rural areas, the exact data cannot be estimated. A systematic review by aarthi*et al.,* have found that there are only 41 eligible studies on estimation of prevalence of RTI. The above study also states that the prevalence of self reported reproductive tract infections is 11 to 72%, whereas it is 7 to 34% in the studies where laboratory methods were used to confirm clinical diagnosis.[18] Hence at situations possible, it is important to support the clinical diagnosis by laboratory based proven diagnosis in all STD centres.

In India, NACP- Phase IV has focused on prevention strategies by condom promotion, targeted interventions for high risk groups and blood safety and prevention and

control of RTI. In 2013-2014, 67.7 lakhs people with RTI were managed as per national protocol.[19]

Mode of Transmission of genital tract infections:

Generally, the agents causing lower genital tract infections enter the body through the mucosal lining due to unprotected sexual intercourse with an infected partner. Most of the infections that are sexually transmitted exhibit "biological sexism" i.e., the anatomical factors make the transmission of infections in higher rate from male to female.[20]Infections occur in women earlier and more severely than men. Women have less ability to get prevention from RTI because of their social conditions, lack of female condoms and also due to larger surface area exposed during intercourse. For instance, in gonorrhea, the risk of getting infection is 25% for men but for women it is 50%.[21]

Factors affecting the spread of genital tract infections in a population:

- Biological factors which include the infectivity of the pathogen, duration of infectivity, presence of co-infections, age and immunological status of the individual.
- Behavioral factors like type of sexual intercourse, number of sexual partners, use of contraceptives.
- Medical factors like accessibility to health care facilities, accessibility of laboratory facilities and availability of appropriate treatment.
- Social and Economic factors like religion, education status, income, occupation and migration.

Etiological agents causing lower genital tract infections:

Lower genital tract infections are caused by various bacteria, viruses, parasites and fungi. The most common among them are discussed as follows:

GENITAL CHLAMYDIASIS:

Chlamydia was first observed in the year 1907, in a conjuctival epithelial cell scrapings obtained from an orang-utan, which has been inoculated with the material from a trachoma infected patient. Chlamydiae are obligate intracellular bacterial pathogen. The genus Chlamydia has three species, *Chlamydia trachomatis*, *Chlamydia psittaci* and *Chlamydia pneumoniae*. *Chlamydia trachomatis* has 15 serologic variants. Serotypes D to K are mostly associated with urethritis, cervicitis, endometritis, salpingitis, infertility, repeated abortions. Joy et al., found 30.8% of STD clinic patients were reported with *Chlamydia trachomatis*.[21] Another study conducted in Punjab, India states that anti-Chlamydial antibodies were present in 68% of women with infertility and 50% of women with bad obstetric history.[22] Many other studies from India have estimated the prevalence of *C. trachomatis* infection to be 23% in gynecology outpatient department (OPD) and 19.9 per cent in STD clinics.[23]

Chlamydia trachomatis presents as asymptomatic infection and hence if left untreated it leads to PID and infertility. Centre for Disease Control and Prevention has recommended screening of women for *C.trachomatis* under the age of 24 years, at least once in a year.[24] In our country screening is not being done due to lack of exact prevalence data and adequate resources. There is no study on the prevalence of *C.trachomatis* from Chengalpattu, a semi-urban area and hence this study is conducted to generate an evidence of infection in this area.

Morphology:

Chlamydiae possess both DNA and RNA and they have cell wall. An unique feature of the species in Chlamydiae is their complex reproductive cycle. There are two forms in the developmental cycle namely elementary body(EB) and reticulate body(RB). The EB is the infective form transmitted from one person to another. EBs gets attached to the target cells and then enter the cells inside a phagosome. Within 8 hrs of entry into the

cell, the EBs gets converted into RBs and gets adapted to intracellular survival and starts its multiplication. They undergo binary fission and produces enormous replicates within the intracellular membrane body called as inclusion body. After 24 h, the RB gets converted to EB and the inclusion then lyses, releasing EBs from the cell.

Laboratory diagnosis:

The endocervix is the preferred site of specimen collection. Endocervical swabs are collected under sterile precautions and immediately stored in the transport medium (-20degC). Studies have shown that 10-20% increase in the recovery of *Chlamydia trachomatis* has been observed if both cervical and urethral specimens are taken when compared to cervical swab alone.[25]

Direct Microscopic Examination:

Chlamydial inclusion bodies can be seen in direct microscopic examination. It is readily stained by Giemsa or Papanicolaou stains. These methods require expert interpretation, not sensitive, helpful only for examination of conjunctival smears and unsuitable for genital smears.

Culture:

As the organism is intracellular, it can be isolated only in living cells. Cell lines helpful for the growth of Chlamydiae are McCoy(Mouse fibroblast line), HeLa 229(Human cervical carcinoma), BHK 21(Baby hamster kidney). Cell culture is difficult to perform and therefore done in laboratories where research process with quality control are being ensured. The sensitivity of the cell culture varies from 70 to 85% depends on the laboratory and the specificity is 100%.[26]

Immunoassays:

Commercially available tests for the rapid detection of Chlamydial antigen have improved the management of sexually transmitted Chlamydial infection. Enzyme Immunoassay and Immunofluorescence are the two widely used methods. Serological tests are not routinely done for the diagnosis of uncomplicated genital infections and it is not recommended for the screening of asymptomatic individuals. The sensitivity of enzyme immunoassay is 62 to 96 per cent and specificity is of 86 to 99 per cent in comparison to cell culture.[27]It is suitable for laboratories where cell culture cannot be performed. However, multiple studies across the world including India have reported that there is poor sensitivity of ELISA in comparison to PCR.Abida Malik et al., reported that the sensitivity and specificity of ELISA in detection of C.trachomatis was 48% and 92.9% respectively.[28]

Molecular methods:

The gold standard test for diagnosing *Chlamydia trachomatis* infection is the detection of nucleic acid in samples i.e., Nucleic Acid Amplification Test(NAAT).[29]The most common are the Amplicor CT/NG test for both *Chlamydia trachomatis* and *Neisseria gonorrheae* and CobasAmplicor Chlamydia trachomatis, Genprobe, BD probe. Endocervical specimens are marginally more sensitive than urine in women but urine is more sensitive than urethral swabs in asymptomatic men. The true specificity of NAAT test is 99 %.[30] The exact chlamydial load in the genital tract can be detected by quantitative real-time PCR and can vary from 10 to over a million organisms/ml of the discharge. This has a role in the interpretation of test results where there is no differentiation between the people with high and low Chlamydia loads in the performance of different nucleic acid amplification tests. The Chlamydial load has an impact with the clinical symptoms, the transmissibility of infection, and the risk of developing chronicity, therefore the role of quantification in the diagnosis is important.

Treatment:

Tetracycline 500mg four times daily for a period of seven days or single 1gm dose of azithromycin is equally effective. Till now, no drug resistance has been reported.

CANDIDIASIS:

First known description of oral Candidiasis was found in the writings of Hippocrates and Galen. Since then, the incidence of Candida infections affecting various systems of the body has risen abruptly. Vulvovaginal candidiasis is characterized clinically by pruritis, pain, curdy white vaginal discharge. It is more common in pregnancy, diabetes mellitus and patients on chronic antibiotic therapy. Some workers have estimated that 75% of adult females will get atleast one single episode of vulvovaginal candidiasis during their lifetime.[31] In India, only two community based studies have been conducted in which laboratory-confirmed vulvovaginal candidiasis had been reported. Bang et al. diagnosed vulvovaginal candidiasis in 35% ,out of 650 reproductive age group women living in Maharashtra, and Prasad et al. diagnosed vulvovaginal candidiasis in 10% , out of 451 married women in Tamil Nadu.[32,33]

Morphology:

Candida are yeast like fungi, small, thin walled Gram positive ovoid cells with pseudohyphae that reproduces by budding. Till date, there are 163 anamorphic species of Candida but among them, twenty are considered to be significant pathogens.

Laboratory diagnosis:

The difficulty in diagnosis Candida as a pathogen is due to the colonization in the body sites. *Candida sp.*, can be a part of the normal vaginal flora of an asymptomatic women. Hence the isolation of organism in culture should be correlated well with the clinical symptoms and signs.

Direct Microscopy:

Saline wet mount and 10% KOH mount helps in detecting yeast cells. On direct Gram staining, Gram positive budding yeast cells and presence of pseudohyphae can be visualized. The presence of pseudohyphae in the direct smear indicates tissue invasion rather than colonization. Calcoflour white staining can also be used to visualize and to increase the chance of identifying the fungal elements.

Fungal Culture:

The most common media used for isolating Candida is Sabourards dextrose agar (SDA). The media is inoculated and incubated at 37degC. Within 3 to 4 days, creamy coloured, smooth and pasty colonies are visualized.

Species identification:

1. Germ tube test – a colony of the cultured candida is treated with sheep or normal human serum and incubated at 37°C for 2 hours. The germ tube formation will be seen in *Candida albicans* and *Candida dublinensis.*
2. Sugar fermentation and assimilation test
3. Chlamydospore formation – the culture is streaked in a cornmeal agar or ricestarch agar with a coverslip overlay and incubated at 25°C; it shows large, highly refractile, thick walled, terminal chlamydospores after 2 to 3 days of incubation.
4. CHROMagar Candida – CHROMagar is a rapid, plate based test for the identification of candida species based on the morphology and colour production.

Immunodiagnosis:

The serological tests which are used currently lack specificity and sensitivity. Antigen based tests to detect cell wall mannan or cytoplasmic components are mainly used in the serodiagnosis of systemic candidiasis.

Treatment:

The azole creams like clotrimazole, miconazole, ketoconazole are applied locally for the mucocutaneous lesions. Nystatin is used as suspension of 2,00,000 units/ml for the resistant mucosal lesions. Amphotericin B is given for the systemic lesions as intravenous infusion

Nowdays, the *non albicans Candida* sp., are in rise of causing infections and they pose a threat by being resistant to antifungal drugs. Hence it is advised to perform antifungal susceptibility testing as per the Clinical Laboratory Standard Institute (CLSI) guidelines M27-A to promptly treat these infections.

TRICHOMONIASIS:

Trichomoniasis is caused by the protozoan *Trichomonasvaginalis*. It was first observed by Donne in 1836 from the purulent genital discharge of a female. The organism is not a colonizer and transmission almost always occurs through sexual contact. It is mostly asymptomatic and if symptomatic they present with complaints of vaginal discharge which are copious, yellow green frothy discharge, vulvar irritation, dysuria, dyspareunia and abnormal fishy vaginal odour. On speculum examination, punctuate mucosal hemorrhages of the cervix gives 'strawberry vagina' appearance. In a study conducted by Madhivannan et al., 2005, out of 898 participated women, 8.5% had Trichomoniasis.[34]

Morphology:

Trichomonas belongs to the family Trichomonadidae. It is a genital flagellate protozoan. It differs from the other flagellates as they lack the cystic stage. Only trophozoite stage exits. It is a pear shaped, motile organism residing in the vagina and urethra of women and urethra, seminal vesicle and prostate of men. It bears five flagella – four anterior flagella and one lateral flagella. The axostyle runs down the middle of the

trophozoite and ends in the posterior pole. It has a single nucleus with a central karyosome. It binds to the vaginal epithelium by the enzymes secreted by the trophozoites like adhesions, proteolytic enzymes and erythrocyte binding proteins etc.,

Laboratory diagnosis:

Microscopy:

Identification is by examination of wet mount of vaginal and urethral discharges. Chance of isolation is more if there is multiple and repeated sampling.

Saline wet mount - The discharge collected and diluted with a drop of saline and examined for motile organism under low power with reduced illumination. The jerky movement of motile trophozoites and pus cells can be seen which has the sensitivity of approximately 60%.

Permanent Stain – Giemsa stain and Papanicolaou stain are helpful to demonstrate the morphology of trophozoites.Acridine Orange(AO) fluorescent stain – rapid and sensitive when compared to wet mount. The sensitivity and specificity of AO staining method is 52% and 98% respectively (S.Mirrett*et al.,)*[35]

Culture :

Culture is the gold standard method for the diagnosis of Trichomoniasis. Culture has a higher sensitivity than wet mount of 75-85% and a specificity of 100%. [36]Specimen should be processed and inoculated immediately. Various culture media used are Lash's cyteinehydrolysate serum media, Diamond`s trypticase yeast maltose media, csyteine peptone liver maltose media. Kupferberg`s STS medium ,Modified Diamond medium is the most sensitive culture method. Cultures should be incubated for a period of 3 to 7 days or even longer. Wet mount should be prepared from the culture to demonstrate the motile trophozoites.

In a study conducted by Razia et al.,(2015), different methods for the laboratory diagnosis of *Trichomonasvaginalis* was compared. The sensitivity of saline wet mount was 58.8%, when compared with culture as gold standard. 8.1% (out of 835 patients)were found to be positive for trichomoniasis by culture in Kupferberg medium.[37] Although, culture is considered gold standard for the diagnosis, but laboratory facilities for culture are not available at all places. There is a delay in the diagnosis, as it takes 2 to 7 days for the culture and thus it may lead to loss of follow up of patient.

Immunological assays:

Antigen detection in the vaginal secretion is more sensitive than microscopy. A rapid dipstick test based on the principle of immunochromatography is available with result in 10 minutes and sensitivity of 83% and specificity of 99%.[38] ELISA detecting Trichomonial antigen is also available with a sensitivity of 89% and specificity of 97%. [39] ELISA detecting antibody is not useful as antibodies persist for longer time and it is difficult to differentiate between the current and past infection.

Molecular methods:

The highly sensitive method is molecular testing like Polymerase Chain Reaction using *T.vaginalis* specific beta tubulin genes.

Miscellaneous tests:

Vaginal pH analysis:

Vaginal pH is raised. But it is not specific as it is also raised in bacterial vaginosis.

Whiff test (Amine test):

When a drop of 10% KOH is added to the frothy vaginal discharge, the fishy odour is accentuated due to the production of amine. It is also positive in bacterial vaginosis. In more than 75% cases of trichomoniasis, the whiff test is positive.[40]

Treatment:

The drug of choice is Metronidazole 2g, single dose or a 7 days course of 500mg bd. The expected cure rate is 90%. Treatment should be given to both the sexual partners. Resistance to metronidazole is rare but it has been reported. Safe sexual practices like use of condoms till infection gets cleared is advised.

BACTERIAL VAGINOSIS:

Bacterial vaginosis (BV) is caused by abnormal growth of bacteria in the vaginal flora with the replacement of normal indigenous microbial flora. This condition was first described by Gardner and Dukes in the year 1955. It is the leading cause of RTI accounting for about 48% of cases.(41) The predisposing factors for the bacterial vaginosis are having multiple sexual partners, when having a new sexual partner or when doing repeated douching. The clinical presentations are homogenous thin vaginal discharge with fishy odour and lower abdomen pain. The labia and vulva are not erythematous or oedematous like candidiasis or trichomoniasis. The causative agents for bacterial vaginosis are *Gardnerellavaginalis, Mobiluncus spp., Peptostreptococci, Prevotella* and *Porphyromonas*.(42) It is usually a polymicrobial infection. Vaginal pH gets altered as the normal lactobacilli are being depleted by the overgrowth of abnormal pathogens in bacterial vaginosis.

Gardnerella is a normal microbial flora which on excess proliferation causes Gardnerellavaginalis infection. It acts as a predisposing factor for miscarriages and preterm deliveries. There is also increased risk of endometritis and salpingitis among women with BV.(43)

Bacterial vaginosis is a major public health concern in India and research studies on BV are low when compared to other RTI studies. Madhivannan et al,(2005) reported that 19.1% of women were infected with bacterial vaginosis in his study and Bhalla P et

all.,(2007) reported that 32.8% were infected with bacterial vaginosis in his population. [44,45]With limited resource laboratories like in rural areas, the diagnosis of bacterial vaginosis is challenging and there is a need for point of care tests.

Laboratory diagnosis:

A wet mount prepared from the vaginal discharge usually reveals clue cells, which are the vaginal epithelial cells studded with coccobacilli. In almost 90% of the cases, clue cells are visible. The clue cell was named by Herman Gardner because they provided a clue to the diagnosis of this condition. The sensitivity and specificity of clue cells in wet mount is 81% and 99%, provided the count of clue cells should be more than 20% .[46]Diagnosis of Bacterial vaginosis was first done by Amsel et al., and he formulated a criteria to distinguish the non specific vaginitis from the BV. [47]The diagnostic criterion of Amsel is as follows:

- Homogenous vaginal discharge
- Whiff test positive
- Clue cells
- Vaginal pH > 4.5

(At least presence of three out of these four criteria is enough to make the diagnosis of BV)

Another criteria which is based on direct Gram staining alone with scoring system was devised by Nugent et al. He made scoring system ranged from 0 to 10 based on the following tabulation criteria. The sensitivity and specificity is 90% and 94% respectively.[48] Nugent scoring is mainly based on observer skill and knowledge.

NUGENT SCORING

Lactobacilli	Score	Gardnerella, Bacteroides	Score	Curved GNB	Score	Sum
≥ 30	0	0	0	0	0	0
5 – 30	1	< 1	1	< 1	1	3
1 – 4	2	1 – 4	2	1 – 4	1	5
< 1	3	5 – 30	3	5 – 30	2	8
0	4	≥ 30	4	≥ 30	2	10

INTERPRETATION OF NUGENT SCORE

Score (N)	Additional criteria	Report
0 – 3		Smear not consistent with Bacterial Vaginosis
4 – 6	When clue cells are not present	
4 – 6	When clue cells are present	Smear consistent with Bacterial Vaginosis
≥ 7		

Treatment:

The drug regimen for treating BV is oral metronidazole 500mg twice a day for a period of 7 days. Vaginal preparations with 0.75% metronidazole gel or 2% clindamycin cream are also effective. For patients who is not responding to the antimicrobial drugs, intravaginal boric acid 600mg at bedtime gives symptomatic relief. Pregnant women with BV should be treated with oral metronidazole or oral clindamycin. Topical agents is not

advised for pregnant females as it has adverse effects like prematurity. There is no protocol to give treatment for the male sexual partners and hence it is not recommended.

GONORRHEA:

In 1879, Neisser discovered the bacterium gonococcus and it was then proven as the causative agent of gonorrhea. Gonorrhea is a sexually transmitted infection that has its history since medieval period. British parliament has enacted a law in the year 1611 to ensure the spread of infection as it was a major public health problem then. It was initially called as clap as the infected person experienced a clapping sensation during micturition. Later it was renamed after the discovery of causative agent, *Neisseria gonorrhea*. The incidence of gonococcal infections has declined significantly worldwide, yet in developing countries it still remains a public health problem. The exact surveillance data is difficult to assess because of variable diagnostic criteria. Studies conducted in Africa showed that non ulcerative STI like gonorrhea are an independent risk factor in the transmission of HIV when compared to ulcerative STI. In 2003, it was second to *Chlamydia trachomatis* among the STI`s in the United States, with 335,104 cases of gonorrhea reported.[(49)] But the prevalence of gonorrhea has been declining. In a study conducted by Prasad et al., at rural Tamil Nadu,(2005) there was no laboratory diagnosed case of gonococcal infection.[(50)]

Morphology:

Neisseria gonorrhoeae is a gram negative diplococci, non motile, non sporingcocci, kidney shaped with concave surface facing each other. It is catalase positive, oxidase positive. It ferments glucose but not maltose, sucrose or lactose. It has proteins, lipooligosaccharides and phospholipids in its outer membrane. Pili mediatesgonococcal attachment to the columnar epithelial cells. Another surface protein helpful in the adherence is the Opa – Opacity associated protein.

In addition to the antigenic structures, host factors are also important in the pathogenesis of infection. Activation of phosphatidyl choline – specific phospholipase C and sphingomyelinase by the organism resulting in the release of ceramide and diacylglycerol, paves a way for the entry of *N.gonorrhoeae* into the epithelial cells. The patient presents with cervicitis, urethritis, proctitis and conjunctivitis. It can also spreads to local sites causing complications like salpingitis, endometritis and tuboovarian abscess, epididymitis in males. Disseminated gonococcemia is rare.

Laboratory diagnosis:

Direct Microscopy:

Gram staining of urethral exudates from men or endocervical swab for women shows gram negative intracellular diplococcic which is highly specific and sensitive in diagnosing gonococcal urethritis. But it is only 50% sensitive in diagnosing gonococcal cervicitis. Polymorphonuclear cells are seen in the Gram stain in an abnormally elevated number which gives a clue to the diagnosis. Sood et al.,(2009) figured out that direct microscopic technique is best with symptomatic men giving high sensitivity of approximately 95% and specificity of 99%. [(51)]

Culture:

The culture of gonococci is quite difficult in basal media. Endocervical swab should be collected with Dacron or rayon or charcoal impregnated swabs. As gonococci is highly susceptible to drying , temperature or toxic substances like cotton swabs, it should be inoculated immediately in a selective medium like Modified Thayer Martin medium. If plates cannot be incubated immediately, it can be kept in room temperature upto six hours provided it is inside the candle extinction jars. The organism needs CO2 for its growth. The isolate is confirmed with biochemical reactions and any gonococci isolated should be tested for antimicrobial susceptibility testing according to CLSI guidelines.

Culture is the "Gold Standard" for the definitive diagnosis of gonorrhoea. The sensitivity of culture is 85−95% in case of acute infection and the specificity is 100%, whereas in chronic infections it is 50% sensitivity.[52] Although, the gonococcal culture methods are well described, there are certain disadvantages in practical usage. They are specimen transports, fastidiousness of the organism, and laboratory techniques. In addition, multiple steps like transport medium, method of inoculation and optimal conditions of incubation are necessary for the processing of specimens, and hence the quality assurance varies with the laboratory performance. Also, at least 48-72 hours is needed for culture and thereby resulting in delays in diagnosis and treatment.

Molecular methods:

In the recent years, nucleic acid probe tests are widely in use for the direct detection of *N.gonorrhoeae* in urogenital specimens. NAATs include Roche Amplicor,Gen-Probe APTIMA Combo2 (detects *Chlamydia trachomatis* also) and BD ProbeTec ET. When compared with NAATs, gonoccocal culture has sensitivity from 85 to 95% for acute infections and may become as low as 50% for females with chronic infection.[53]

Treatment:

Single dose regimens of Ceftriaxone and Cefixime are the mainstays of treatment modality for uncomplicated gonococcal infection of genitourinary system. Since co infection with *Chlamydia trachomatis* is common, the treatment regimen should include a single 2gm dose of azithromycin also.

SYPHILIS:

Syphilis, caused by *Treponemapallidum subsp., pallidum* is a sexually transmitted infection causing a chronic systemic disease. *T.pallidum* can penetrate the intact mucous membrane or through the minute abrasions in the skin and enters the lymphatics and blood. It has three stages of clinical presentation. In primary syphilis, single painless chancre

(hard chancre) appears in the genital region. After 1 to 3 months of the occurrence of primary lesion, secondary syphilis stage starts. Due to rapid multiplication of the spirochaetes, it disseminates through the blood and the patient may presents with meningeal, ocular, osseous or gastrointestinal involvement. In tertiary stage, cardiovascular and neurological involvement like tabesdorsalis, general paralysis of insane occurs. Eventhough the advent of penicillin therapy has significantly reduced the incidence of syphilis, it still remains as a global health problem. The number of new infections occurring globally is estimated at nearly 12 million per year. Arpita et al., found that among 570 cases attended the STI clinic from the year 2005 to 2009, 42 (7.36%) cases were found to have syphilis. (54)

The causative agent of syphilis, *Treponemapallidum*, was discovered in the year 1905 by Schaudinn and Hoffmann. The word pallidum refers to the pale staining nature of syphilis. It is a thin, delicate spirochaete which is of size about 10µm× 0.1 to 0.2µm with ten regular spirals. It is actively motile with rotator movement around the long axis and backward and forward movement and flexion of the body.

Laboratory diagnosis:

The lesions are highly infectious. Hence care must be obtained while collecting the specimens from the lesions. The lesion is gently scraped and gently pressure is given to the undersurface of the lesion and the exudates are collected. Serum samples have to be collected for serological evaluation.

Direct Microscopy:

Wet films are prepared from the exudates and it is examined under dark ground illumination. *T.pallidum* is identified by its slender spiral structure and its slow motility. A concentration of 10^4 /ml in the exudates is needed to visualize the *T.pallidum* in a wet mount. Silver impregnation staining technique can also be used.

Direct fluorescent antibody test for *T.Pallidum*(DFA – TP) : Smears from the infective lesions is fixed with acetone. DFA-TP test is done using the fluorescent tagged anti *T.pallidum* antiserum.

The direct microscopic techniques become positive one to three weeks before serological test and are most useful in the diagnosis of primary, secondary and early congenital syphilis.

Serological test:

Non Specific Tests include Venereal Disease Research Laboratory (VDRL)/ Rapid Plasma Reagin (RPR) test. These tests use the cardiolipin antigen extracted from beef heart with added lecithin and cholesterol. These tests are recommended for screening as they give rapid results. The sensitivity of VDRL in diagnosing Primary syphilis is 78%, secondary syphilis is100%, latent syphilis is 95%, late syphilis is 71% [(55)]. The specificity of the test is 98 to 100%. Specific tests for *T.pallidum* includes *TreponemaPallidum*Immobilisation(TPI), Fluorescent treponemal antibody(FTA) test, *Treponemapallidum*hemagglutination assay(TPHA) and Enzyme immunoassays. TPI test is the gold standard test and FTA-ABS is the standard reference test. The sensitivity of TPHA is 85 to 100% and the specificity is 98 to 100%.[(56)]

Treatment:

Benzathine penicillin G - 2.4mU i.m single dose is given in primary and secondary stages of syphilis. In patients with history of allergy to penicillin, Tetracycline HCl – 500mg qid or doxycycline – 100mg bd can be given. In tertiary stage with neurosyphilis, aqueous crystalline penicillin G 18 to 24mU/d IV is given.

HERPES SIMPLEX VIRUS:

Herpes Simplex Virus (HSV) is a linear double stranded DNA virus. It produces a wide variety of infections which involve the local mucocutaneous surfaces causing painful lesions to systemic manifestations affecting central nervous system and sometimes visceral organs. Exposure to HSV through oral or genital sexual contact permits the entry of the virus through the skin abrasions or minor trauma. The virus then enters the epidermal cells and multiplies rapidly. First episode herpetic lesions presents with fever, pain, itching, painful micturition, discharge per vaginum and associated inguinal lymphadenopathy. But the presentation is usually subclinical. Then it enters to the sensory or autonomic nerve endings like trigeminal ganglia in case of HSV I and sacral nerve root ganglia in case of HSV II and remains latent. Virus then spreads to other mucocutaneous surfaces and there is increased frequency of new lesions with primary genital and orolabial HSV infection. Once infected the patient presents subclinical infection and later recurrent infections are more common when the immune condition of the patient gets altered.

Infection with HSV I is acquired more frequently than HSV II. The major problem with HSV II infections are unidentified carrier state which acts as a source of threat by silent transmission of infection. The frequent reactivation of the latent stage of the virus from the genital tract led to continuous spread of virus throughout the world. Study conducted at JIPMER, India by Devinder et al., found that 115 (85.1%) were seropositive for HSV-2 from a total sample size of 135.[57] Pregnant women with genital herpes lead to preterm delivery, neonate with HSV meningitis and septicemia. International studies have found that 1:3200 children born have neonatal herpetic infection every year. [58]

Laboratory diagnosis:

Direct Microcopy:

The base of the genital lesion is scraped off and the smear is stained with Giemsa or Toluidine blue stain. The smear reveals multinucleated giant cells with intra nuclear

inclusion called Cowdry type A bodies. When electron microscopy is available, the diagnosis is fast and reliable. From the fluid exudates from vesicle, the virions are visible in electron microscopy and it is easy to interpret. The diagnosis can be made within an hour of specimen collection.

Virus isolation:

Scrapings or Swabs collected and transported in a suitable condition are inoculated in the cell culture lines like human diploid fibroblast lines, Vero cell lines and cytopathic effects can be seen as early as 16 hours with a median time of 2 to 3 days. Almost 95% cases can be diagnosed within a period of seven days, but the cultures should not be discarded until 14 days. The main advantage of viral culture is the detection of even low titres of virus.

Serology:

IgM Antibodies to HSV infections occurs in 4 to 7 days after infection and reaches a peak in 2 to 4 weeks. It can be detected by neutralization tests, immunofluorescent technique and Enzyme linked Immunosorbent Assay (ELISA). The drawback of serological tests is IgG antibodies persist for lifelong with fluctuations and it is helpful only for research purposes. Recently developed type specific IgG ELISA detects HSV antibodies accurately with no cross reactivity. The sensitivity and specificity of the tests varies from 95% to 99%.[59]

Molecular methods:

Polymerase Chain Reaction method is sensitive and specific in the diagnosis. It is the widely used method for diagnosis of HSV meningitis. According to Slomka MJ et al., the sensitivity of PCR vs Cell culture is 80.9%, whereas the specificity is 100%.[60]

Treatment:

Acyclovir (200mg 5 times/day or 400mg tid) is the drug of choice for genital herpetic lesions. It is a nucleoside analog which inhibits the viral DNA synthesis. The drug suppresses the severity of the clinical manifestations and it reduces the recurrent infections. However, the virus remains latent in the sensory ganglia throughout the life. Acyclovir resistant strains have been identified. A study conducted by Centre for Disease Control and Prevention (CDC) states that about 5 % of the HSV II isolates obtained from HIV patients were found to be resistant for acyclovir.[61]

HEPATITIS VIRUS:

Viral hepatitis is caused by any one of the following agents namely Hepatitis A, B, C, D and E virus. The clinical presentation of infection by hepatitis viruses varies from asymptomatic infections to mild anicteric illness or it may present as severe prolonged jaundice or may end up in acute fulminant hepatitis, cirrhosis, malignancy. The mode of transmission for Hepatitis B and Hepatitis C infections is sexual, blood transfusion and through needle sticks injuries. Hence screening for HBV and HCV infections are mandatory during blood transfusion, during surgeries, during pregnancy and if the person is diagnosed with any of the sexually transmitted disease. About 10 to 20% of the total population has chronic carrier state of HBV infection.Both HBV and HCV have significant association with the causality of hepatocellular carcinoma that appears years after infection.

HEPATITIS B VIRUS:

HBV belongs to the family Hepadnaviridae. The virion is spherical in structure with an envelope enclosing a capsid and partly double stranded DNA genome. The surface proteins of the envelope are known as hepatitis B surface antigen (HBsAg). The capsid has icosahedral symmetry and it has dimers of core protein (HBcAg). In addition to above

antigens, a soluble virus protein is found in the blood of some infected individuals which is known as HBeAg. These main antigens induce corresponding antibodies i.e., Anti-HBsAg, Anti-HBeAg, Anti-HBcAg which are helpful for the diagnosis of infection and carrier state. HBV DNA polymerase is helpful in the early diagnosis. Cappel R et al., have found that the enzyme has been detected even in HbsAg negative cases.

India accounts for 10 to 15% of the HBV carriers all over the world. The estimated prevalence of HBV carriers in India is around 40 million as estimated by various studies. NajmaJaved et al., found 10.46% of HbsAg positivity among women of reproductive age group in their study.[62] In another study, it was estimated that the prevalence of HbsAg positivity among the pregnant females is around 0.9 to 6.3%. [63]Vertical transmission of HBV to fetus causes low birth weight, prematurity and chronic complications. In women with acute hepatitis, the transmission rate is aroung 90%, whereas the chronic carrier state without immunoprophylaxis, the rate ranges from 10 to 20%.

Laboratory diagnosis:

Even before the appearance of jaundice, the HBsAg can be detected in the serum and Anti-HBcAg is the first antibody to appear. Anti-HBsAg appears last in the course of infection and its presence indicates a state of complete recovery and immunity to reinfection.

The useful detection methods are ELISA for HBV antigens and antibodies and PCR for the viral DNA analysis. The sensitivity of HEPALISA used in India is around 99%.[64] Rapid card test based on immunochromatography principle is also widely used but the sensitivity differs based on the manufacturer.

Treatment:

IFN-alpha2b is given subcutaneously at a dose of 5 million units daily or 10 million units thrice weekly for 16 to 24 weeks. Oral lamivudine is given as 100mg/day for a period of 12 to 18 months and it is effective in reducing the HBV DNA levels.

HEPATITIS C VIRUS:

HCV is a member of family Flaviviridae, which has a positive sense ssRNA. WHO estimates that 3% of the world's population is infected with HCV. Occult HBV infections (33%) are seen in patients with chronic HCV liver disease, where HBsAg cannot be detectable in serum because of its lower levels. The co-infections of Hepatitis infections with HIV are more common. There is no data about HCV infection rate in reproductive age group women, as it is not a part of routine screening method. But studies of HCV infection in pregnant women are available which shows 0.5% (FI Buseri et al.,)[65] and 5% (TehniyatIshaq et al.,)[66] In a meta analysis, the pooled transmission rate of vertical transmission of HCV to neonate was 5.8%, whereas the carriers with HIV co infections, the rate is 10.8%.

Laboratory diagnosis:

ELISA and immunoblot tests are available for detecting antibodies to core, envelope and NS3 and NS4 non structural proteins. ELISA has higher sensitivity when compared to the immunoblot rapid card tests. The limitations of immunoassays are inability to distinguish among acute, chronic or resolved infections. Nucleic acid based assays are helpful in detecting the presence of circulating HCV RNA and for genotyping. The viral load assays are helpful in the monitoring of antiviral therapy. The sensitivity of ELISA in detecting antibodies differs according to the manufacturer. In a comparative study, the HEPALISA sensitivity is estimated as 100% and the specificity is 98 to 100%.[67]

Treatment:

Interferon-Alpha2a or 2b given at the dose of 3 million units thrice weekly for 12 to 24 months is effective. Ribavirin can also be given orally at a concentration of 1000-1200mg daily for 6 to 12 months.

MISCELLANEOUS BACTERIA CAUSING RTI:

The microbial flora of the female genital tract is difficult to understand because of complexity of microbial colonization. Sometimes, even the endogenous organisms are the source of infections Eg: Bacterial vaginosis and many times the exogenous organisms like *Escherichia coli, Klebsiellapneumoniae* gets introduced in the host without causing any pathogenesis. Studies by Bartlett et al., have identified that the following organisms are the frequent colonizers of the female genital tract. These include *Diphtheroids, Lactobacilli, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus species, Escherichia coli, Proteus sp,Klebsiellasp, Pseudomonas sp.* The ability of these organisms to become pathogenic is governed by the interactions between endogenous and exogenous environment. Some antimicrobial substances produced by the pathogenic organisms may cause destruction of endogenous flora and hence it may leads to infections. Although *lactobacilli* are the principal regulator of the flora, the commensal flora of other bacterial load alters the regulatory process and thus paves a threat for recurrent infections.

Many studies have estimated the bacterial causes of vaginitis and reported that Escherichia coli, Klebsiella sp., Proteus sp., Pseudomonas sp., Staphylococcus aureus and Streptococcus sp., are the most common bacterial agents affecting the lower genital tract. Samia S Khamees have estimated 21.8% Staphylococcus aureus, 14.2% Escherichia coli and 13.6% Klebsiella species infections in his study. Kouamouo et al have estimated 34.9% of Escherichia coli, 23.8% of Enterobacter sp., 14.8% of Staphylococcus sp., 9% each of Citrobactersp and Streptococcus sp., 5.3% of Proteus sp., and 3.2% of Klebsiella sp.[(68)]The ascending infection from the lower genital tract causes pelvic inflammatory disease (PID) and it was substantiated by Spencer et al., in his study.

Complications and sequel of RTI:

Many RTI are asymptomatic and its get unnoticed by most of the women. Gerbase et al., estimated that 70 to 75% of women infected with *Chlamydia trachomatis* had no

symptoms suggestive of RTI. But the complications of such infections are dramatic. 40% of Chlamydial infections end up in Pelvic Inflammatory Disease (PID) which in turn leads to infertility, ectopic pregnancy and repeated fetal loss.

Syphilis can present with localized chancres, left if untreated can cause systemic manifestations like aneurysm, meningitis, stroke, gummas, visual defects, dementia. In pregnant women, the transmission to child leads to mid trimester abortion, still born and baby born with birth defects. Studies estimated that 15 to 40% of syphilitic patients develop late complication, if left untreated.

The temporal association between bacterial vaginosis and complications is an ongoing debate. It is related to increased risk of acquiring other sexually transmitted infections. It has a role in obstretic complications like pre term deliveries, miscarriage, chorioamnionitis, post abortion PID, post abortion endometritis. Several studies have emphazised that altered bacterial vaginal flora is responsible for the above complications (Johnson et al., 1985) and some studies have disapproved that (Alfonsi et al., 2004).[69,70]

Trichomonasvaginalis infections cause localized lesions, which increases the chance of acquiring HIV. In addition, it causes adnexitis, endometritis, pyosalpinx, infertility, low birth weight in pregnant females with TV.

Over all the reproductive tract infections have a direct impact on fertility, the risk of gynecological morbidities including PID, cervical erosions, cervical cancer and chance of transmission of other sexually transmitted infections. In addition to the physical burden, women with RTI suffer from psychological stress because of the symptoms and social stigma. Patel et al. concluded in his study that there is a significant link between vaginal discharge and mental health among the study group. Hence to overcome the physical, mental, psychosocial, economic burden of women due to reproductive tract infections, every nation should implement a protocol of screening, programs directed in the intervention of those diseases and also increase the research projects aimed at reducing the prevalence.

MATERIALS AND METHODS

Women with symptoms and signs suggestive of lower genital tract infections were selected and samples were collected from each individual. A total of 110 samples were collected over a period of one year which included vaginal swab, endocervical swab and blood. The samples were collected and processed as follows:

Study design:

Hospital based cross sectional study

Study place:

1. Department of Microbiology, Chengalpattu Medical College, Chengalpattu Tamil Nadu, India
2. Department of Obstetrics & Gynecology, Chengalpattu Medical College and Hospital, Chengalpattu,Tamil Nadu, India

Study period:

One year (2014-2015)

Study population:

Women of reproductive age group (18-45 years)

Sample size:

110 (It was proposed to include all the women over a period of one year who have fulfilled the inclusion criteria)

Ethical committee:

Ethical committee approval obtained from the institutional ethical committee, Chengalpattu Medical College.

INCLUSION CRITERIA:

- Age group 18 to 45 years
- Women with history of

1. Vaginal discharge
2. Low back ache
3. Abdominal pain
4. Infertility
5. Itching

EXCLUSION CRITERIA:

1. Unmarried women
2. Pregnant women
3. Women who have undergone hysterectomy
4. Women on menstruation
5. Women with diabetes, chronic illness
6. Women who is on antimicrobial therapy
7. Women who do not give consent

Sample collection:

After getting informed consent, brief history was obtained by structured questionnaire related tosociodemographic profile, presenting complaints, past history,

history of contraceptive practices, obstretic history and menstrual history. Procedure for obtaining vaginal and endocervical swabs was explained to the patient before hand. Patients were asked to lie comfortably on the examination couch. In lithotomy position, under aseptic precautions, vaginal discharge was collected for wet mount, then three swabs from posterior vaginal fornix with the help of sterile cotton swabs and one sample from cervical os using Ayer`s spatula was collected.Vaginal discharge was immediately utilized for performing wet mount and Gram staining. The vaginal swabs were transported to the diagnostic microbiology laboratory as early as possible for culture. Sample from cervical os was used for Pap smear.

A sterile swab was then introduced into the cervix to remove the cervical mucus secretions.The endocervical brushes was inserted one by one, 1 to 2 cm into the endocervical canal, rotated against the wall for 10 to 30 seconds, withdrawn without touching the vaginal surfaces and then placed in the appropriate transport medium. Oneendocervical brush was inoculated immediately in Modified Thayer Martin medium and incubated in candle extinction jar. Another endocervical brush was inoculated in a sterile aliquot tube containing 2ml of 99% ethanol for detecting Chlamydial nucleic acid by Real-time PCR. The aliquot tubes were carried in an ice-packed carrier and stored in deep freezer (-20^0C) immediately.

About 5ml of blood was collected from patient for serological examination. By centrifugation, serum was separated from blood sample and used for performing serological tests.

1. DIRECT MICROSCOPIC EXAMINATION:

a) WET MOUNT:

Few drops of discharge is kept on a clean glass slide and mixed with normal saline and overlaid with a coverslip and examined for the presence of pus cells, yeast cells, clue cells and for motile *Trichomonasvaginalis*trophozoites.

- Clue cells – Dysmorphic epithelial cells with distinctive stipple appearance by being covered with bacteria
- *Trichomonasvaginalis* – Pear shaped, trophozoites with jerky movements

b) GRAM STAINING:

Smear was made from vaginal discharge on a clean glass slide, air dried and heat fixed. Gram staining was done and observed for the presence of:

- Epithelial cells
- Pus cells
- Clue cells, if seen Nugent scoring was done
- Gram positive bacilli or cocci
- Gram negative bacilli or cocci
- Intracellular Gram negative diplococci
- Yeast cells

2. MICROBIOLOGICAL CULTURE:

a) AEROBIC CULTURE:

The vaginal swab was inoculated on to a MacConkey agar plate and blood agar plate and incubated at 37^0C for 24 hours with 10% CO_2. The colonies were read on the next day and the isolated organism was identified by Colony morphology, Gram staining, Catalase test, Oxidase test and other biochemical reactions.

Antimicrobial susceptibility testing:

The isolated organism was subjected to antimicrobial susceptibility testing by disc diffusion method using modified Kirby-Bauer technique and interpreted as per CLSI 2014 guidelines. Three to four morphologically similar colonies were suspended in peptone water and incubated at 37°C for 2 hours. The turbidity of the test suspension was standardized to 0.5McFarland Units. The suspension was inoculated in Muller Hinton agar

plate with a sterile cotton wool swab by lawn culture method. After brief drying, the antibiotic discs were placed with sterile precautions, about 6 discs per 100mm plate. Then the plate was incubated at 37°C overnight and interpreted next day as per CLSI 2014, M100-S24, Volume 34, No.1.[71]

PANEL OF DISCS USED FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING OF ENTEROBACTERIACEAE

Disc	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Gentamycin (10µg)	≥15	13-14	≤12
Amikacin (30µg)	≥17	15-16	≤24
Ciprofloxacin (5µg)	≥21	16-20	≤15
Cefotaxime (30µg)	≥26	23-25	≤22
Imipenam (10µg)	≥23	20-22	≤19

PANEL OF DISCS USED FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING OF STAPHYLOCOCCUS AUREUS

Disc	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Penicillin (10 units)	≥29	-	≤28
Amikacin (30µg)	≥17	15-16	≤14
Cefoxitin (30µg)	≥22	-	≤21
Trimethoprim - Sulfamethoxazole (1.25/23.75µg)	≥16	11-15	≤10
Ciprofloxacin (5µg)	≥21	16-20	≤15
Erythromycin (15µg)	≥23	14-22	≤13

PANEL OF DISCS USED FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING OF STREPTOCOCCUS SP.,

Disc	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Penicillin (10 units)	≥24	-	-
Trimethoprim - Sulfamethoxazole (1.25/23.75µg)	≥19	16-18	≤15
Ciprofloxacin (5µg)	≥21	16-20	≤15
Erythromycin (15µg)	≥23	14-22	≤13

b) ANAEROBIC CULTURE:

Blood agar plates inoculated with one of the vaginal swab was incubated in McIntosh Fildes Jar along with anaerobic Gaspak for maintaining anaerobiasis and kept at incubator for 72 hours. After 72 hours, the blood agar plates were read for the presence of colonies. A nutrient agar plate with *Pseudomonas aeruginosa* was used as the anaerobic control.

c) FUNGAL CULTURE:

The other vaginal swab was inoculated in Sabourard`s dextrose agar and incubated at 37^0C. The tube was regularly observed every day for growth upto 3 days and then on every alternate day till 7 days. Candida colonies were identified by colony morphology, Gram staining and further speciation was done.

IDENTIFICATION OF CANDIDA SPECIES:

i. Germ tube test:

A Germ tube is defined as a filamentous extension from a yeast cell that is about half the width and three to four times the length of the mother cell. Germ tube test is

helpful in identification of *Candida albicans* and *Candida dublinensis* from other species of Candida. A light suspension of the test colonies was inoculated in 0.5ml of fresh human serum in a test tube. The tube was incubated at 37^0C for two hours. Exactly at two hours, one drop of the serum was placed in a clean glass side and prepared a mount with the help of cover slip and observed under low and high power for the production of Germ tubes. If there was 30% of the cell producing germ tubes, it was considered as positive. Approximately 5% of the *Candida albicans* may not produce germ tubes.[72]

ii. Dalmau plate culture:

The Dalmau plate culture was done on cornmeal agar and to observe for chlamydospore production in yeasts. It is one of the specific tests for the detection of Candida species. A heavy inoculum of the yeast colony was streaked across the medium by ploughing technique. A coverslip was placed over it and the streak line should be beyond the cover slip. The plates were incubated at 25^0C for 48 hours in a closed, moisturized chamber. After 48hours, the plates were examined under low power and high power for the presence and arrangement of Chlamydospores.

CANDIDA SPECIES	**MORPHOLOGY**
Candida albicans	Large, thick walled, terminal chlamydospores
Candida tropicalis	Blastospores singly or in small groups
Candida kefyr	Abundant pseudohyphae, elongated cells, log in stream appearance
Candida krusei	Elongated cells with tree like arrangement or crossed match stick appearance
Candida parapsilosis	Single blastospores or in small clusters with pseudohyphae
Candida glabrata	No pseudohyphae
Candida dublinensis	Chlamydospore formation

Sugar Fermentation tests:

The test suspension of organism was inoculated in the sugar fermentation media. It is a liquid media containing peptone(1%), sodium chloride(0.5%), filter sterilized sugars 2% each of Glucose, Lactose, Sucrose and Maltose with indicator. It was incubated at 30^0C for 48 hours. When the colour of the medium changes to yellow, it indicates fermentation of sugars producing acid and gas in the Durham`s tube is checked. Production of acid and gas was taken as positive.

Antifungal susceptibility testing:

The candida species isolated was subjected to antifungal susceptibility testing by disc diffusion method on Muller Hinton agar supplemented with 2% glucose and 0.5 ug/ml of methylene blue dye. Five similar looking colonies from 24 hour old culture of Candida sp., was suspended in about 5 ml of normal saline and it was matched to 0.5 McFarland Standards. A sterile swab was dipped into the suspension and made a lawn culture in Muller Hinton agar. Commercial antifungal discs Fluconazole (10µg) and Voriconazole (1µg) were used and the zones of inhibition were measured after 24 hours of incubation at 37° C according to CLSI M44-A, Volume 25, No.15.[73]

PANEL OF DISCS USED FOR ANTIFUNGAL SENSITIVITY TESTING OF CANDIDA SP.,

Disc	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Fluconazole (10µg)	≥19	15-18	≤14
Voriconazole (1µg)	≥17	14-16	≤13

CULTURE FOR NEISSERRIA GONORRHOEAE:

The endocervical swab was inoculated aseptically at the side room in Modified Thayer Martin medium and incubated immediately in a candle extinction jar and carried over to the microbiological laboratory and placed in incubator (36^0C±1) maintaining humidity. Plates were examined after a period of 24 hours and if no growth was visible, it was again incubated upto 48 hours.

If the colonies appear on the selective medium, Gram stain, catalase test and oxidase test was performed. Gram negative diplococci, Catalase positive, Oxidase positive and sugar fermentation tests gives a presumptive identification of *Neisseria gonorrhea.*

3. MOLECULAR METHOD FOR DETECTION OF CHLAMYDIA TRACHOMATIS:

The endocervicalcytobrush after collection was transported in aliquots containing 99% ethanol as per the manufacturer`s instructions (HELINI® biomolecules) and stored in deep freezer (-20^0C)

Protocol for *Chlamydia trachomatis* Real – time PCR assay:

Materials:

HELINI PureFast®Bacterial DNA minispin purification kit, HELINI *Chlamydia trachomatis* Real- time PCR kit, Instrument used was Agilent MX3000P Real time PCR machine

Nucleic acid purification protocol:

1. 200µl of lysis buffer was added to a nuclease free1.5ml centrifuge tube.
2. 200µl of sample added then, after vortex mixed for 2 min.

3. 20µl of Proteinase K was added then, mixed immediately by inverting and incubating at 56^0C for 15min.

4. 300µl of 100% ethanol was then added and mixed well by vortex for 30 seconds. Spinning was done for few seconds to bring down drops to bottom of the tube.

5. Then entire sample was pipette and transferred into the PureFast® spin column. Centrifuge at 12000rpm for 1 min. Discard the flow through and place the column back into the same collection tube.

6. 500µl of wash buffer-1 was then added to the PureFast® spin column. Centrifuged at 12000rpm for 1 min and discarded the flow- through. The column back was placed into the same collection tube.

7. 500µl of wash buffer-2 was added to the PureFast® spin column. Centrifuged at 12000rpm for 1 min and discarded the flow- through. The column back was placed into the same collection tube.

8. Repeated Wash buffer-2 wash step once again.

9. Centrifuged the empty spin column attached with collection tube at 12000rpm for an additional 1 min. This step is essential to avoid residual ethanol. Discarded the collection tube.

10. Transferred the PureFast® spin column into a fresh 1.5ml micro centrifuge tube.

11. 50µl of elution buffer added to the center of PureFast® spin column membrane. Incubated for 2 minute at room temperature.

12. Centrifuged at 12000rpm for 1 min and discarded the PureFast® spin column. Centrifuged the tube now which contains the eluted nucleic acid. It was used directly in PCR and aliquots stored at -80^0C for later analysis.

REACTION MIX:

Components	Endogenous control	*Chlamydia trachomatis*
Probe PCR Master Mix	10 µl	10 µl
Endogenous control primer probe mix	5 µl	--
Chlamydia trachomatis primer probe mix	--	5 µl
Purified DNA sample	10 µl	10 µl
Total reaction volume	25 µl	25 µl

Negative control setup:

Use 5µl of nuclease free water instead of purified DNA sample as negative control.

Positive control setup:

5µl of positive control template provided in the kit.

The PCR vials were centrifuged briefly before placing into thermal cycler.

Amplification protocol:

	Step	Time	Temperature
	Taq enzyme activattion	15 min	95^0C
50 cycles	Denaturation	20 sec	95^0C
	Annealing/Data collection	20 sec	56^0C
	Extension	20 sec	72^0C

Endogenous control = FAM channel (Human RNase P gene)

Chlamydia trachomatis = FAM channel

Real time interpretation was done by the amplification plots and by the ct value.

4. PAP SMEAR:

With the help of Ayre`s spatula, exfoliative cells from the squamocolumnar junction was evenly spread onto a glass slide. Then it was immediately fixed using 95% ethanol. Papanicaloau staining was done and the smear was examined for the presence of:

- Inflammatory cells
- Shift in flora
- Clue cells
- Yeast cells
- *Trichomonasvaginalis*
- Microorganisms
- Malignant cells

5. SEROLOGICAL ASSAYS:

5ml of blood was collected from each patient and the separated serum was used for performing serological tests. Samples were stored in deep freezer (-20^0C) for future reference.

a) RAPID PLASMA REAGIN TEST:

Rapid Plasma Reagin(RPR) test is an in vitro qualitative and semi quantitative test for diagnosis of syphilis.

Principle:

The RPR reagent containing modified VDRL antigen(Cardiolipin antigen, lecithin and cholesterol) and microparticulate carbon particles, flocculate when mixed with serum

containing reagin. RPR is a screening test and the positive tests should be confirmed with specific confirmatory tests.

Procedure:

RPR kit (Span Diagnostics Limited) with reagents were brought to room temperature.

Qualitative test:

The disposable cards are labeled with patient identification number

1. Using disposable dropper, a drop of serum was placed in the card. Positive and negative controls were also tested with each set of test samples.
2. The RPR reagent was gently shaked to resuspend the paricles. A drop of the reagent was added in all circles.
3. The card was placed on the mechanical rotator and rotated for 8 minutes at 100±2rpm
4. Observe for floccules.

Interpretation:

Reactive specimen is indicated by macroscopically visible black clumps against clear background, in contrast non reactive specimen appear to have smooth uniform light gray colour.

Semi quantitative test:

The positive samples were further tested for the titre by semiquantitative assay.

1. 50ul of 0.9% saline solution was kept in the 2nd, 3rd,4th and 5th circles of the card by using a micropipette.
2. 50ul of the sample was added in 1st circle and to the saline in the 2nd circle.
3. 50ul of the diluted sample was aspirated from the 2nd circle and transferred to 3rd circle. This was repeated upto 5th circle.
4. 50ul from the 5th circle was aspirated and stored for further dilutions if needed.
5. RPR antigen was added in each of the wells and then rotated well by placing in a mechanical rotator for 8 minutes at 100±2rpm.
6. Observe for clumping in the circles.

Interpretation:

The results are given as reactive with 1:2, 1:4, 1:8, 1:16 dilution with respect to the clumping observed in the circles. The titre value of ≥1:8 is considered as significant titre.

b) TREPONEMA PALLIDUM HAEMAGGLUTINATION ASSAY:

IMMUTREP® TPHA (Omega diagnostics Ltd.,) is a specific and sensitive passive haemagglutination test for the detection of antibodies to *Treponemapallidum* in serum and CSF. It is the confirmation test for the diagnosis of syphilis.

Principle:

The assay constituents consists of *T.pallidum* sensitized formolised tanned fowl erythrocytes; unsensitised formalized tanned fowl erythrocytes, diluents and control sera. When diluted positive samples are mixed with sensitized erythrocytes, antibody to the sensitizing antigen causes agglutination of the cells. The cells form a characteristic pattern of cells in the bottom of the microtitre plate. If there is no antibody, they form a compact button in the well.

Procedure:

All the sample and reagents were brought to room temperature before use.

Qualitative test:

1. For a single test 4 wells are needed in the microtitre plate
2. The diluents was dispensed into the microtitration plate as follows: 25µl in rows 1,3 and 4; 100 µl in row 2.
3. 25 µl of the sample was added in the well in row 1. It was mixed well and then 25 µl was transferred from row 1 to row 2, then from row 2 to row 3 and then 25 µl from row 3 was discarded.
4. Then 25 µl was transferred from row 2 to row 4 and mixed well. 25 µl was discarded from row 4.
5. 75 µl of the well mixed control cells were added to row 3.
6. 75 µl of the well mixed test cells were added to row 4.
7. Now the final dilutions in the row 3 and 4 are 1/80
8. It was covered and left at room temperature for 60 minutes.
9. After one hour, the wells were examined for agglutination patterns.

Interpretation:

- Agglutinated cells form an even layer at the bottom of the well and it is declared as reactive.
- Non agglutinated cells form a compact button in the centre of the well and is declared as non reactive.

Quantitative procedure:

1. The dilutions were prepared in the microtitre plate.
2. Doubling dilutions were done starting from row 1.
3. 75 µl of the well mixed control cells were added to row 1.
4. 75 µl of the well mixed test cells were added to row 2 to 8.
5. Final dilutions in row 1 and row 2 were 1/80.
6. The plate was covered and left at room temperature for 60minutes.

Interpretation:

The starting dilution was 1/80; hence the highest dilution which showed agglutination was taken as titre and the dilution was calculated accordingly.

c) MICROWELL ELISA TEST FOR THE DETECTION OF HEPATITIS B SURFACE ANTIGEN:

HEPALISA is an invitro qualitative detection test for Hepatitis B surface antigen (HBsAg) in human serum or plasma.

Principle:

HEPALISA is a solid phase Enzyme Linked ImmunosorbentAssay(ELISA) based on the direct sandwich principle. The microwells are coated with monoclonal antibodies with high reactivity for HBsAg. The samples are added in the wells followed by addition of enzyme conjugate. This leads to the formation of sandwich complex wherein HBsAg is sandwiched between the antibody and antibody HRPO conjugate. Unbound conjugate is washed off by wash buffer. The amount of bound peroxidase is proportional to the concentration of HBsAg in the sample. When substrate buffer and chromogen are added, a blue colour develops. The intensity of developed blue colour is proportional to the

concentration of HBsAg in the sample. To end the enzyme substrate reaction, stop solution is added and a yellow colour develops finally which is read at 450nm spectrophotometrically.

Procedure:

1. The test kit (J.Mitra&Co.Pvt. Ltd.) and samples were brought to room temperature.
2. The HEPALISA strips was fitted in the stripholder and labeling was done.
3. 100µl of negative control was added in A1 and B1 wells.
4. 100µl of positive control was added in C1 and D1 wells.
5. The samples were added as 100 µl from E1 wells.
6. Then 50µl of working enzyme conjugate was added to each well. And the plate was covered and incubated at $37^0C \pm 1^0C$ for 60 minutes.
7. The wash buffer was diluted with distilled water to 1:25 dilution.
8. After one hour, the plate was taken from incubator and washed six times with working wash buffer by ELISA washer.
9. The wells were dried and 100µl of working substrate was added in all the wells.
10. The plate was covered with aluminium foil and incubated at room temperature (20 to 25^0C) for 30 minutes in dark.
11. 100µl of stop solution was then added to each well and mixed gently.
12. The reaction was read at 450nm in ELISA reader.

Calculation of results:

- Positive control: PC value or its average should be more than 0.5, if not the test is considered as invalid.

- Negative control: NC value must be <0.150 .
- Cut off value : Formula = NCx + 0.1 (NCx is mean absorbance OD of the negative control)

Interpretation of results:

- Test specimens with absorbance value less than the cut off value were reported as non reactive.
- Test specimens with absorbance value greater than or equal to the cut off value were reported as reactive.

d) MICROWELL ELISA TEST FOR THE DETECTION OF ANTIBODIES TO HEPATITIS C VIRUS :

HCV Microlisa is an in-vitro qualitative third generation enzyme linked immunosorbent assay for the detection of antibodies against HCV(anti-HCV).

Principle:

HCV Microlisa utilizes a combination of antigen with the sequence of both HCV structural and non structural antigens like CORE, E1, E2, NS3, NS4 and NS5. Antibodies to HCV if present in the sample bind to the immobilized HCV antigens on the microwell during the incubation period. The microwells are thoroughly washed with wash buffer and then enzyme conjugate added. The bound antigen-anti HCV-enzyme conjugate complex is formed. Now the substrate is added which leads to the development of blue colour indicating Ag-Ab reaction has occurred. Finally, stop solution is added to end the reaction and optical density is read photometrically.

Procedure:

The kit with reagents(J.Mitra&Co.Pvt. Ltd.) were brought to room temperature.

1. 100µl Negative Control was added in well No.A-1. It is ready to use solution and hence no dilution was needed
2. 100µl Positive Control was added in B-1, C-1 & D-1 wells.
3. 100µl Sample Diluent was added in each well, starting from E-1 well followed by addition of 10µl sample.
4. The well plate was covered and incubated at $37^0C \pm 3^0C$ for 30± 2 minutes.
5. Working wash solution and working conjugate solution were prepared as per the manufacturer`s instructions.
6. After the incubation is over, the wells were washed 6 times with working wash solution using ELISA washer.
7. Then 100 µl of Working Conjugate Solution was added in each well.
8. The well plate was covered and incubated at $37^0C \pm 3^0C$ for 30± 2 minutes.
9. The working substrate solution was prepared in last 5 minutes of incubation and kept protected from light.
10. After the incubation is over, the wells were washed 6 times with working wash solution by ELISA washer.
11. Now, 100 µl working substrate solution was added in each well.
12. The well plate was covered and incubated at room temperature in dark for 30 minutes.
13. 100 µl of stop solution was then added.
14. With the help of ELISA READER, read absorbance at 450 nm .

Calculation of results:

- Positive control: PC value or its average should be more than 0.5, if not the test is considered as invalid.

- Negative control: NC value must be <0.150 .
- Cut off value : Formula = PC$\bar{x}$ × 0.23

Interpretation of results:

- Test specimens with absorbance value less than the cut off value were reported as non reactive.
- Test specimens with absorbance value greater than or equal to the cut off value were reported as reactive.

e) ENZYME IMMUNOASSAY FOR DETERMINATION OF IgG ANTIBODIES TO HERPES SIMPLEX VIRUS TYPE 2:

DS-EIA-ANTI-HSV-2-G-FAST (DSI S.r.l. Italy) is for determination of IgG antibodies to Herpes Simplex Virus type 2 in human serum or plasma.

Principle:

Microtiter wells as a solid phase are coated with HSV-2 antigens. During incubation with positive test sera, HSV type 2 specific antibodies bound to the immobilized antigens. The microwells are washed to remove the unbound serum proteins. Horseradish peroxidase conjugated anti-human IgG antibodies are dispensed in the washed wells. This results in the formation of enzyme linked immune complexes. The microwells are washed to remove the unbound conjugate and then substrate is added. The colourless substrate is hydrolysed to a coloured end product in the presence of peroxidase. The intensity of the colour is proportional to the amount of antibodies present in the patient`s serum.

Procedure:

1. The samples were diluted with the given sample diluent at 1:100 ratio.
2. The controls were ready to use and hence they were not diluted.

3. 100µl of negative control, 100µl of positive control and 100µl of samples were added in the wells and incubated at room temperature for 15 minutes.
4. The wells were washed with working wash buffer for 5 times with ELISA washer.
5. 100µl of conjugate was pipetted in each well.
6. The well plate was incubated at room temperature for 15 minutes.
7. The wells were washed with working wash buffer for 5 times with ELISA washer.
8. 100µl of TMB-substrate was added in each well.
9. The well plate was incubated at room temperature for 5 minutes in dark.
10. 100µl of stop solution was added into each well and the results were read by microplate reader at wavelength of 450nm.

Calculation of results:

Cut – Off value = average OD of the negative control + 0.250
(0.250 = Co-efficient defined by the manufacturer)

Interpretation of results:

- The result of serum analysis is considered as positive, if OD/Cut-off > 1.1
- The result of serum analysis is considered as negative, if OD/Cut-off<1.0

STATISTICAL ANALYSIS

Data was formulated in terms of frequency distribution for different variables. As the data are categorical variable, Fischer test was employed as test of significance for testing associations. Multivariate logistic regression model for statistically significant predictors of lower genital tract infections were also tested. The data was analyzed using Epi-Info software (7.1.0.6 version; Center for disease control, USA) and Microsoft Excel 2010.

RESULTS

Women with symptoms and signs suggestive of lower genital tract infections were selected and samples were collected from each individual. A total of 110 samples were collected over a period of one year which included vaginal swab, endocervical swab and blood. The samples were processed and the results are shown as follows.

TABLE 1: AGEWISE DISTRIBUTION OF STUDY GROUP (n=110)

Age (years)	No. of patients	Percentage (%)
18-20	4	3.64
21-25	48	43.64
26-30	37	33.64
31-35	11	10
36-40	8	7.27
41-45	2	1.81
TOTAL	110	100

Age wise distributions of the subjects were analyzed. The range of age was 20 to 43 years. The median age was 26. The majority (43.63%) of the study population were in the age group of 21 to 25 years, followed by the age group of 26 to 30 years (33.64%).

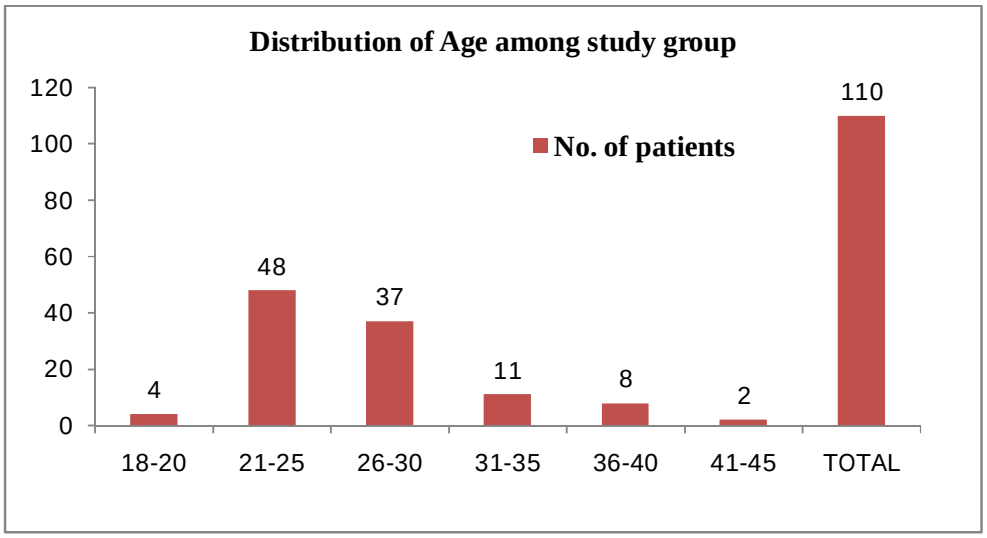


TABLE 2: TYPES OF RESIDENCE AMONG THE STUDY GROUP (n=110)

Residence	**N**	**%**
Rural	84	76.36
Semiurban	26	23.64
Urban	0	0
Total	110	100

Among the study group, 84 (76.36%) belonged to rural area and 26 (23.64%) belonged to semi urban area.

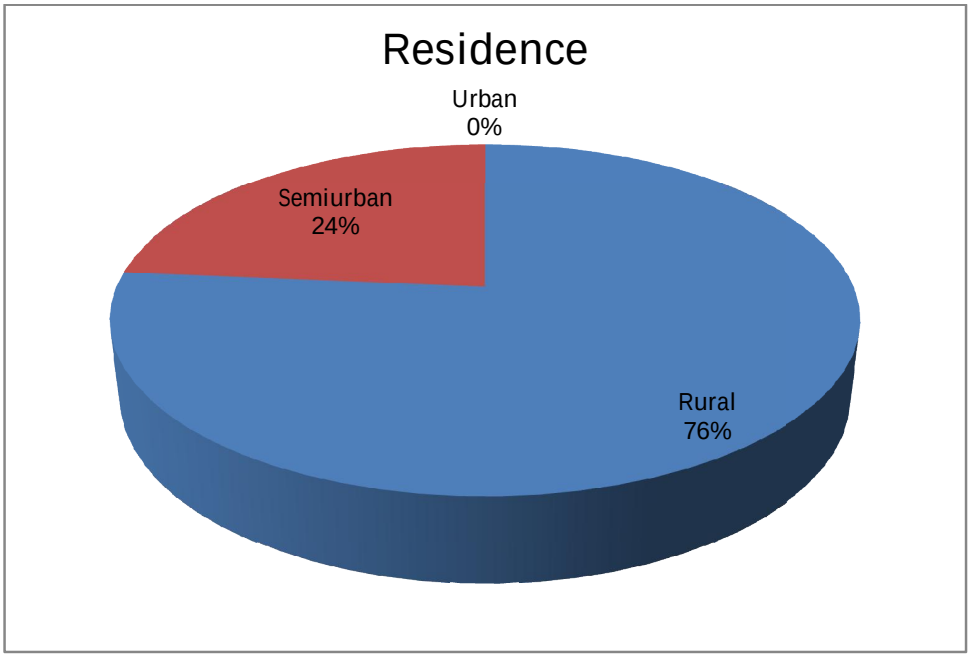


TABLE 3: DISTRIBUTION OF OCCUPATION STATUS AMONG THE STUDY GROUP (n=110)

Occupation (According to Kuppusamy scale)	No of subjects	%
Unemployed	74	67.27
Unskilled	29	26.36
Semi skilled	3	2.73
Skilled	1	0.91
Clerical, shopowner, Farmer	1	0.91
Semi professional	2	1.82
Professional	0	0
Total	110	100

74 out of 110 (67.27%) of the study subjects were unemployed, 29 out of 110 (26.36%) were unskilled workers followed by 3 out of 110 (2.73%), who were semiskilled workers.

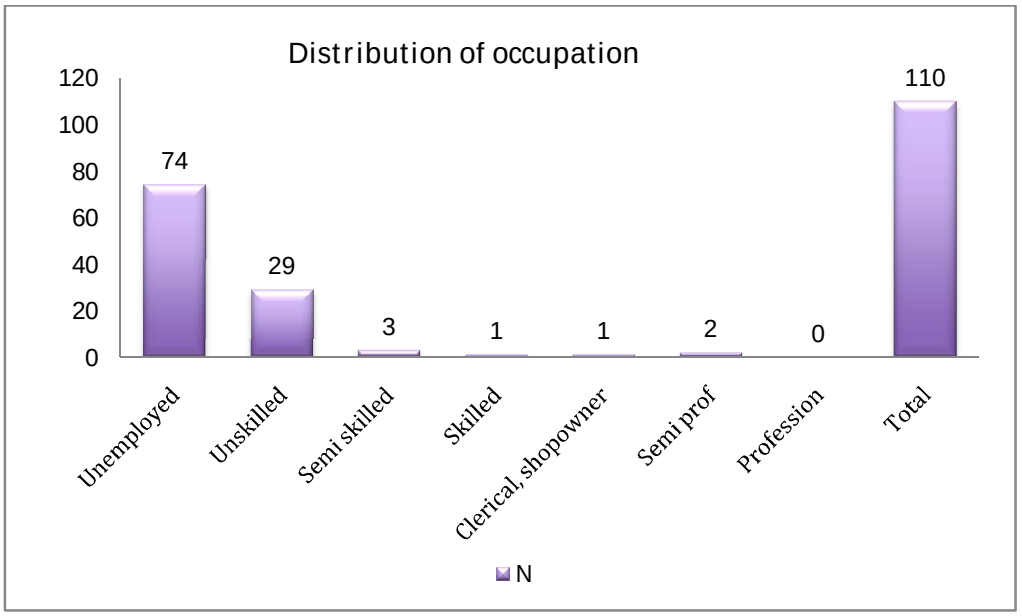


TABLE 4: PARITY WISE DISTRIBUTION OF STUDY GROUP (n=110)

Parity	No. of subjects (n)	Percentage (%)
Nulliparous	10	9.09
Abortion/Still born	3	2.73
One child (L1)	34	30.9
Two child (L2)	54	49.1
>Two child (>L2)	9	8.18
Total	110	100

The majority of the study group (49.1%) had two children. 9.09% were nulliparous women and 2.73% had history of abortion/still born.

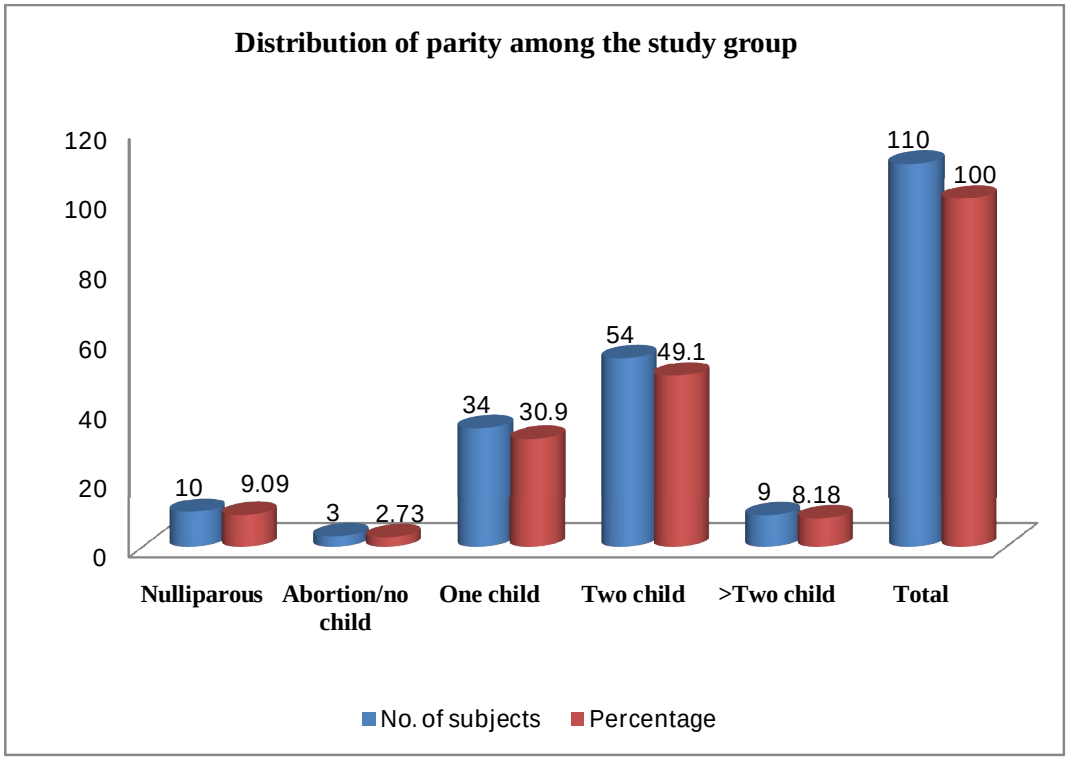


TABLE 5: SYMPTOM WISE DISTRIBUTION OF STUDY GROUP (n=110)

Symptoms	Present		Absent		Total	
	No. of subjects	%	No. of subjects	%	No. of subjects	%
Discharge per vaginum	82	74.55	28	25.45	110	100
Lower abdomen pain	62	56.36	48	43.64	110	100
Itching	26	23.64	84	76.36	110	100
Burning micturition	19	17.27	91	82.73	110	100

Most of the study group population presented with more than one symptom.Discharge per vaginum (82, 74.55%) was the major presenting complaint, followed by abdomen pain (56.36%), itching (23.64%) and then burning micturition (17.21%).

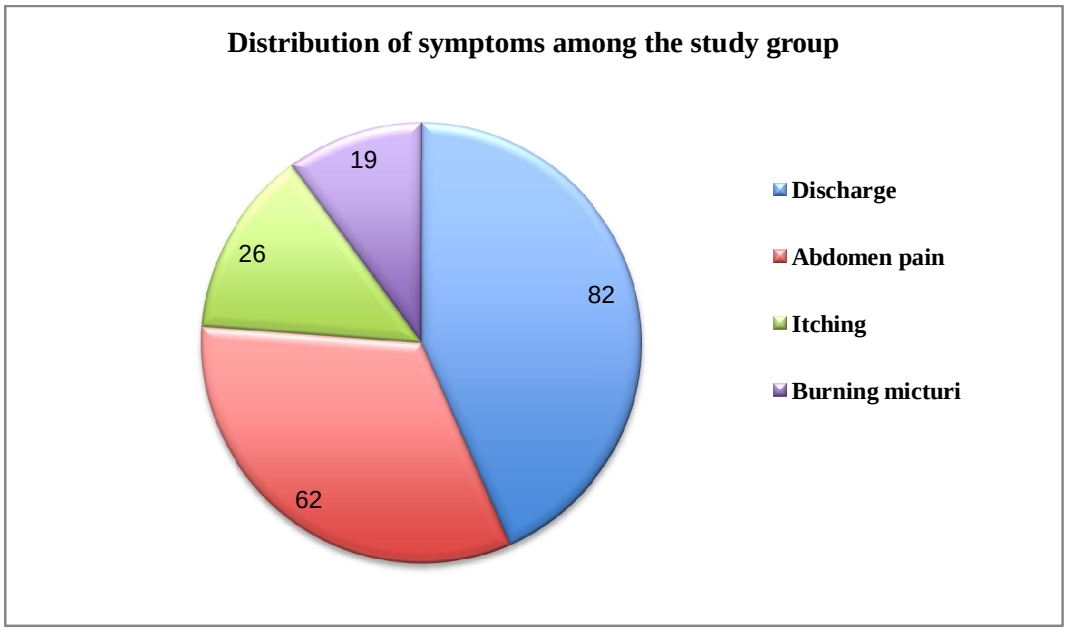


TABLE 6: DISTRIBUTION OF SYMPTOMS AMONG DIFFERENT AGE GROUPSIN THE STUDY POPULATION

Symptoms	Age ≤30yrs		Age > 30yrs		Total	
	No.	%	No.	%	No.	%
Discharge per vaginum	65	79.27	17	20.73	82	100
Lower abdomen pain	50	80.65	12	19.35	62	100
Itching	21	80.77	5	19.23	26	100
Burning micturition	16	84.21	3	15.79	19	100

Most of the study group population presented with more than one symptom. Discharge per vaginum (65 out of 82, 17 out of 82) and lower abdomen pain (50 out of 62, 12 out of 62) were the common symptoms in both study group of ≤30years and >30years respectively.

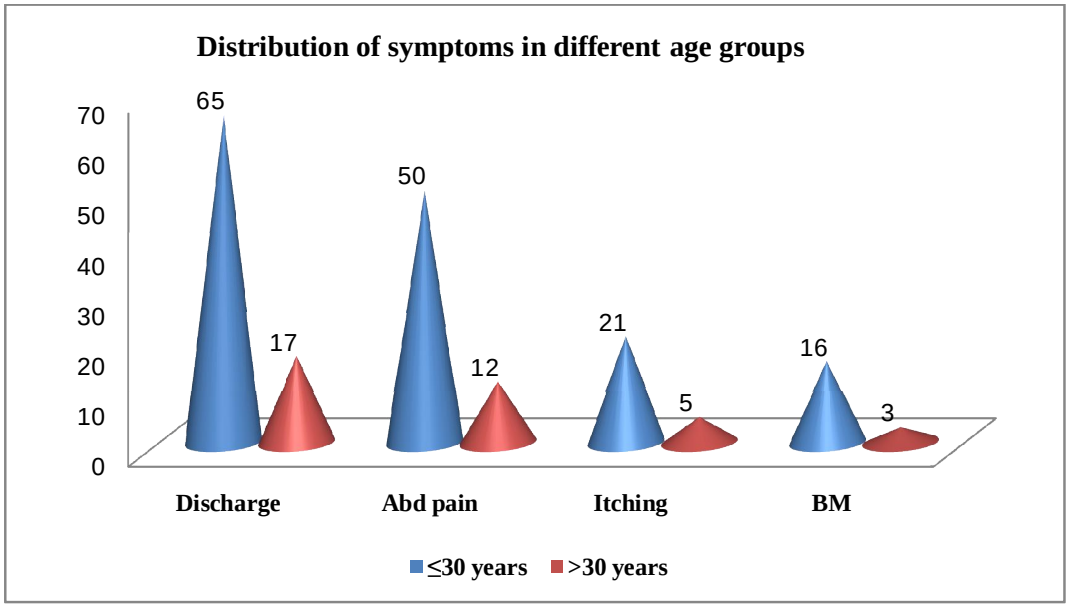


TABLE 7: DISTRIBUTION OF SYMPTOMS IN RELATION WITH PARITY

Parity	Discharge per vaginum (n=82)		Lower abdomen pain (n=62)		Itching (n=26)		Burning Micturition (n=19)	
	No.	%	No.	%	No.	%	No.	%
Nulliparous	9	10.97	5	8.06	5	19.23	2	10.53
Abortion/Still born	3	3.65	2	3.23	1	3.85	0	0
One child	27	32.93	17	27.42	9	34.62	6	31.58
Two child	35	42.68	33	53.23	9	34.62	9	47.37
>2 child	8	9.75	5	8.06	2	7.69	2	10.53
Total	82	100	62	100	26	100	19	100

Each subject presented with multiple complaints. Commonest symptom in relation with all types of parity in the study group population was discharge per vaginum (82) and lower abdomen pain (62).

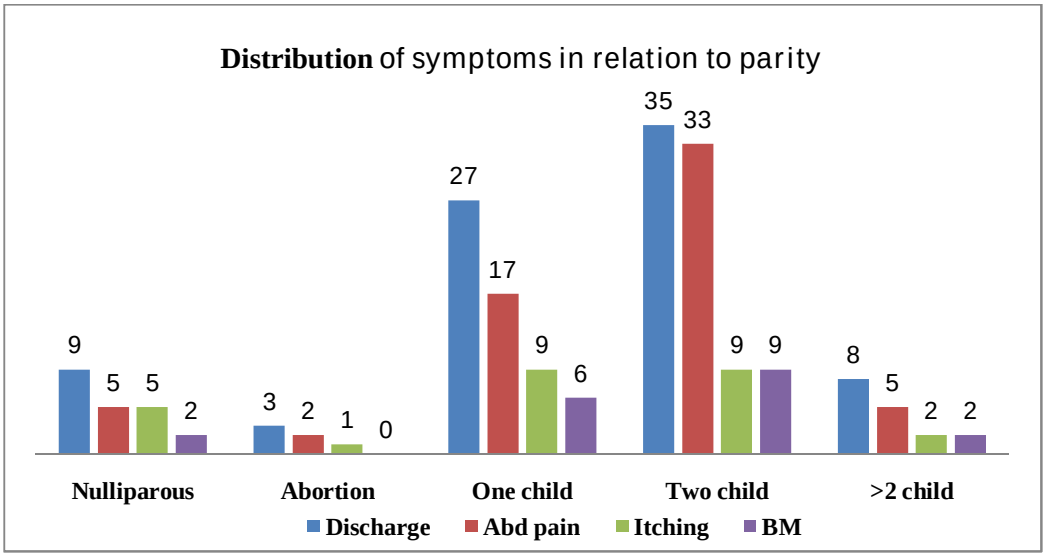


TABLE 8: DISTRIBUTION OF CLINICAL SIGNS AMONG THE STUDY GROUP (n=110)

Signs	No of patients	%
Discharge per vaginum	44	40
Redness	5	4.55
Erosions	3	2.73
Nodule	1	0.9
Multiple signs	16	14.55
No clinical sign	41	37.27
Total	110	100

Among the 110 subjects, 40% had Discharge per vaginum, 37.27% had no clinical sign, 14.55% had multiple signs like redness, erosions and nodule. All the study subjects who presented with symptoms of discharge per vaginum were not presented with the clinical sign as discharge per vaginum.

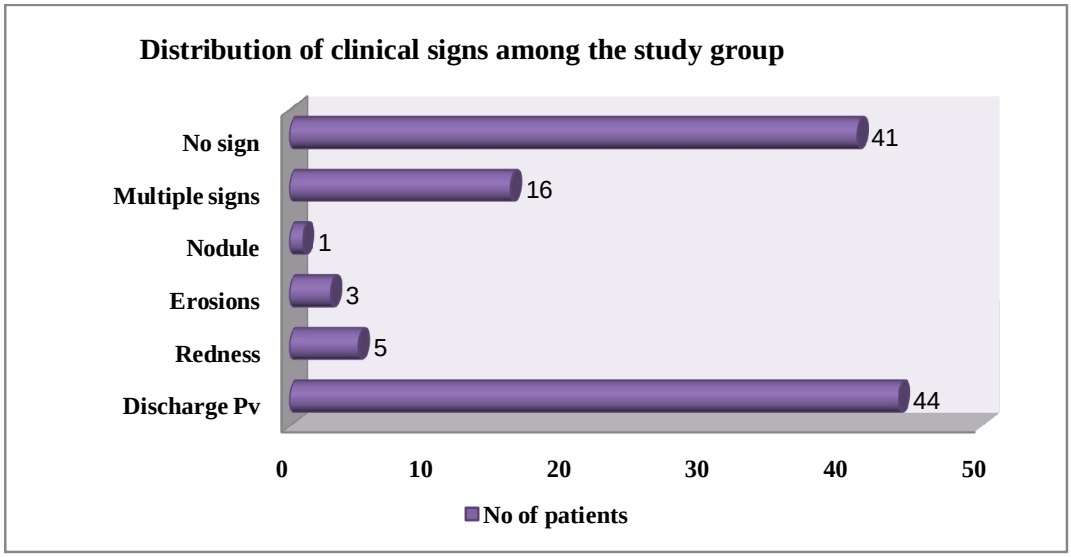


TABLE 9: DISTRIBUTION OF PAP SMEAR FINDINGS AMONG THE STUDY GROUP (n=110)

Pap smear	No. of subjects	Percentage
NILM	72	65.45
Inflammation	17	15.45
Yeast cells	15	13.64
Clue cells	3	2.73
TV	3	2.73
Total	110	100

Among 110 subjects screened by pap smear, 61.82% had Negative for Intraepithelial Lesion or Malignancy (NILM), 15,45% had inflammation, 13.64% had yeast cells, 6.36% had clue cells and 2.73% had Trichomonasvaginalis. Pap smear finding correlated well with the etiological agents identified by microscopy and culture.

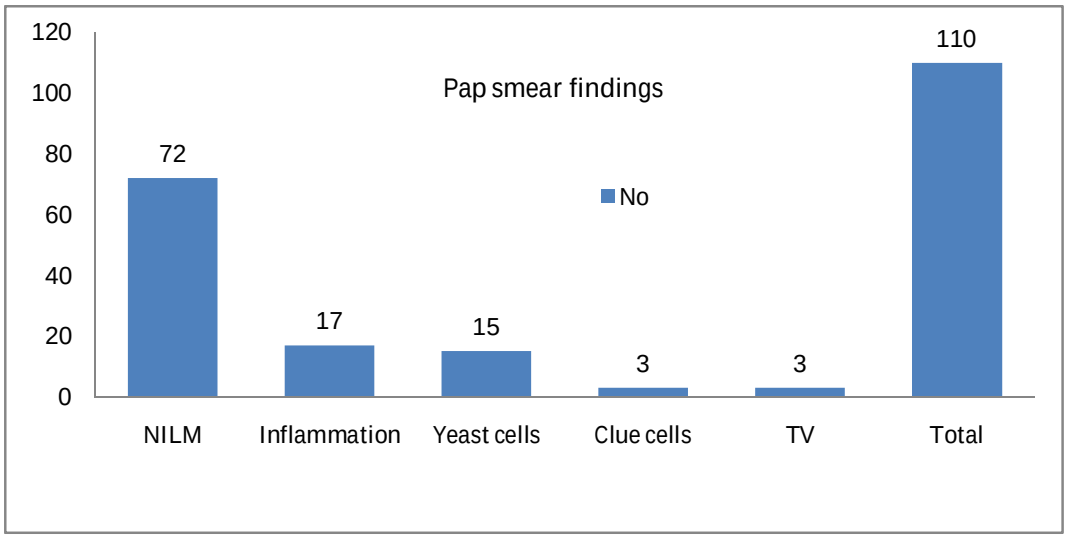


TABLE 10: LABORATORY DIAGNOSED CASES OF LOWER GENITAL TRACT INFECTIONS AMONG THE STUDY GROUP (n = 110)

Laboratory diagnosis of lower genital tract infection	No. of isolate	%
Positive	43	39.09
Negative	67	60.91
Total	110	100

Among 110 samples, laboratory diagnosis of lower genital tract infections was positive in 43 subjects (39.09%).

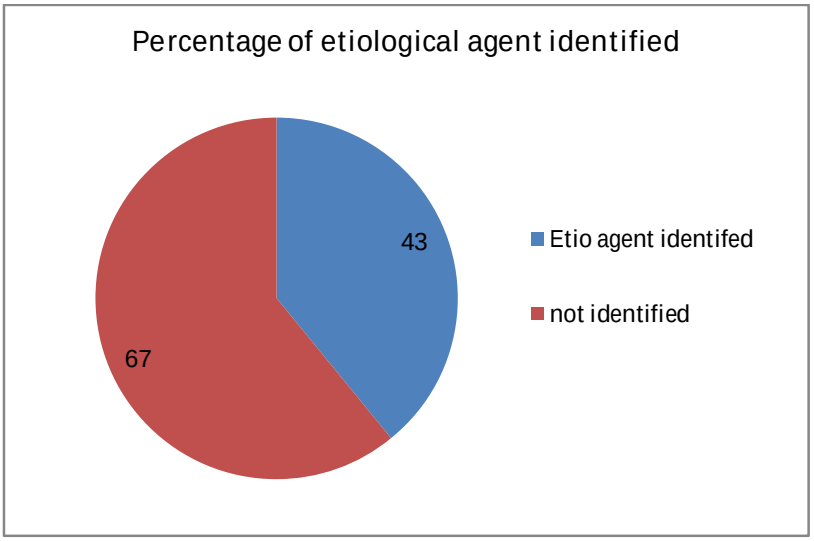


TABLE 11: DISTRIBUTION OF LABORATORY DIAGNOSED CASES WITH RESPECT TO TYPE OF INFECTIONS (n = 43)

INFECTION	No. of subjects	%
Single infection	36	83.72
Mixed infection	7	16.28

Among 43 subjects who had laboratory findings for lower genital tract infections, 36 (83.72%) presented with single infection and 7(16.28%) were presented with mixed infections.

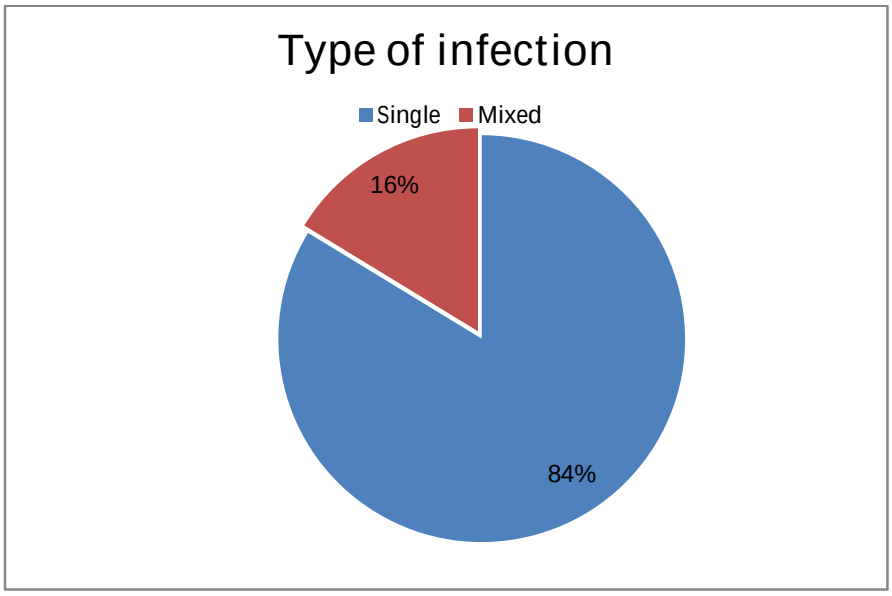


TABLE 12: DISTRIBUTION OF STUDY GROUP WHO HAD LABORATORY FINDINGS FOR LOWER GENITAL TRACT INFECTIONS

Type of infections	Cases positive for laboratory diagnosis	No. (n=43)	%
Single Infections	Candida sp.,	12	27.91
	Chlamydia trachomatis	5	11.63
	Trichomonasvaginalis (TV)	3	6.97
	Bacterial vaginosis (BV)	3	6.97
	Staphylococcus aureus	1	2.33
	Streptococcus sp.,	1	2.33
	Escherichia coli	7	16.28
	Klebsiellapneumoniae	4	9.30
Mixed Infections	Candida+Chlamydia	1	2.33
	Candida+Staphylococcusaureus	1	2.33
	Candida+Escherichia coli	2	4.65
	Candida+Chlamydia+Escherichia coli	1	2.33
	Chlamydia+Citrobacterkoseri	1	2.33
	Chlamydia+Klebsiellapneumoniae	1	2.33
	Total	43	100

Among the study group with single infections, Candida sp., was the most common agent (12, 27.91%) causing lower genital tract infections followed by Gram negative bacilli infections (11, 25.57%). Among the study group with mixed infections, Candida sp and C.trachomatis were the most common agents presented as mixed infections with other organisms.Laboratory diagnosis of bacterial vaginosis (6.97% positivity) was based on Nugent`s score.

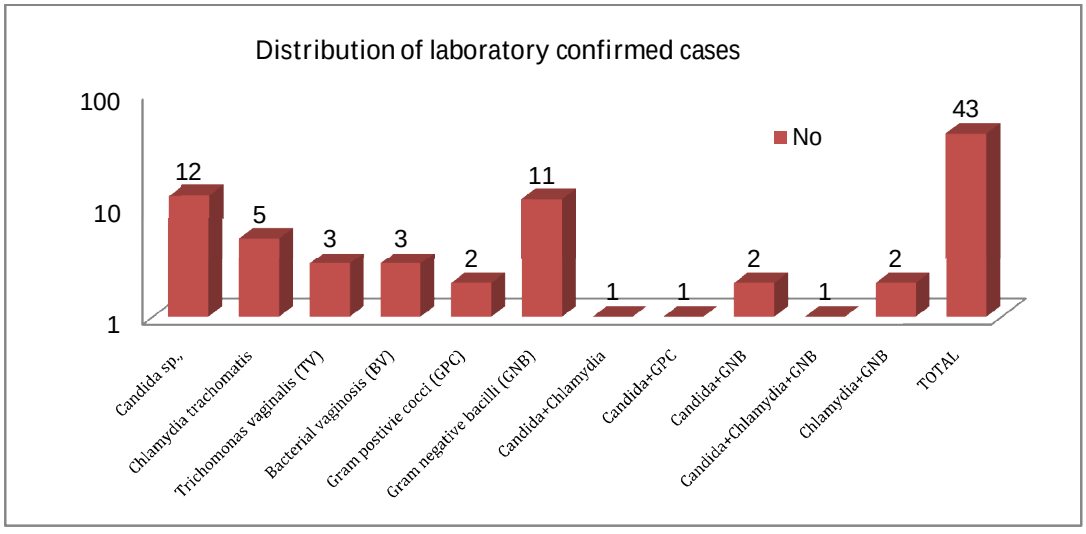


TABLE 13: DISTRIBUTION OF LOWER GENITAL TRACT INFECTIOUS AGENTS IDENTIFIED BY VARIOUS MICROBIOLOGICAL METHODS (n = 48)

Organisms	No. of isolates	Percentage
Candida sp.,	17	35.42
Chlamydia trachomatis	9	18.75
Trichomonasvaginalis	3	6.25
Staphylococcus aureus	2	4.17
Streptococcal sp.,	1	2.08
Citrobacterkoseri	1	2.08
Klebsiellapneunomiae	5	10.42
Escherichia coli	10	20.83
Total	48	100

Candida sp.,(17, 35.42%) was the most common organism identified followed by Escherichia coli (10, 20.83%). 18.75% of the samples were positive for C.trachomatis infection.

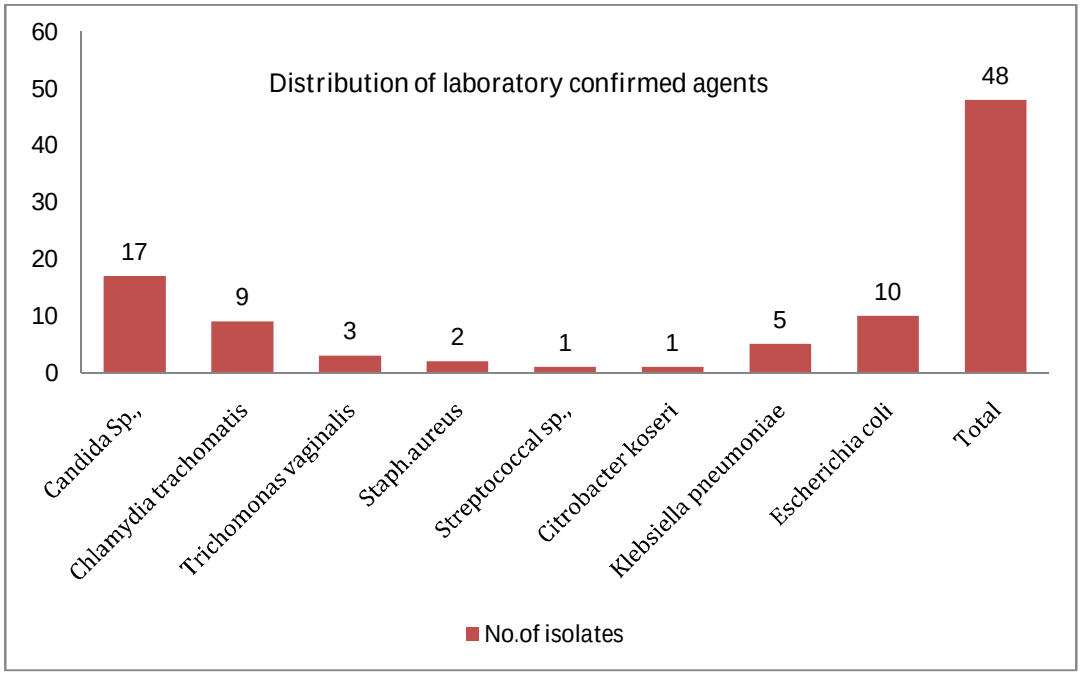


TABLE 14: DISTRIBUTION OF DIFFERENT SPECIES AMONG THE CANDIDA ISOLATES (n=17)

Candida sp.,	No. of isolates	Percentage
Candida albicans	8	47.05
Candida glabrata	3	17.65
Candida tropicalis	3	17.65
Candida kefyr	3	17.65
Total	17	100

Among the isolated Candida sp., Non albicans species of Candida (52.95%) was the commonest organism followed by Candida albicans (47.05%).

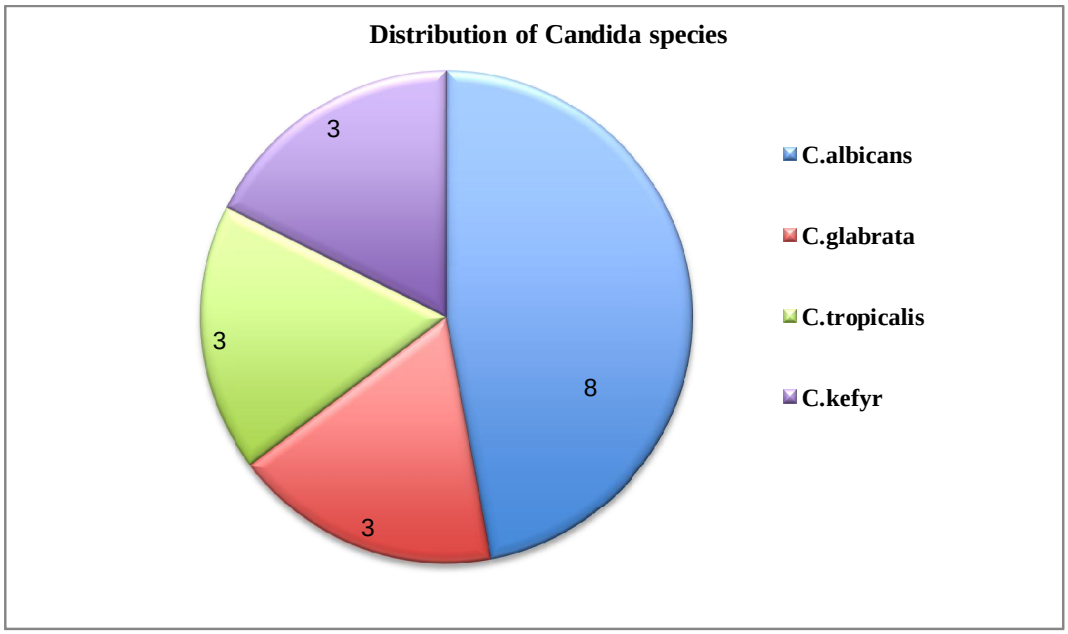
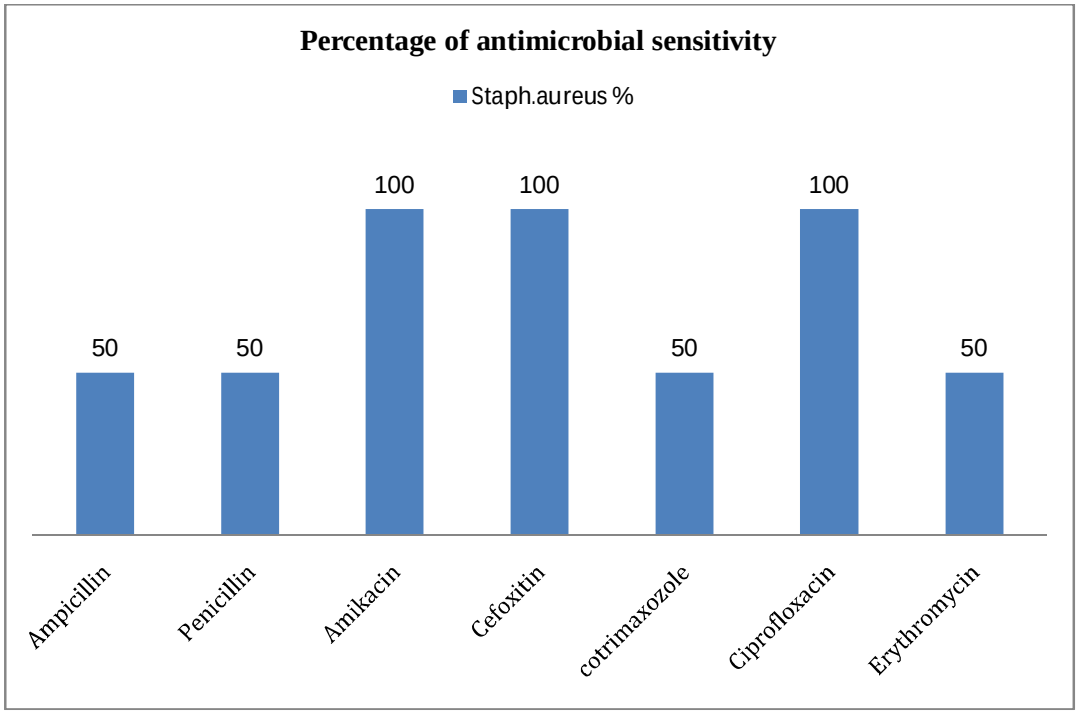


TABLE 15: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM POSITIVE COCCI INFECTION

Drugs	Staphylococcus aureus (n=2)		Streptococci sp., (n=1)	
	S	%	S	%
Ampicillin	1	50	1	100
Penicillin	1	50	1	100
Amikacin	2	100	NA	NA
Cefoxitin	2	100	NA	NA
Cotrimaxozole	1	50	1	100
Ciprofloxacin	2	100	1	100
Erythromycin	1	50	1	100

Staphylococcus aureus isolates had 100% sensitivity to amikacin, ciprofloxacin and cefoxitin, whereas 50% of the isolates were sensitive to ampicillin, penicillin, erythromycin and cotrimaxozole.

Streptococcus sp., had 100% sensitivity to ampicillin, penicillin, ciprofloxacin, cotrimaxozole and erythromycin.



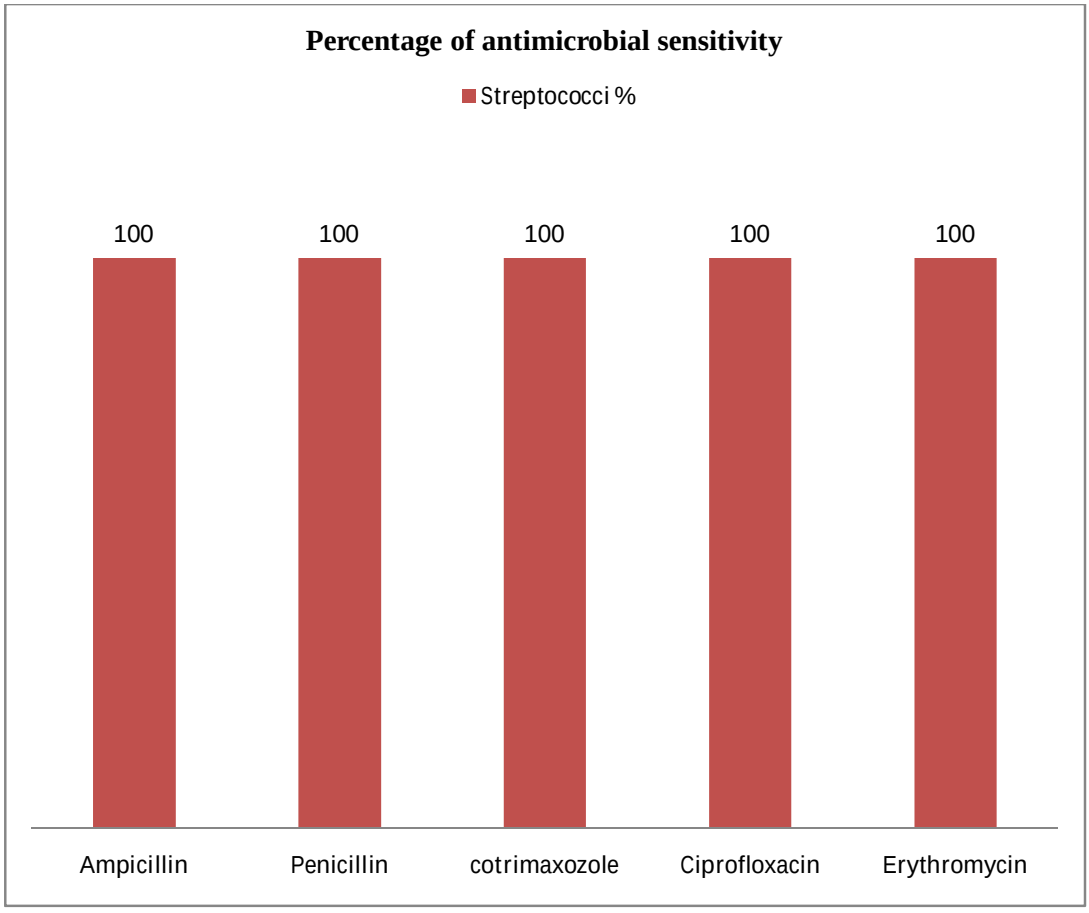

Percentage of antimicrobial sensitivity
Streptococci %
100
100
100
100
100
Ampicillin
Penicillin
cotrimaxozole
Ciprofloxacin
Erythromycin

TABLE 16: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACILLI INFECTION

Drugs	Escherichia coli (n=10)		Kleb. pneumoniae (n=5)		Citrobacterkoseri (n=1)	
	S	%	S	%	S	%
Gentamycin	7	70	3	60	1	100
Amikacin	10	100	5	100	1	100
Ciprofloxacin	7	70	3	60	1	100
Cefotaxime	7	70	5	100	1	100
CefotaximeClavulanic acid	10	100	5	100	1	100
Imipenam	10	100	5	100	1	100

All Gram negative isolates were 100% sensitive to amikacin, cefotaximeclavulanic acid and imipenam.

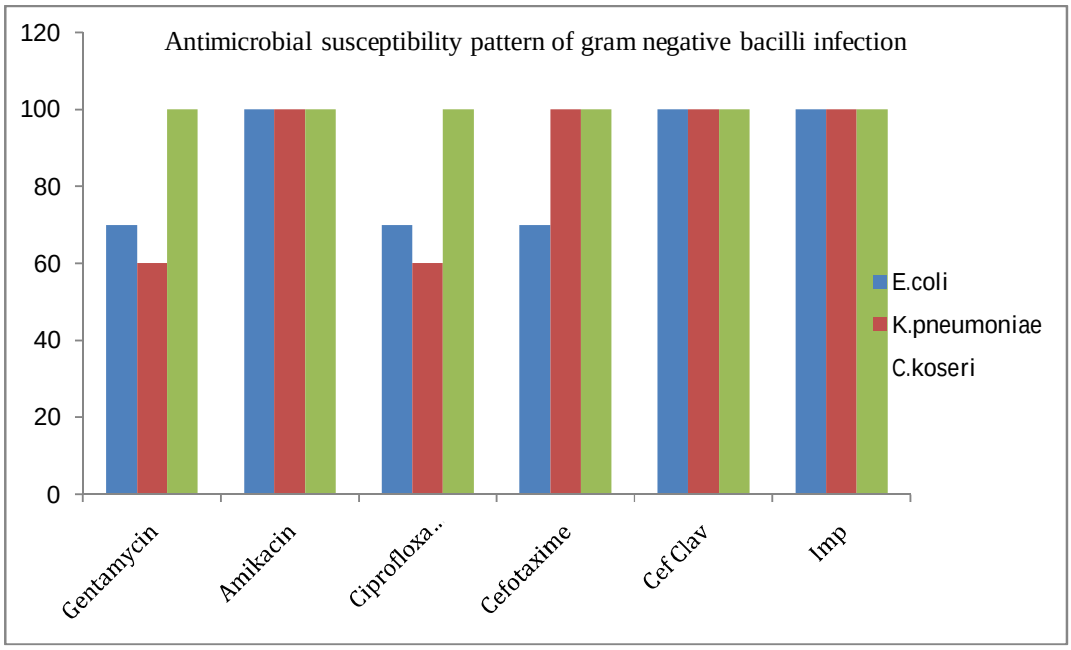


TABLE 17: ANTIFUNGAL SUSCEPTIBILITY PATTERN OF ISOLATED CANDIDA SP.,

Antifungal drug	C.albicans (n=8)		C.glabrata (n=3)		C.tropicalis (n=3)		C.kefyr (n=3)	
	No.	%	No.	%	No.	%	No.	%
Fluconazole (25ug)	8	100	3	100	3	100	3	100
Voriconazole (1ug)	8	100	3	100	3	100	3	100

All the Candida isolates have 100% sensitivity to fluconazole (25ug) and voriconazole (1ug).

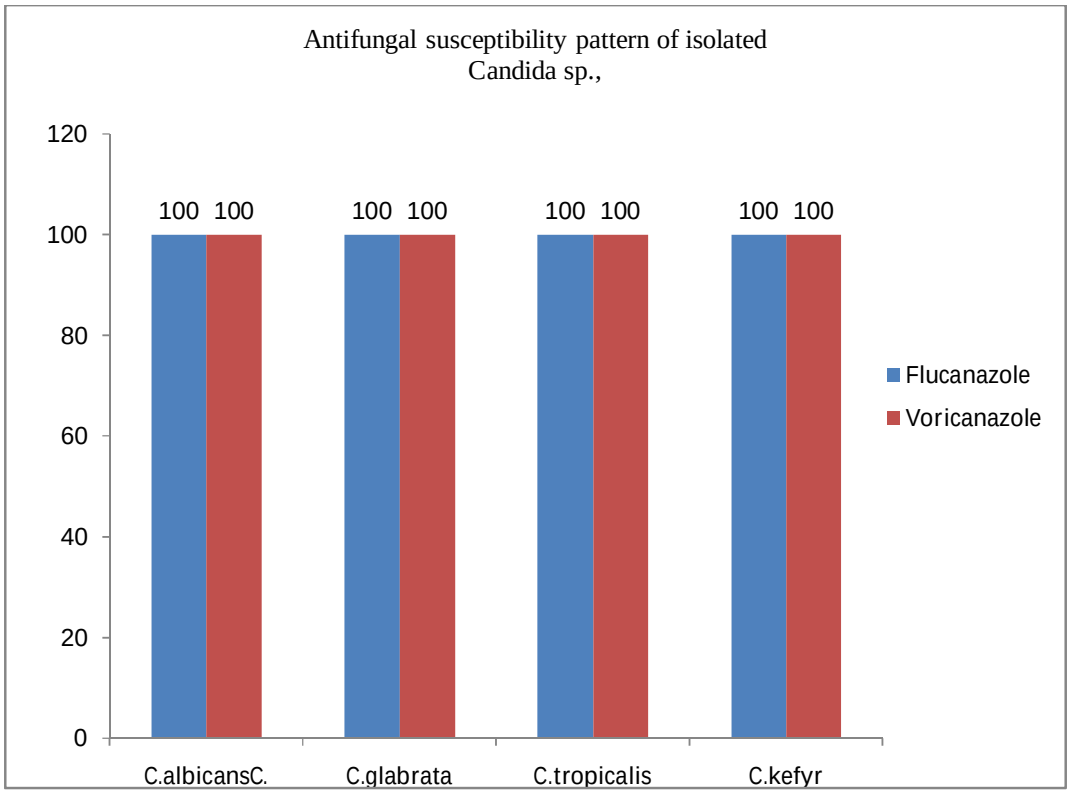


TABLE 18: DISTRIBUTION OF LABORATORY DIAGNOSED CASES IN RELATION TO AGE GROUP

Age	Candida sp.,		Chlamydia trachomatis		Trichomonas vaginalis		Bacterial vaginosis		Aerobic bacteria		Poly microbial	
	No	%	No	%	No	%	No	%	No.	%	No	%
≤ 30 years	9	75	5	100	2	66.67	3	100	7	53.85	7	100
> 30 years	3	25	0	0	1	33.33	0	0	6	46.15	0	0
Total	12	100	5	100	3	100	3	100	13	100	7	100

Candida sp., was the commonest agent (75%) causing lower genital tract infections in study group ≤ 30 years and aerobic bacterial agents were the commonest agent among > 30 years age group study population. Whereas, Poly microbial infections were seen only in age group≤ 30 years. Over all, lower genital tract infections were common among women ≤ 30 years with significant p value (0.0037)

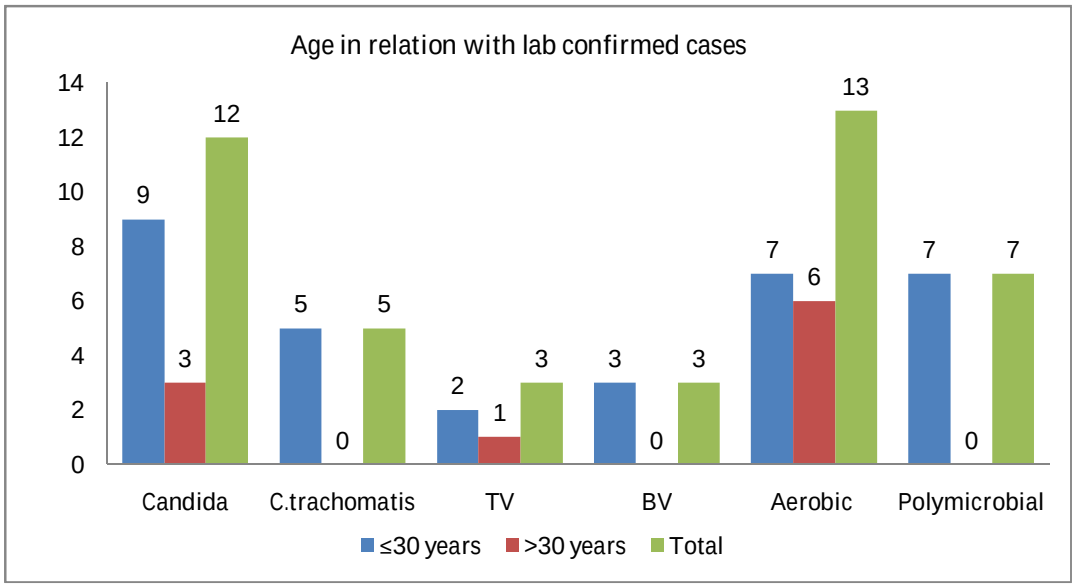


TABLE 19: DISTRIBUTION OF LABORATORY DIAGNOSED CASES IN RELATION WITH PARITY

PARITY	Candida Species		Chlamydia Trachomatis		Trichomonas vaginalis		Bacterial Vaginosis		Aerobic bacteria		Poly microbial	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Nulliparous	1	8.33	0	0	1	33.33	1	33.33	0	0	2	28.56
Abortion	0	0	0	0	0	0	1	33.33	1	7.69	1	14.29
One child (L1)	5	41.67	2	40	0	0	1	33.33	4	30.77	3	42.86
Two child (L2)	4	33.33	3	60	2	66.67	0	0	5	38.46	1	14.29
>2 child (>L2)	2	16.67	0	0	0	0	0	0	3	23.08	0	0
Total	12	100	5	100	3	100	3	100	13	100	7	100

Candida sp., was the most common agent affecting all the parity groups with majority of study group belonging to L1 (41.67%). Aerobic bacteria was commonly identified among all groups of parous women (L1, L2,>L2).Over all, lower genital tract infections were common among the women with multi parity with significant p value (0.0797)

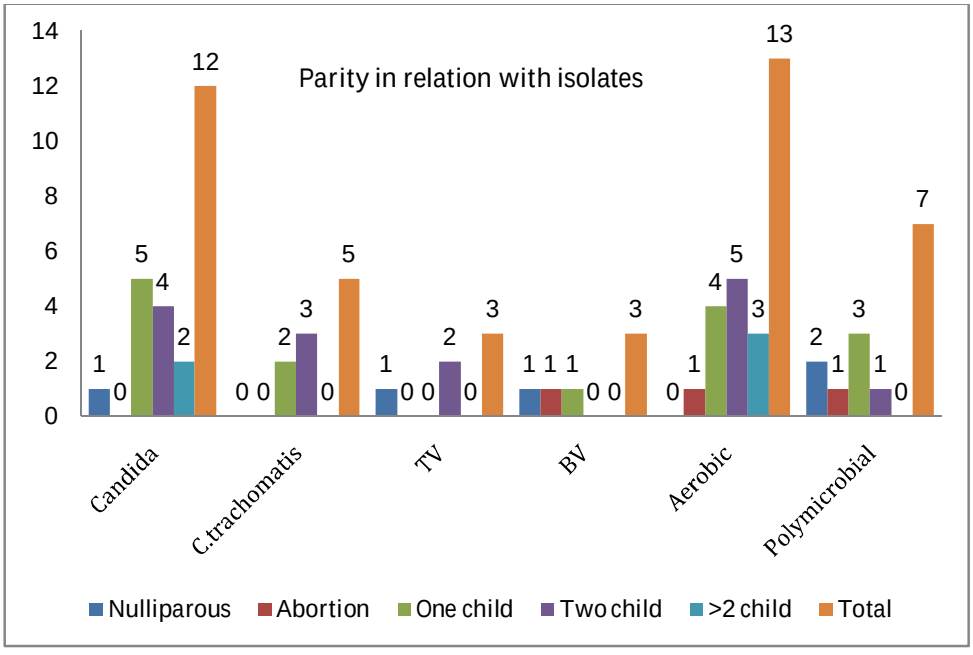


TABLE 20: SIGNIFICANCE OF SYMPTOMS AND SIGNS IN LOWER GENITAL TRACT INFECTIONS

Variables		Positive lab findings	%	Negative lab findings	%	Total	%
SYMPTOMS	Discharge	37	86.05	45	67.16	82	74.55
	Abdomen pain	23	53.49	39	58.21	62	56.36
	Itching	18	41.86	8	11.94	26	23.64
	Burning Micturition	10	23.26	9	13.43	19	17.27
SIGNS	No sign	6	13.95	35	52.24	41	37.27
	Discharge PV	35	79.55	9	20.45	44	40
	Redness	5	100.00	0	0.00	5	4.55
	Erosions	3	100.00	0	0.00	3	5.45
	Nodule	0	0.00	1	100	1	1.82
	Multiple sign	13	81.25	3	18.75	16	14.55

The symptom of Discharge per Vaginum and Itching acts a significant predictor for lower genital tract infections with p value of 0.0423 and 0.0005 respectively. All the clinical signs are statistically significant for the development of lower genital tract infections.

TABLE 21: SIGNIFICANCE OF CONTRACEPTION IN LOWER GENITAL TRACT INFECTIONS

Contraception	Positive laboratory diagnosis (n=43)		Negative laboratory diagnosis (n=67)		Total (n = 110)	
	No.	%	No.	%	No.	%
Nil	12	27.91	19	28.36	31	28.18
Barrier	1	2.33	3	4.48	4	3.64
IUCD	9	20.93	3	4.48	12	10.91
Tubectomy	21	48.84	42	62.69	63	57.27
Total	43	100	67	100	110	100

Among the subjects with lower genital tract infectious agents identified, 9 out of 12 (20.93%) were IUCD users with significant p value (0.0107).

TABLE 22: DISTRIBUTION OF CO INFECTIONS AMONG THE STUDY GROUP

Co infection	Positive laboratory diagnosis (n=43)		Negative laboratory diagnosis (n=67)		Total (n=110)	
	No.	%	No.	%	No.	%
HBV	0	0	2	2.99	2	1.82
HCV	0	0	0	0	0	0
HSV 2	17	39.53	11	16.42	28	25.45
HIV	0	0	0	0	0	0
Syphilis	0	0	1	1.49	1	0.91

HSV – 2 was the commonest co infection among both the laboratory confirmed and not confirmed cases, 39.53% and 16.42% respectively. P value was significant (0.0127) for association between HSV-2 co infections in lower genital tract infections. Note: HIV screening was done for high risk groups as a screening modality in ICTC centre and the results were taken with informed consent obtained from the subjects.

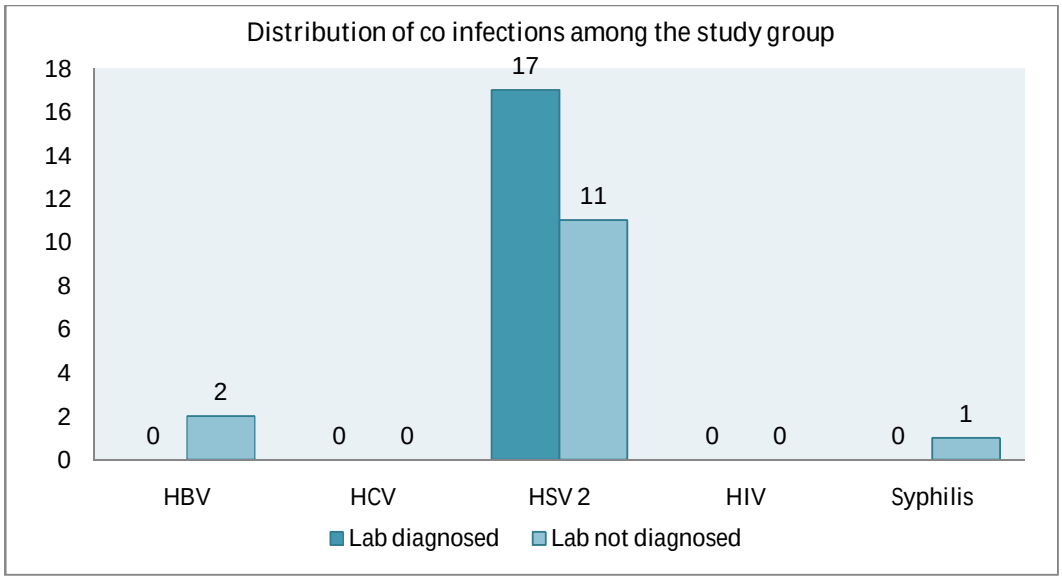


TABLE 23: MULTIVARIATE LOGISTIC REGRESSION MODEL FOR STATISTICALLY SIGNIFICANT PREDICTOR OF LOWER GENITAL TRACT INFECTIONS

Independent Variables	RTI Positivity		
	Odds Ratio	95% Confidence Interval	P value
Age < 30 years	0.6482	0.2486 to 1.6901	0.3753
Parity > 2	1.3900	0.4336 to 4.4555	0.5796
Abortion	5.2703	1.0117 to 27.4546	0.0484
Contraception	1.0226	0.4361 to 2.3978	0.9591
Semi Urban Residence	1.4654	0.6022 to 3.5662	0.3997
Unemployment	0.6818	0.2778 to 1.6736	0.4032
Spouse Occupation- Unskilled	0.4259	0.1631 to 1.1126	0.0815
Presence of Co Infection	3.3287	1.3673 to 8.1033	0.0081

On multivariate analysis of independent variables, the risk factors for developing genital tract infections were observed.Abortion has 5 times higher risk (5.2703 odds) and associated co infections positive people has 3 times higher risk (3.3287 odds) of developing lower genital tract infections

FIGURE 1: SPECIMEN COLLECTION KIT

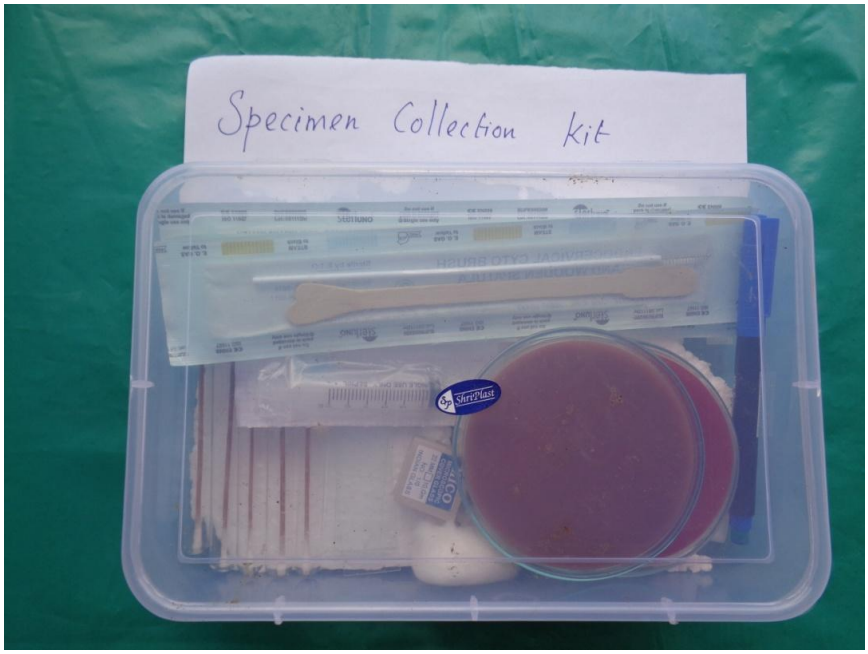


FIGURE 2: ENDOCERVICAL CYTOBRUSH

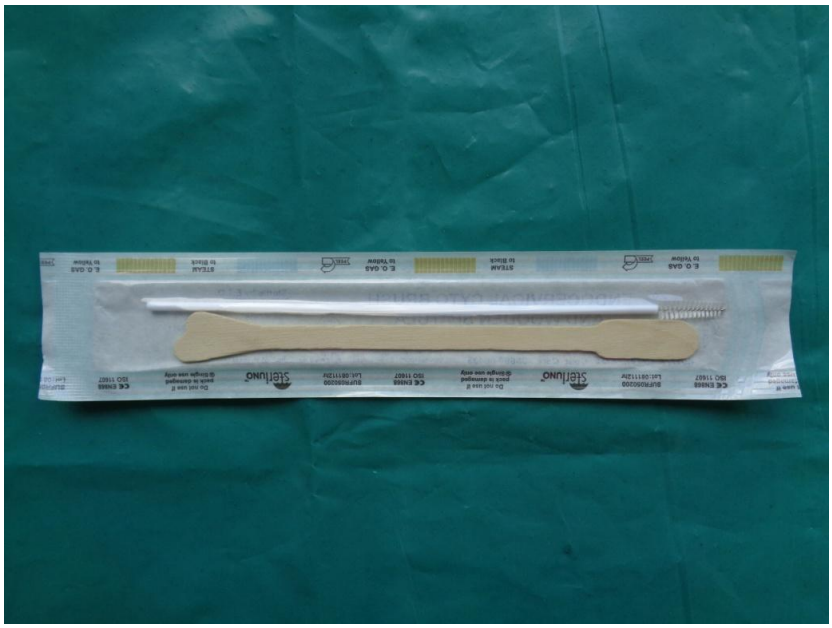


FIGURE 3: TRANSPORT MEDIUM USED FOR DETECTION OFCHLAMYDIA TRACHOMATIS

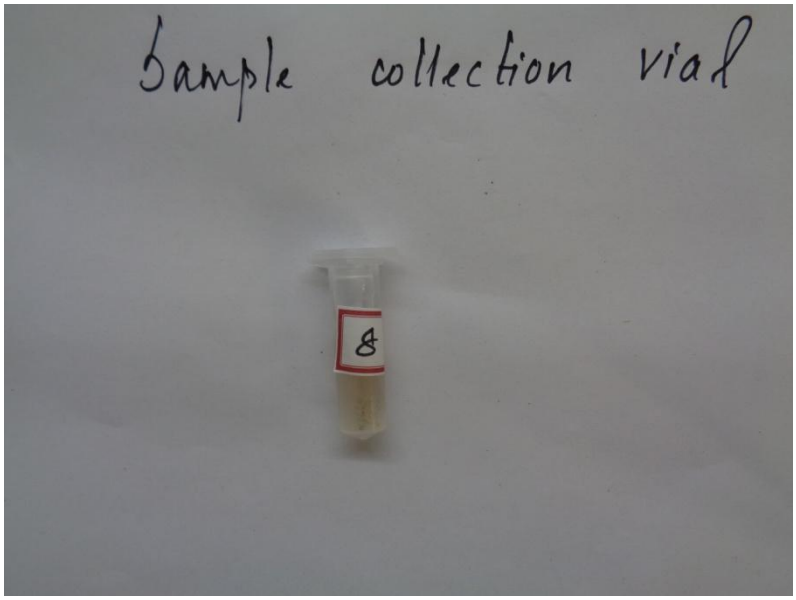


FIGURE 4: WET MOUNT OF VAGINAL DISCHARGE SHOWING TRICHOMONAS VAGINALIS

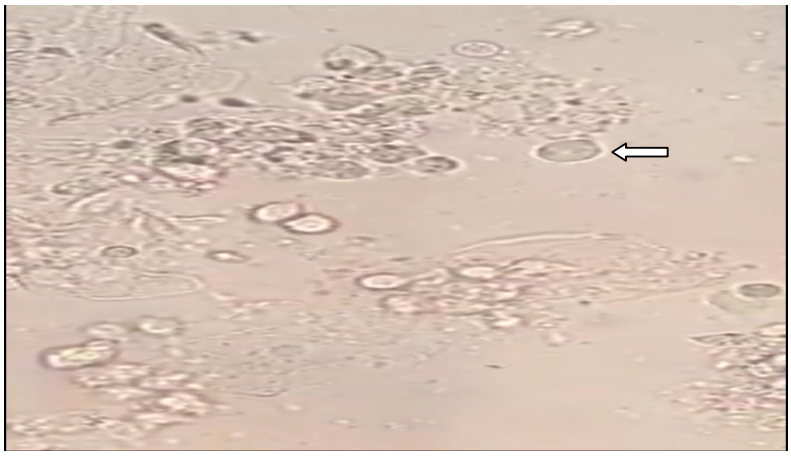


FIGURE 5: DIRECT GRAM STAINING OF VAGINAL DISCHARGE SHOWING PLENY OF PUS CELLS

FIGURE 6: DIRECT GRAM STAINING SHOWING PUS CELLS, SPERM AND GRAM NEGATIVE BACILLI

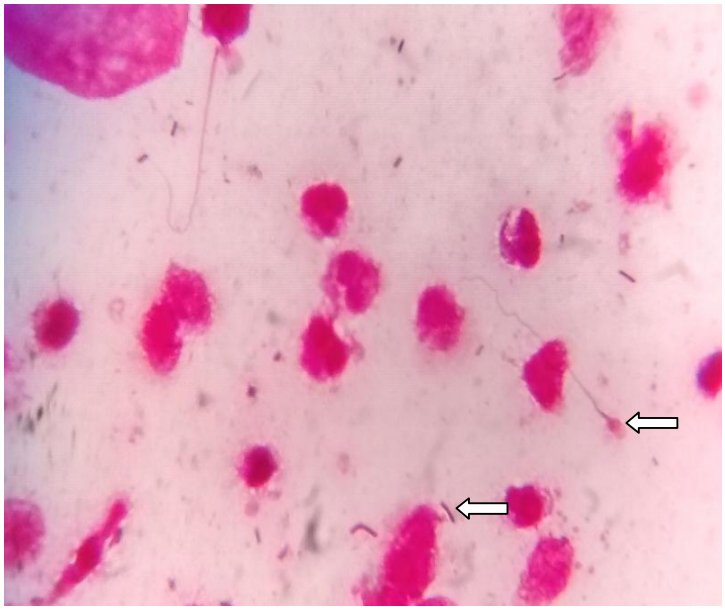


FIGURE 7 : DIRECT GRAM STAINING SHOWING GRAM POSITIVE BUDDING YEAST CELLS

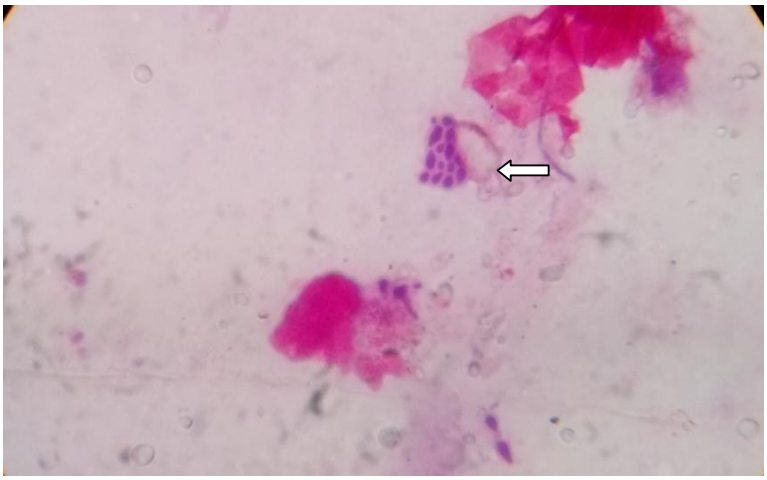


FIGURE 8: DIRECT GRAM STAINING SHOWING CLUE CELLS

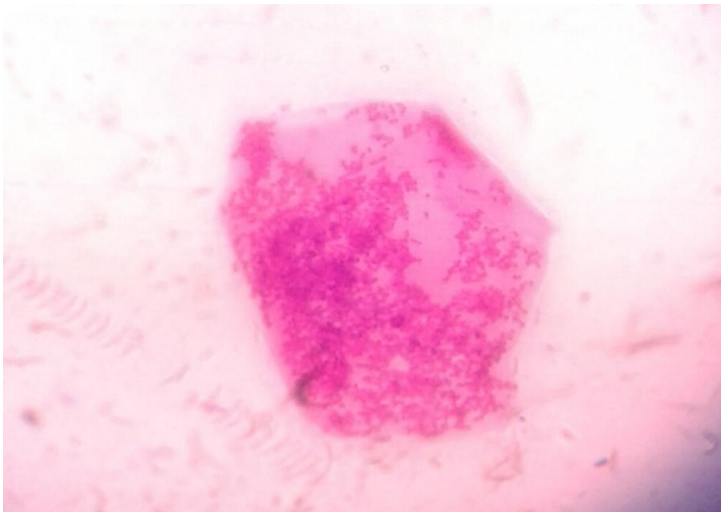


FIGURE 9: PAP SMEAR SHOWING TRICHOMONAS VAGINALIS

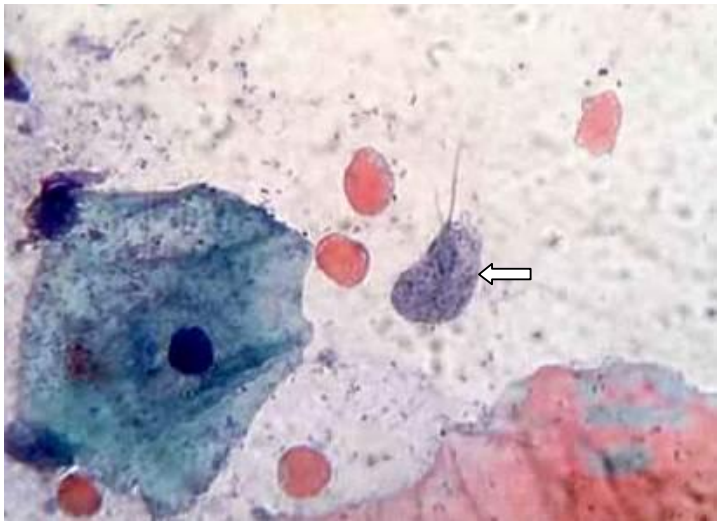


FIGURE 10: PAP SMEAR SHOWING YEAST CELLS

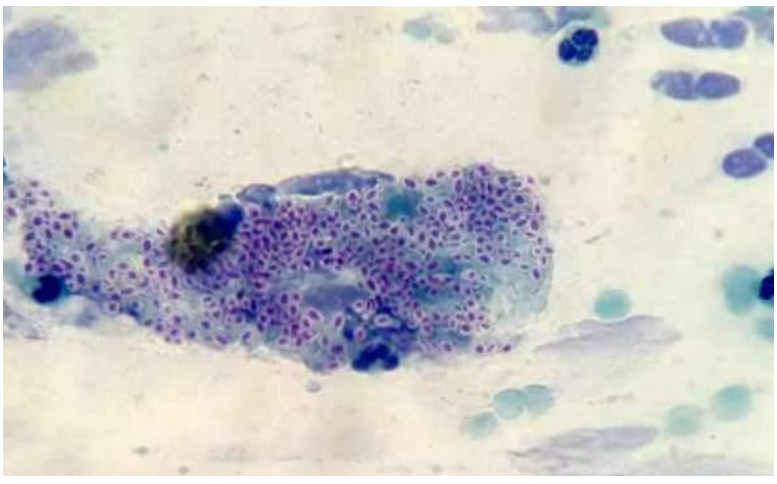


FIGURE 11: COLONY MORPHOLOGY OF CANDIDA IN SABOURAUD`S DEXTROSE AGAR

FIGURE 12: GERM TUBE TEST POSITIVE FOR CANDIDA ALBICANS

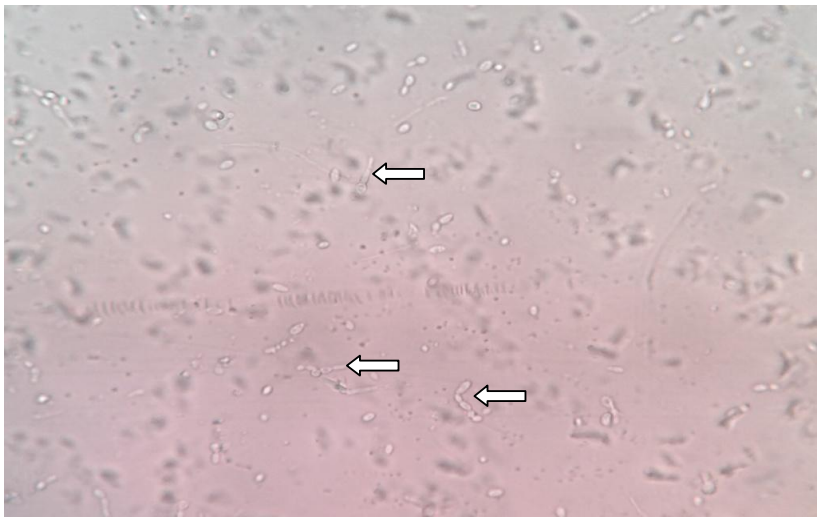


FIGURE 13: GRAM STAINING OF CANDIDA SPECIES GROWN IN SABOURAUD`S DEXTROSE AGAR

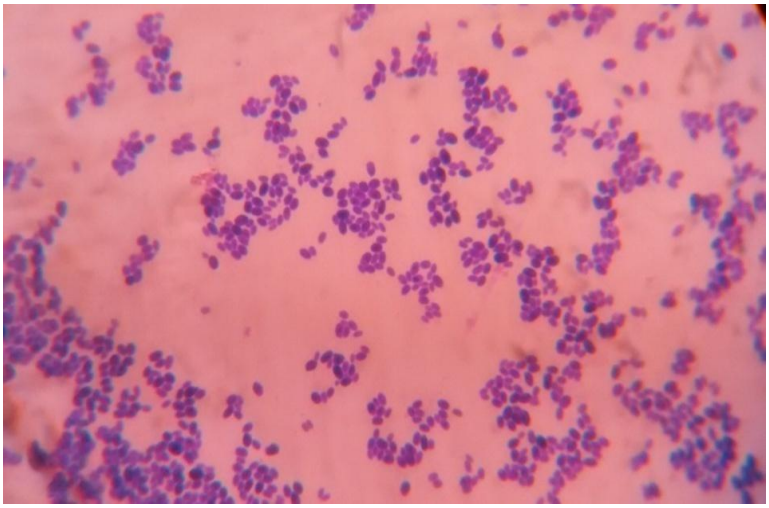


FIGURE 14: DALMAU PLATE CULTURE TECHNIQUE DONE FOR SPECIATION OF CANDIDA

FIGURE 15: DALMAU PLATE CULTURE ON CORN MEAL AGAR SHOWING FEATURES OF CANDIDA GLABRATA

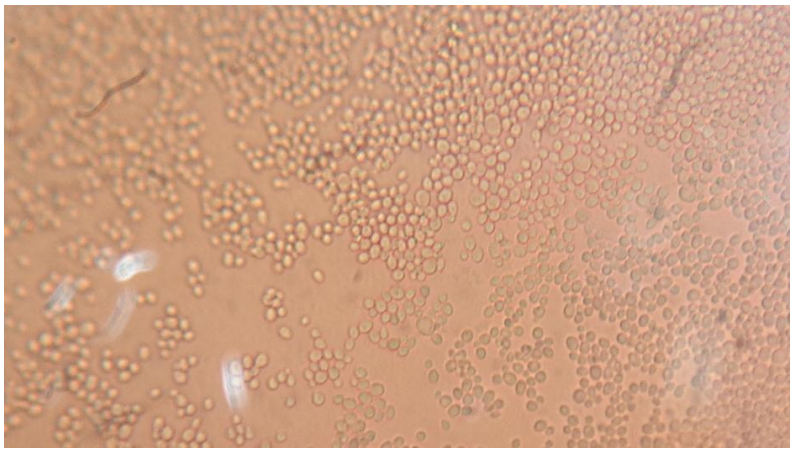


FIGURE 16: DALMAU PLATE CULTURE ON CORN MEAL AGAR SHOWING FEATURES OF CANDIDA KEFYR

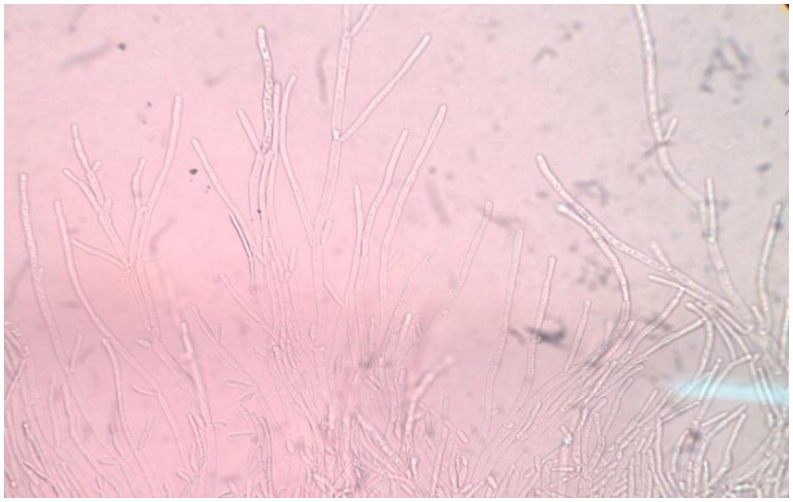


FIGURE 17: DALMAU PLATE CULTURE ON CORN MEAL AGAR SHOWING FEATURES OF CANDIDA TROPICALIS

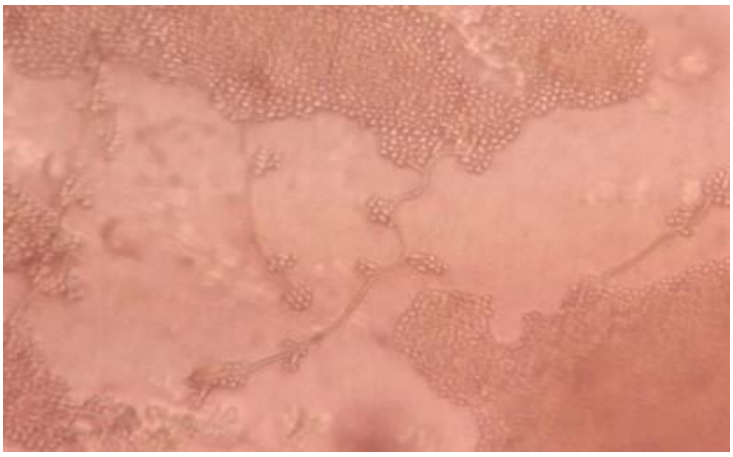


FIGURE 18: ANTIFUNGAL SUSCEPTIBILITY TESTING FOR CANDIDA TROPICALIS

FIGURE 19: PROTOCOL RUN FOR REAL TIME PCR ASSAY FOR DETECTION OF CHLAMYDIA TRACHOMATIS

MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Consolidated Report
C:\Users\Admin\Desktop\2015-Custom services\Dr.Malathi, Chegalpatu\Production - 01-06-2015.mxp
Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x8
Run date: June 01, 2015

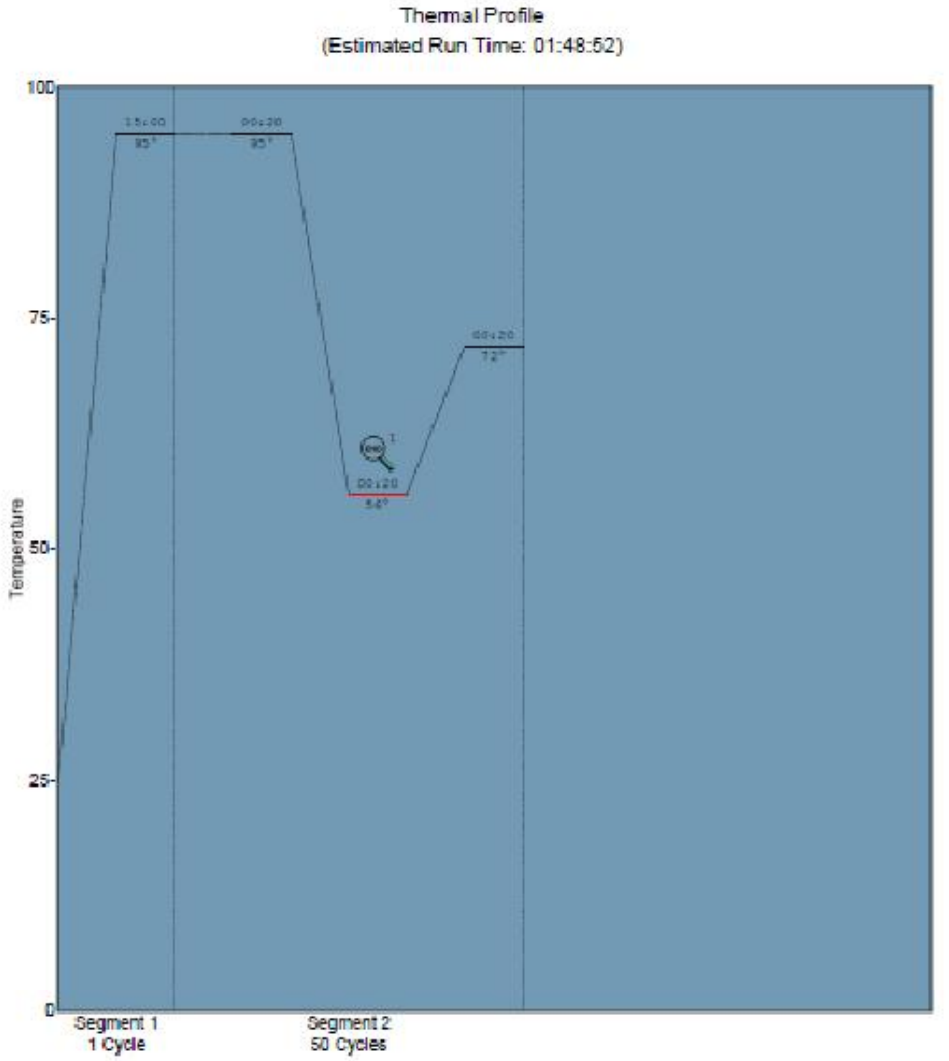


FIGURE 20: AMPLIFICATION PLOT OF REAL TIME PCR ASSAY FOR DETECTION OF CHLAMYDIA TRACHOMATIS

MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Consolidated Report
C:\Users\Admin\Desktop\2015-Custom services\Dr.Malathi, Chegalpatu\Production - 01-06-2015.mxp
Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x8
Run date: June 01, 2015

Amplification Plots

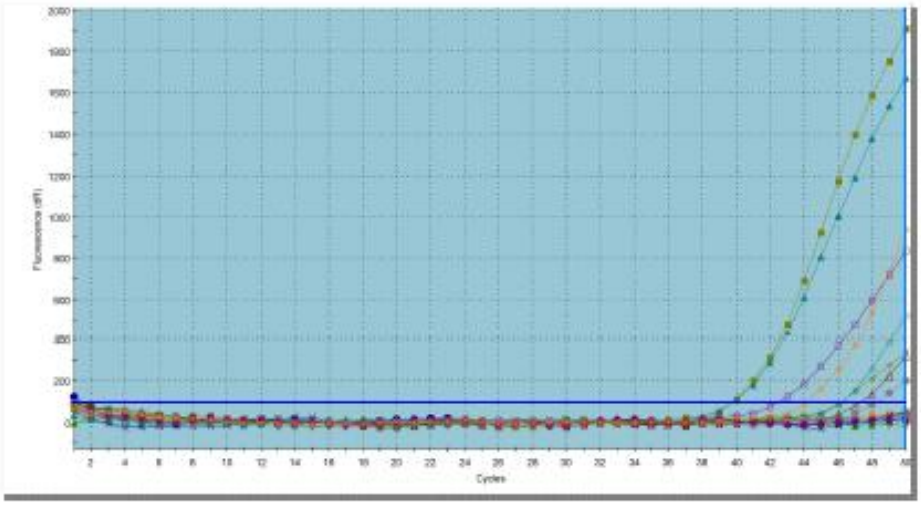


FIGURE 21: RAPID PLASMA REAGIN TEST FOR SYPHILIS

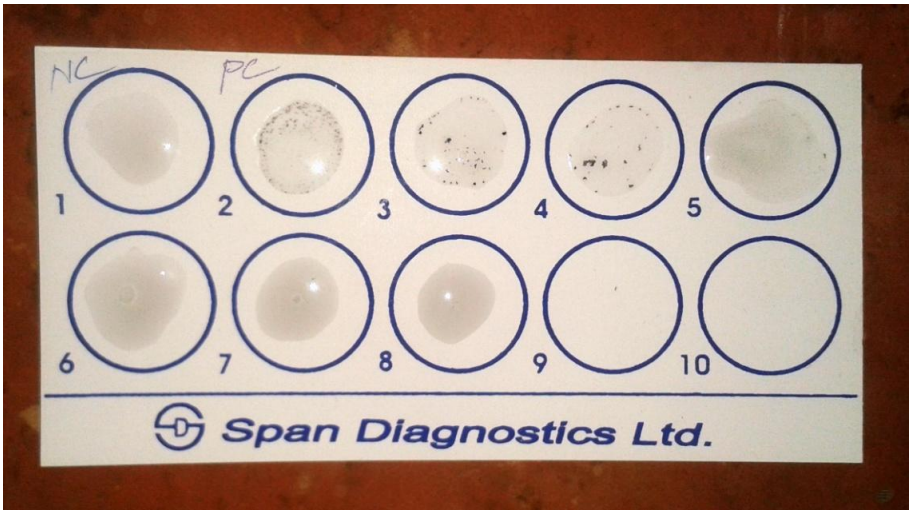


FIGURE 22: TREPONEMA PALLIDUM HAEMAGGLUTINATION ASSAY TEST FOR SYPHILIS

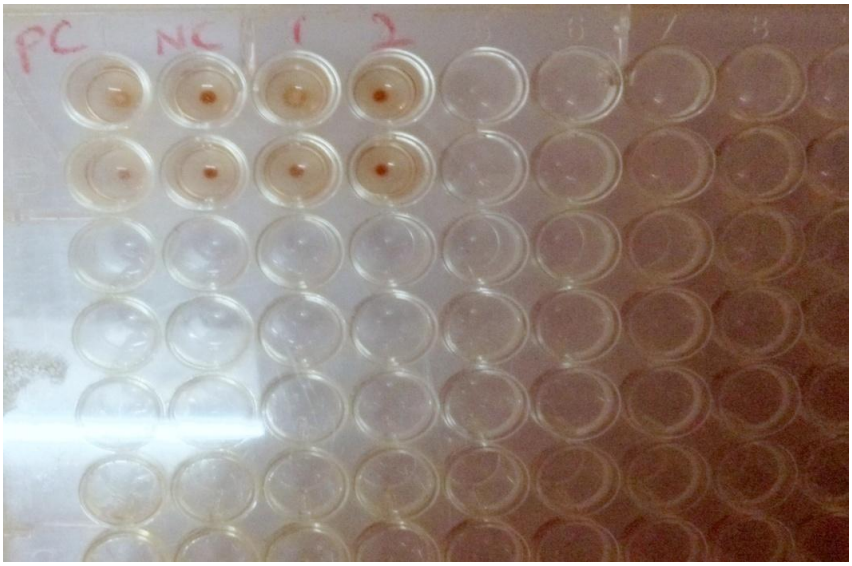


FIGURE 23: HEPALISA FOR THE DETECTION OF HEPATITIS B SURFACE ANTIGEN

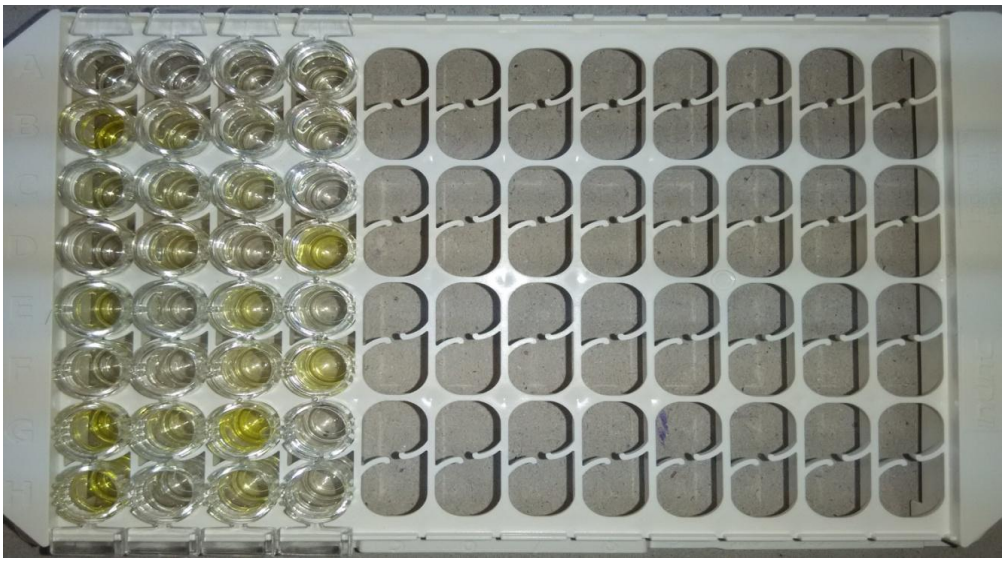
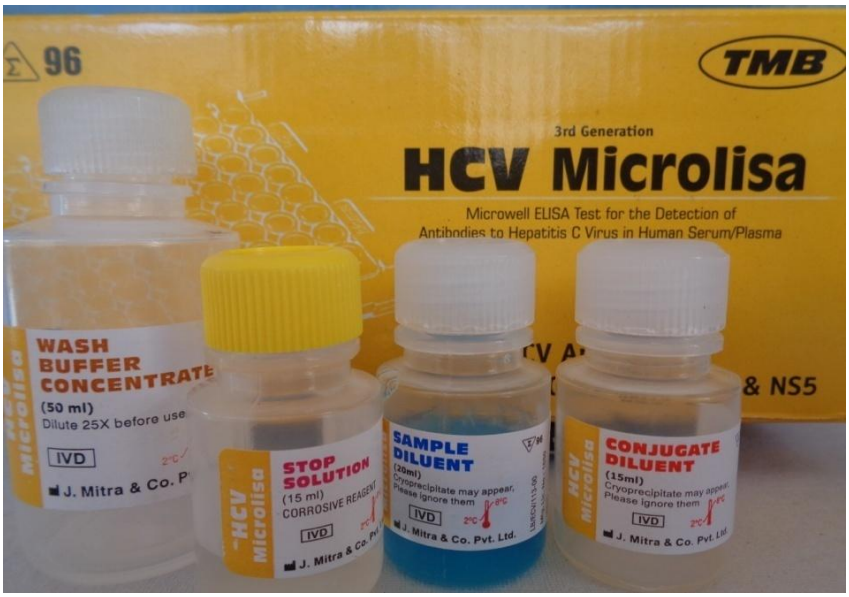


FIGURE 24: MICROWELL ELISA FOR THE DETECTION OF ANTIBODIES

TO HEPATITIS C VIRUS



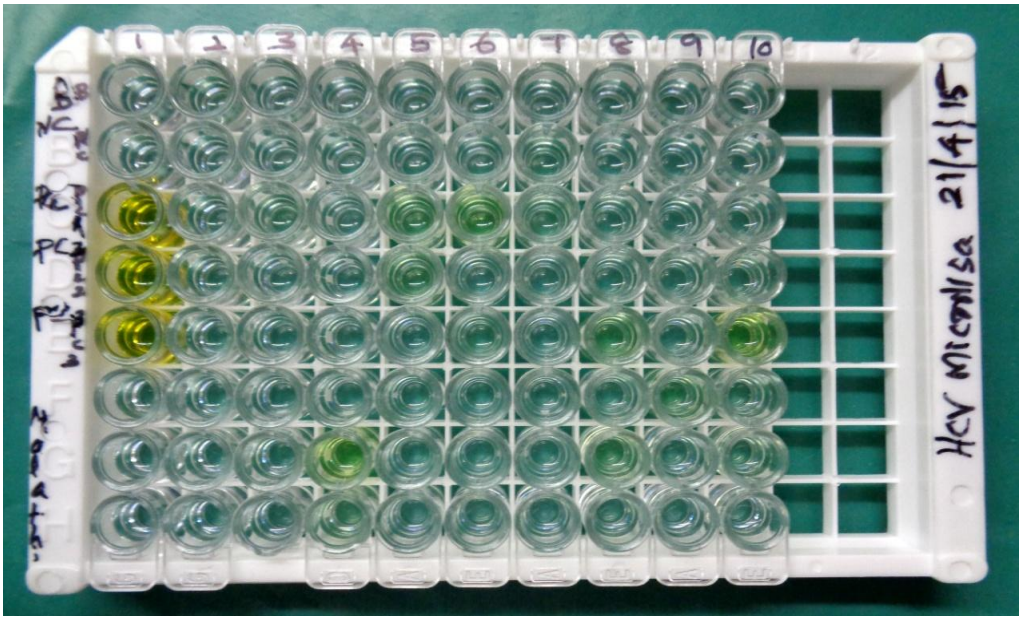
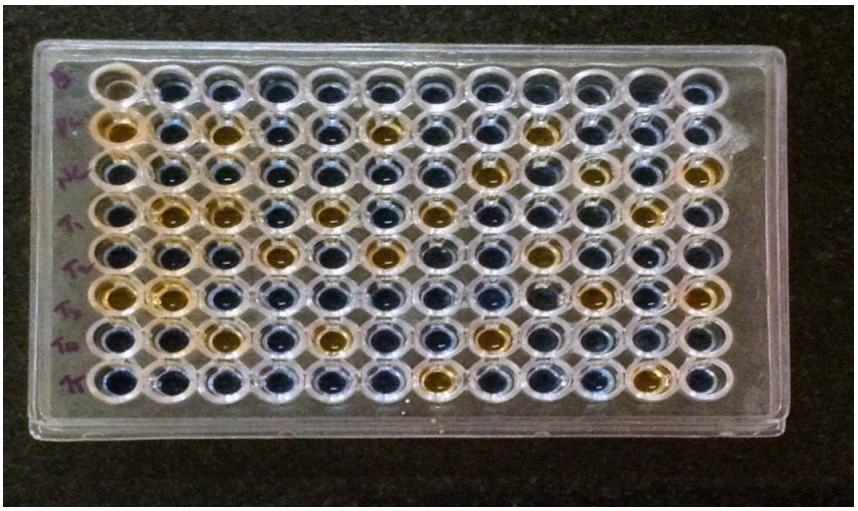


FIGURE 25: ELISA DETECTION OF IgG ANTIBODIES TO HERPES SIMPLEX TYPE 2 VIRUS



DISCUSSION

This study was conducted at the Department of Microbiology, Chengalpattu MedicalCollege in association with Department of Obstetrics and Gynecology, Chengalpattu Medical College and Hospital. A total of 110 subject who presented with the symptoms and signs of lower genital tract infections during the one year study period and who satisfied the inclusion criteria were included in the study.

Our country is in a state of epidemiological and demographical transition where morbidity and mortality rates may differ from previous demographic studies. In the last few years, community based studies on lower genital tract infections have been carried out in various parts of our country. Yet, South Indian studies are not widely available.

The socio demographic profile has a direct impact on the occurrence of lower genital tract infections and it was analyzed in our study. Age is one of the pivotal vulnerable factors in genital tract infections. The range of age group in our study population was from 18 to 45 years (Table 1) with the majority of age group between 21-25years (43.64%) followed by 26-30 years (33.64%). The predominant age distribution of 20-29 years is in accordance with other community based studies conducted by Shalini S *et al.*,2011 (74.3%) and Savita Sharma *et al.*, 2009 (63.6%).[74,75] Half of all the genital tract infections occur in this age group of 15 to 24 years and in some nations, 60% of all new infections occurred among these age group individuals (Brabin*et al.*,1995).[76]This increased trend in relation to age is because they belonged to more sexually active age when compared to other age groups.

As our study was conducted in a semi urban area, 76.36% of the study population belonged to rural community and 23.64% belonged to semi urban community (Table 2). In a review article by GayatriDesi*et al.*, the prevalence of symptoms of genital tract infections

was 39.3% and 33.6% in rural and urban India respectively.[77]Krishna ray *et al*., reported 74.8% in rural India and 68.1% in urban India.[78]Various studies have also depicted that there was no major difference in the prevalence of lower genital tract infections in rural and urban areas.

An increasingly observed factor which determines the morbidity is the occupation status of the individual and spouse. 67.27% were homemakers and the remaining study subjects (32.73%) were employed (Table 3). Majority of them were belonging to unskilled occupation (26.36%). Similar findings were observed in a study conducted by Prasad *et al*., in Tamil Nadu in which 55% were performing household chores and 77.5% in another study by Neeraja*et al.,* 2009.[33,79] The occupation status has a prime role in determining the occurrence of genital tract infections based on migration, duration of contact with partners, emotional bonding with partners, skill, knowledge and awareness about genital tract infections.

Age, residence and occupation are the hinges for determining the awareness of women about gynecological morbidities, decision making capacities and treatment seeking behavior in a rural society with stigma and discrepancies towards genital tract infections.

Another strong association is attributed to parity and genital tract infections. In our study, about 49.1% who presented with symptoms of lower genital tract infections belonged to L2 group (Table 4). Our study result correlated with study conducted by Sangeetha S Balamurugan*et al*., who have reported that the prevalence of RTI was 33% among women with more than one children and it gets increased on increasing parity.[80] The chance of getting a reproductive tract infections were 1.5times more among women who had more number of pregnancies than with fewer pregnancies as evidenced by study conducted by Prasad *et al.,*[33] There is also a strong link between the nature of deliveries and history of abortion, unsafe practices during delivery and factor for developing genital tract infection with significant p value of 0.0797 (Table 19)

A large number of studies have correlated well with contraceptive usage and genital tract infections. 9 out of 12 IUCD users (20.93% among the laboratory diagnosed cases) in our study (Table 20) were proven for laboratory diagnosed genital tract infection with significant p value (0.0107). Wasserheit*et al*., have estimated that women who were using IUCD have seven times increased risk of Pelvic inflammatory disease (PID) than with no contraceptive usage.[81] Similarly a South Indian study by Bhatia *et al*., showed there was 2.5 times increased risk for IUCD users in developing PID.[82] This has correlated well with our study results. The reasons for IUCD and genital tract infections are mainly attributed to the IUCD tail which facilitates the ascent of infectious organisms and also there is a change in the cervicovaginal flora which could favor for bacterial vaginosis. Out of 63 subjects who underwent permanent sterilization, 21(48.84%) presented with laboratory findings suggestive of lower genital tract infections. Even though it sounds to be clinically significant, statistical significance could not be obtained. This is in accordance with study conducted by Prasad *et al*., (23%) [33] The reason for increased number of infections in PS cases is due to lack of usage of barrier methods and increased sexual exposure.

Among the symptoms of lower genital tract infections identified in our study (Table 5, 6, 7), Discharge per vaginum (74.55%) was the major presenting complaint followed by lower abdomen pain (56.36%), Itching (23.64%) and burning micturition (17.21%). Anjanaverma*et al*., have reported that the prevalence of symptoms of lower genital tract infections were around 41.8% in rural Delhi, in which 41.8% discharge PV, 10% lower abdomen pain, 3% burning micturition and 1% dyspareunia .[83] National Family Health Survey -3 concluded that prevalence of these symptoms among Indian women ranged between 11 to 18% in nationwide studies. In some other studies by Rani *et al*., and Prasad *et al*., the range varied from 40 to 57%. [33,84] M.L.S Prabha*et al.,* found that the sensitivity and specificity for diagnosing a genital tract infection by vaginal discharge was 58.95% and 55.13% respectively.[85] For lower abdomen pain, the sensitivity was very low (14.49%), but specificity of 76.63%. This correlated well with our findings that in

laboratory diagnosed subjects, discharge and itching were found to be significant with p values 0.0423 and 0.0005 respectively (Table 21). Since our study was a hospital based study with lesser sample size and it included only symptomatic women, the percentages of individual symptoms seems to be in an increased way, when compared with community based studies.. As discharge per vaginum occurs also as a physiological phenomenon and the perception of symptoms differs from individual to individual, the reliability of patient`s history is lacking in genital tract infections. There is a necessity to do laboratory based diagnosis to overcome this drawback and to estimate the exact prevalence data.

The distribution of clinical signs (Table 8, 21) in our study has shown that out of 82 subjects who had discharge per vaginum as the primary complaint, only 44 presented with white discharge per vaginum on speculum examination. Among 44 subjects with vaginal discharge, only 30 (68.18%) was diagnosed with lower genital tract infections by laboratory methods. This warrants that symptoms based treatment has a drawback and it should be given only after proper speculum examination and laboratory evaluation of each individual to avoid unnecessary medication and improper estimation of prevalence data. Out of 41 subjects who presented with no characteristic clinical sign, 6 (13.95%) were diagnosed with lower genital tract infection by laboratory method. This signifies that the subjects may have taken a course of empirical treatment or the infection per se presented without any clinical signs. The correlation of these findings could not be obtained as there is lack of statistical data for individual clinical signs. On analyzing individual clinical signs, statistical significance was obtained in our study.Sangeetha S Balamurugan*et al*., 2012 have estimated 72.4% positivity for clinical signs in symptomatic women with RTI.[80] Hence it is advisory to screen all the reproductive age group women with feasible laboratory method at regular intervals to prevent sequelae.

The cervical region is both a sentinel target for genital tract infection and carcinoma. Women presenting with symptoms and signs suggestive of RTI should be evaluated by Pap to distinguish between malignancy and infection, as cervical cancer is the

second most common cause of cancer among women in our country. Moreover, chronic pelvic infections leads to inflammatory changes that adds up the risk for developing into malignancy. In our study, the findings of the routine cytological screening of women with leucorrhea were taken and the scoring was done according to Bethesda system. None of them had malignant changes, whereas 15.45% had inflammatory changes, 13.64% had yeast cells, 2.73% had clue cells and 2.73% had *Trichomonasvaginalis*(Table 9). In a study conducted by Smith et al., 16.2% of Bacterial vaginosisand 31% candidiasis were identified by Pap smear.[86] The proportion is lesser in our study as there is lesser sample size when compared to other studies. 88.23% of Candidiasis, 100% of bacterial vaginosis, 100% of *Trichomonasvaginalis* cases diagnosed by microbiological methods has been identified readily by Pap smear in our study. Data from Lions –FSH project, Palakad figured out that 41.5% inflammation, 6.5% Candidiasis, 6.2% *Trichomonasvaginalis* was identified by Pap smear in their control suburban population. The above finding implies that Pap smear can also be used as a screening modality in resource poor settingsfor identifying genital tract infections.

Out of 110 vaginal swabs which were subjected to wet mount microscopy, Gram staining, aerobic and anaerobic culture, fungal culture and the endocervical swabs which were subjected to culture for *N.gonorrhea* and Real time PCR assay for the detection of C.*trachomatis*, 43(39.09%) of the cases were positive for any one of the agents commonly causing lower genital tract infections (Table 10). This correlates well with a study conducted in rural area of Vellore district in Tamil Nadu by Prasad *et al.*, which showed 53% of women reported gynecological symptoms and 38% had laboratory findings for genital tract infections. [33] M.L.S Prabha*et al.*, have estimated the prevalence of 33.1% in their study population.[85] Kosambiya*et al.*, conducted a similar study at Surat with comparison of rural and urban prevalence showing 53% and 69% respectively.[86] The higher prevalence in urban areas is due to knowledge and awareness about genital tract infections and easy accessibility to tertiary care centres.

The findings of laboratory diagnosed cases were evaluated and it was estimated that in our study about 83.72% had single infection and 16.28% had mixed infections(Table 11). Among the individuals with single infections, the distributions were 27.91% *Candida sp*., 11.63% *Chlamydia trachomatis*, 6.97% *Trichomonasvaginalis*, 6.97% Bacterial vaginosis, 2.33% *Staphylococcus aureus*, 2.33% *Streptococcus sp.*, 16.28% *Escherichia coli* and 9.30% *Klebsiellapneumoniae* infections(Table 12). The mixed infections had various combinations with *Candida sp.*, and *C.trachomatis*.Prasad et al., figured out that 10% of vaginal candidiasis, 18.2% of bacterial vaginosis, 1.8% *Chlamydia trachomatis* and 12.9% *Trichomonasvaginalis* as laboratory diagnosed infection rate.[33] M.L.S.Prabha et al., estimated 14.3% bacterial vaginosis, 6% Candidiasis, 3% *Chlamydia trachomatis*, 1% *Trichomonasvaginalis* and 8.8% of secondary bacterial infections.[85] The differences in the figures in our study is due to lesser sample size, hospital based study while all the comparative study groups are community based studies.

Bacterial vaginosis, as such is a clinical term diagnosed by Amsel criteria. The sensitivity and specificity of Amsel criteria is 37% and 99% respectively (Beverely E sha*et al.*,). [87] We have estimated 6.97% of BV cases by the gold standard laboratory diagnostic method i.e.,Nugent scoring (Table 12). There is a low level of estimation in our study when compared to other studies (RohitChawla et al., 32.86% and TamonudModak et al., 24%). [88.89] Since only laboratory method was employed, correlation between Amsel criteria method and Nugent scoring method could not be obtained in our study. The consequences of BV include abortion, still birth, preterm deliveries and co infections are also more common. 100% of bacterial vaginosis cases affected the women with age group ≤30 years. This correlated well with SaradaTiyaggura et al., who have estimated that the majority of bacterial vaginosis affects ≤30 years women (53.1%). Hence in addition to screening of high risk groups, all child bearing age group and pregnant women should be screened for BV to avoid such complications.

The agents causing the lower genital tract infections were analyzed individually and it was estimated that 35.42% were *Candida sp* (Table 13). Among them, 47.05% *Candida albicans*, 17.65% *Candida glabrata*, 17.65% *Candida tropicalis*, 17.65%*C. kefyr* (Table 14). There is slightly increased prevalence when compared to other studies. Jindal et al., estimated 23.4% culture positive for *Candida sp.*, with 74.4% of *Candida albicans*,11% *Candida glabrata*, 6% *Candida tropicalis*, 3.6% *Candida krusei* and 2.43% *Candida parapsilosis* and *Candida guillermondi* each.[90] Anisahmad et al., estimated 20.47% prevalence of *Candida sp.*, with *Candida glabrata* 36.7%, *Candida parapsilosis* 10.2%, *Candida tropicalis* 2.8%, *Candida kefyr* 1.8% and *Candida krusei* 1.4%. [91] 75% of *Candida* isolates affected ≤30years which gets correlated with a study conducted by SujithD.Rathod et al., 2012. [92] Vulvovaginal candidiasis (VVC), as such is not a sexually transmitted disease but the majority of women presenting with leucorrhea is diagnosed with VVC. *Non albicans* species of *Candida* is in increased prevalence for the recent years. The risk of recurrent infections is more common if the infection is not properly treated. Also, there is increased prevalence of resistant strains among *Candida sp.*, due to inadvertent usage of antifungal treatment (Zahra salehi et al.,).[93] It is advised to perform antifungal susceptibility testing for all candida isolates to give appropriate treatment and to avoid the raising antifungal resistance strains. In our study, according to CLSI disk diffusion zone interpretation method, all the isolates (100%) were sensitive for Fluconazole (25ug) and Voriconazole (1ug). Quindos*et al.* study showed that the sensitivity of *Candida* species to fluconazole and ketoconazole was 90.2% and 91.4% respectively.[94] Many more studies have estimated the resistance pattern and concluded that it is advisable to do antifungal susceptibility testing in order to battle against the emerging antifungal drug resistance.

Chlamydia trachomatis (18.75%) was the proportion obtained from our study (Table 13). The prevalence rate of *C.trachomatis* by PCR is 7.04% (Dwibedi*et al.*,) and 23% (Daman Saluja*et al.,)*[95,96] The increased proportion of *Chlamydia trachomatis* in

our study is attributed to the molecular diagnostic technique employed i.e., Real-time PCR detection method which has higher sensitivity when compared to other methods. Jaton K *et al*., have identified that the sensitivity and specificity of Real time PCR is 95.7% and 100% respectively.[97] As *Chlamydia trachomatis* presents as asymptomatic infection, untreated genital tract infection can lead to ramifications for the childbearing age group women. It is a must to screen for all the child bearing age group women for *C.trachomatis* infection with cost effective methods. The available diagnostic techniques like ELISA are not a reliable indicator and at least in resource rich areas, molecular methods are advisable to estimate the exact prevalence of *C.trachomatis* infection.Similar to findings observed by Ugboma*et al*., and Mawak*et al.*, *Chlamydia trachomatis* (100%) affected the age group ≤30 years in our study (Table 18).This finding is evidenced by Corbeto et al.,2010 in his study.[98,99] The reasons for the significance of this age group and *C.trachomatis* infections is due to early coitarche, inconsistent use of barrier methods and multiple sexual partners. There is a need to educate the young sexually active females to avoid risky sexual practices and to undergo periodic screening for *C.trachomatis*. Health programmes should be implemented to screen the clinically silent *C.trachomatis* infections at an early age (<25 years) to safeguard the reproductive health of women.

6.25% of lower genital infections were contributed by *Trichomonasvaginalis* in our study (Table 13). This is in accordance with 8.5% estimated by Madhivannan*et al.,*[44] As TV is a treatable infection, the consequences like preterm deliveries, transmissibility to sex partner, increased exposure for other sexually transmitted infections can be prevented when appropriate etiological diagnosis is obtained. In developing countries, the screening modalities are mainly aimed at high risk groups leaving behind the study population belonging to community, where the exact prevalence data is submerging. In our study, only wet mount method was used as the modality of diagnosis of TV. The sensitivity of wet mount method is 55% (Sood s *et al.*,)[100] ShivkumarShetkarhave estimated that the prevalence of TV by wet mount and broth culture method was 0.5% and 3% respectively. Accordingly, there is an underestimation of the TV cases in our study and if broth culture method has been employed, the actual data could be obtained. However, our estimation

reflects the methodology employed in a developing country like India, where wet mount is the universal method of screening (NACO guidelines).

Among the aerobic bacterial infections, 6.25% (4.66% as single infection, 1.59% as mixed infection) caused by Gram positive cocci and 33.33% (25.58% as single infection, 7.75% as mixed infection) by Gram negative bacilli (Table 13). The difference between colonization and infections was established by the presence of active symptoms, presence of pus cells in wet mount and Gram staining, presence of inflammation in Pap smear findings and remission of symptoms following treatment. *Staphylococcus aureus* isolates showed 100% sensitivity to amikacin, ciprofloxacin and cefoxitin, whereas only 50% isolates showed sensitivity to ampicillin, penicillin, erythromycin and cotrimaxozole. *Streptococcus sp.*, had 100% sensitivity to ampicillin, penicillin, ciprofloxacin, cotrimaxozole and erythromycin (Table 15) The gram negative isolates were 100% sensitive to amikacin, cefotaximeclavulanic acid and imipenam (Table 16). The above results were substantiated by Samia S Kamees and J.Kouamouo*et al.,* in their studies.[68]

In our study, the age group ≤30 years were more prone for poly microbial infections (16.28%) with significant p value of 0.0037 (Table18). This is in accordance with a study conducted by Richard Omorogie*et al.*, 2010 where the prevalence of mixed infections was 25.16%.[101] The findings of parity analysis in our study showed that mixed infections were equally distributed in almost all the types of parity groups.

On analyzing the subjects with laboratory diagnosed lower genital tract infections, HSV 2 was the predominant co infectious agent (39.53%) with significant p value (0.0127). The positivity of HBV (2.99%), Syphilis (1.49%) and HSV 2 (19.42%) were noted in laboratory undiagnosed cases of our study group (Table 22). This differs from other research studies like Devinder*et al.*, (82.9% HSV 2 infection) and Madhivannan*et al.*,(11.3% HSV 2 infection). [57,44] This variation in the data is supported by a global review narrated by Smith and Robinson who suggested that HSV-2 prevalence depends upon residence, population subgroup, age and sex.

In our study group, there was nil clinical sign (i.e., Chancre) suggestive of primary syphilis. Serological screening for syphilis revealed 1.49% positivity in the study population as detected by RPR which was further confirmed by TPHA. This finding is corroborative with 1.5% of syphilis cases identified by Prasad *et al.,*[33]

Women of child bearing age have a risk of transmission of HBV to their offspring and hence it is a must to screen all the women for HBV infection and carrier state. 2.99% of our study group was positive for HBV. This is an underestimate when compared with NajmaJaved (14.07%) and Yu Zhang (9.51%), which is mainly due to lesser sample size.[62]

A number of studies have been conducted to demonstrate the prevalence of HBV, HSV, HIV and syphilis among reproductive age group women. Many studies have postulated a strong association between HSV-2 positivity and HIV/Syphilis infections. However, no such association was made in our study due to different set of population involved in various studies. The vulnerability of women to acquire HSV, HBV, HIV, HCV and syphilis is due to lack of knowledge about the infection rates, insufficient access to prevention, inability to adopt safer sex due to male dominance and lack of easy availability of female condoms. Hence targeted interventions should be aimed at prevention strategies through information and health education.

The significance of individual risk factors for developing lower genital tract infections was finally compiled and evaluated in our study by multivariate logistic regression analysis (Table 23). It showed abortion has 5 times higher risk (5.2703 odds) and associated co infections positive people has 3 times higher risk (3.3287 odds) of developing lower genital tract infections.

SUMMARY

This study conducted at the Department of Microbiology, Chengalpattu Medical College, Chengalpattu aimed at analyzing the microbiological profile of lower genital tract infections revealed the following findings.

- Out of 110 subjects, 43 subjects (39.09%) were identified with laboratory findings for lower genital tract infections.
- Lower genital tract infections were common among women with age ≤ 30 years and parity belonging to L2 group.
- Women who were IUCD users (20.93%) were more affected with lower genital tract infections.
- Discharge per vaginum (86.05%) and Itching (41.86%) were the significant symptoms seen in the 43 subjects who had laboratory findings for lower genital tract infections.
- All the clinical signs were found to be statistically significant for diagnosing lower genital tract infections.
- Out of 43 laboratory diagnosed cases, 83.72% presented with single infection and 16.28% with mixed infections.
- 6.97% cases of Bacterial Vaginosis were estimated in our study.
- On analysis of individual agents causing lower genital tract infections, 35.42% *Candida sp.*, 20.42% *Escherichia coli,* 10.42% *Klebsiellapneumoniae*, 6.25% *Trichomonasvaginalis*, 4.17% *Staphyloccocusaureus,* 2.08% each of *Streptococcus sp.,* and *Citrobacterkoseri* was identified in our study.

- 18.75% of *Chlamydia trachomatis* was identified as etiological agent in genital tract infections by Real time PCR assay.
- Out of 17 *Candida sp.*, 47.05% was *Candida albicans* and 17.65% each of *Candida glabrata, Candida tropicalis, Candidakefyr.*
- *Staphylococcus aureus* isolates had 100% sensitivity to amikacin, ciprofloxacin and cefoxitin (MSSA).
- *Streptococcus species* had 100% sensitivity to ampicillin, penicillin, ciprofloxacin, cotrimaxozole and erythromycin.
- All Gram negative isolates were 100% sensitive to amikacin and imipenam.
- All the Candida isolates had 100% sensitivity to fluconazole and voriconazole.
- Pap smear findings revealed 13.64% yeast cells, 2.73% each of Bacterial Vaginosis and *Trichomonasvaginalis* which correlated well with microscopy and culture.
- On screening for co infections, out of 110 subjects, 25.45% HSV-2, 1.82% HBV, 0.91% Syphilis positivity was observed in our study. Among them, 39.53% HSV-2 positivity were laboratory diagnosed cases for lower genital tract infections.
- Abortion has 5 times higher risk (5.2703 odds) and associated co infections positive people has 3 times higher risk (3.3287 odds) of developing lower genital tract infections.

CONCLUSION

This study conducted at the Department of Microbiology, Chengalpattu Medical College, Chengalpattu, aimed at analyzing the microbiological profile of lower genital tract infections had revealed that accurate diagnosis of lower genital tract infections can be established by laboratory based methods. The findings of our study have thrown some light on the dark shades of stigmatized society, where the estimation of exact prevalence of genital tract infections is cumbersome.

A complex interplay of factors like sexual promiscuity, unprotected sexual behavior, stigma towards genital tract symptoms and non availability of screening modalities at all health centres contributes to increased occurrence of genital tract infections in our country. Community based studies should be conducted at regular intervals to estimate the socio-demographic transition status of genital tract infections all over the country. This focus helps in timely intervention by formulating new policy and programmes to improve the reproductive health of women.

As *Chlamydia trachomatis* is the most common cause of treatable RTI worldwide and it presents mostly as asymptomatic infection, screening is mandatory for all the child bearing age group women to avoid consequences like PID and infertility. Hence it is recommended to take a prudent action on implementing a nationwide screening programme for *Chlamydia trachomatis.*

To conclude, laboratory diagnosis for women with symptoms and signs is suggested when compared to syndromic approach. Screening for sexually transmitted infections by serological methods are mandatory in women with symptoms and signs of genital tract infections. In addition, Pap smear can be used as a reliable indicator for screening genital tract infections in resource poor areas.

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ABBREVIATIONS

RTI	–	Reproductive Tract Infections
STD	–	Sexually Transmitted Disease
HIV	–	Human Immunodeficiency Virus
HBV	–	Hepatitis B Virus
HCV	–	Hepatitis C Virus
HSV	–	Herpes Simplex Virus
HPV	–	Human Papilloma Virus
TV	–	TrichomonasVaginalis
BV	–	Bacterial Vaginosis
PID	–	Pelvic Inflammatory Disease
RPR	–	Rapid Plasma Reagin
TPHA	–	TreponemaPallidumHaemagglutination
ELISA	–	Enzyme Linked ImmunoSorbent Assay
PCR	–	Polymerase Chain Reaction
NACO	–	National AIDS Control Organisation
WHO	–	World Health Organisation
CDC	–	Centre for Disease Control

APPENDIX

STAINS AND REAGENTS:
GRAM STAINING

- Methyl violet (2%) - l0g Methyl violet in l00ml absolute alcohol in 1litre of distilled water (primary stain)
- Grams Iodine l0g Iodine in 20g KI (fixative)
- Acetone (Decolourising agent)
- Carbolfuchsin 1% Secondary stain

PAPPANICOLAOU STAIN:

- Ethyl alcohol 80 to 82%
- Water 9 to 10%
- Methyl alcohol 4 to 5%
- Isopropyl alcohol 4 to 5%
- Eosin- Y <1.0%
- Phosphotungstic acid <1.0%
- Fast green <0.1%

CULTURE MEDIA:

1. Mac Conkey agar:

Peptone - 20g

Sodium taurocholate- 5g

Distilled Water - 1 ltr

Agar - 20g

2% neutral red in 50% ethanol - 3.5ml

10% lactose solution - l00ml

Dissolve peptone and taurocholate in water by heating. Add agar and

dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix.Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

2. Blood agar (5% sheep blood agar):

Peptone- l0g

NaCl- 5g

Distilled water- 1Ltr

Agar - 10g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

3. **Modified Thayer Martin Medium:**

Approximate formula per litre

Pancreatic digest of Casein – 7.5g

Agar	- 12 g
Selected Meat peptone	- 7.5g
Hemoglobin	- 10g
Corn starch	- 1 g
IsoVitaleX Enrichment	- 10ml
Dipotassium phosphate	- 4 g
V-C-Ninhibitor	- 10ml
Monopotassium phosphate	- 1g
Trimethoprim lactate	- 5mg
Sodium chloride	- 5g
Free water	- 500µl

4. Sabouraud's dextrose agar:

Dextrose - 40g

Peptone- l0g

Agar - 20g

Distilled water- l000ml

pH = 5.5

Sterilise by autoclaving at 121ºC for 20 min

5. Corn Meal Agar:

Corn meal infusion - 2gms/litre

Agar - 15gms/litre

Final pH of 6±0.2 at 25°C

6. **Muller Hinton Agar:**

Beef infusion solids – 4gms/litre

Starch – 1.5gms/litre

Casein hydrolysate – 17.5 gms/litre

Agar – 15gms/litre

Final pH – 7.4±0.2

Suspend 38g in 1 litre of distilled water, bring to the boil to dissolve the medium completely and sterilize by autoclaving at 121°C for 15 minutes.

MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION:

1. Oxidase Reagent

Tetra methyl p-phenylenediaminedihyrochloride- 1% aqueous solution.

2. Catalase

3% hydrogen peroxide

3. Indole test

Kovac's reagent

Amyl or isoamyl alcohol 150ml

Para dimethyl amino benzaldehyde 10g

Concentrated hydrochloric acid 50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4. Christensen's Urease test medium

Peptone 1g

Sodium chloride 5g

Dipotassium hydrogen phosphate 2g

Phenol red 6ml

Agar 20g

Distilled water 1 ltr

10% sterile solution of glucose l0ml

Sterile 20% urea solution l00ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in aflask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube themedium as slopes.

5. Simmon's Citrate Medium

Koser's medium 1 ltr

Agar 20g

Bromothymol blue 0.2% 40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

6. Triple Sugar Iron medium

Beef extract 3g

Yeast extract 3g

Peptone 20g

Glucose 1g

Lactose l0g

Sucrose l0g

Ferric citrate 0.3g

Sodium chloride 5g

Sodium thiosulphate 0.3g

Agar 12g

Phenol red 0.2% solution l2ml

Distilled water 1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube.

Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

7. Peptone water fermentation test medium.

To the basal medium of peptone water, add sterilised sugars of 1%

indicator :bromothymol blue with Durham's tube.

Basal medium peptone water

Sugar solutions:

Sugar 1ml

Dislilled water l00ml

pH = 7.6.

8. Mannitol motility medium

Agar 5g

Peptone 1g

Potassium nitrate 1g

Mannitol 2g

Phenol red indicator

Distilled water l000ml

pH 7.2

9. Hugh &Leifson's Oxidation –Fermentation test:

Peptone 2g

Sodium chloride 5g

D-glucose 10g

Bromothymol blue 0.03g

Agar 3.0g

Dipotassium phosphate 0.30g

Distilled water 1L

pH =7.1

Basal medium is autoclaved.1% of sterile sugar solutions is added to the basal medium.Dispense into sterile test tubes without slant.

10. Coagulase test:

Fresh human plasma (sterile)

INSTITUTIONAL ETHICS COMMITTEE

CHENGALPATTU MEDICAL COLLEGE , CHENGALPATTU

APPROVAL OF ETHICAL COMMITTEE

To

Dr.M.Malathi,
MD Microbiology,
(2nd Year)
Chengalpattu Medical College,
Chengalpattu.

Dear Dr.

The Institutional Ethical Committee of Chengalpattu Medical College reviewed and discussed your application to conduct the clinical / dissertation work entitled

"MICROBIOLOGICAL PROFILE OF REPRODUCTIVE TRACT INFECTION(RTI) AND SEXUALLY TRASMITTED INFECTION (STI) IN WOMEN OF REPRODUCTIVE AGE GROUP WITH SPECIAL REFERENCE TO CHLAMYDIA TRACHOMATIS BY SEMINESTED PCR ASSAY IN A TERTIARY CARE HOSPITAL".

ON 11.06.2014

The following documents reviewed

1. Trial protocol, dated _____version no
2. Patient information sheet and informed consent form in English and / or
   vernacular language.
3. Investigators Brochure, dated _____version
4. Principal Investigators current CV
5. Investigators undertaking

The following members of the Ethics committee were present at the meeting held on

Date 11.06.2014 Time 11.30 Noon Place Chengalpattu Medical College

Approved _____ Chairman Ethics Committee

11/6/14 Member secretary of Ethics Committee.

PATIENT PROFORMA

NAME:

AGE:

IP/OP NO.:

ADDRESS:

OCCUPATION:

HUSBAND OCCUPATION:

SOCIOECONOMIC STATUS:

SPECIMEN COLLECTED: ENDOCERVICAL SWAB
VAGINAL SWAB
BLOOD – 5 ml

HISTORY OF PRESENT ILLNESS:

MARITAL HISTORY:

PARITY HISTORY:

MENSTRUAL HISTORY:

STERILISATION HISTORY:

PAST HISTORY (CONTACT HISTORY):

LABORATORY EVALUATION FORM

VAGINAL SWAB:

- MICROSCOPIC EXAMINATION:
- BACTERIAL CULTURE:
- FUNGAL CULTURE:

ENDOCERVICAL SWAB:

- REAL TIME PCR ASSAY :

SERUM SAMPLE:

- SYPHILIS :
- HBV:
- HIV :
- HCV :
- HSV :

INFERENCE:

PATIENT CONSENT FORM

STUDY DETAIL:

"MICROBIOLOGICAL PROFILE OF LOWER GENITAL TRACT INFECTIONS IN WOMEN OF REPRODUCTIVE AGE GROUP WITH SPECIAL REFERENCE TO CHLAMYDIA TRACHOMATIS BY REALTIME PCR ASSAY IN A TERTIARY CARE HOSPITAL "

STUDY CENTER:

CHENGALPATTU MEDICAL COLLEGE & HOSPITAL, CHENGALPATTU

PATIENT NAME: PATIENT AGE:

IDENTIFICATION NUMBER:

PATIENT TO TICK ( )THESE BOXES

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask the question and all my questions and doubts have been answered to my satisfaction.I understand that my participation in the study is voluntary and that I am free to withdraw at anytime without giving any reasons, without my legal rights being affected.I understand that investigator, regulatory authorities and the ethics committee will not need my permission to look at my health records both in respect to the current study and any further research that may be conducted in relation to it, even if withdraw from the study, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from the study.

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperative with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or wellbeing or any unexpected or unusual symptoms.

I hereby give consent to participate in this study. I hereby give permission to undergo complete clinical examination and diagnostic test.

Signature/Thumb impression: Place: Date:
Patient name and address:

Signature of the investigator: Place: Date:
Study investigator's name:

சுயஒப்புதல்படிவம்

ஆய்வுசெய்யப்படும்தலைப்பு :

MICROBIOLOGICAL PROFILE OF LOWER GENITAL TRACT INFECTIONS IN WOMEN OF REPRODUCTIVE AGE GROUP WITH SPECIAL REFERENCE TO CHLAMYDIA TRACHOMATIS BY REALTIME PCR ASSAY IN A TERTIARY CARE HOSPITAL

ஆய்வுசெய்யப்படும்இடம் :
பங்குபெறுபவரின்பெயர் :
பங்குபெறுபவரின்வயது :
பங்குபெறுபவரின்எண் :

மேலேகுறிப்பிட்டுள்ளமருத்துவஆய்வின்விவரங்கள்எனக்குவிளக்கபட்டுள்ளது.நான்இவ்வாய்வில்தன்னிச்சையாகபங்கேற்கின்றேன்.எந்தகாரணத்தினாலோ,
எந்தசட்டசிக்கலுக்கும்உட்படாமல்நான்இவ்வாய்வில்இருந்துவிலகிக்கொள்ளலாம்என்றும்அறிந்துகொண்டேன்.

இந்தஆய்வுசம்பந்தமாகவோ,
இதைசார்ந்துமேலும்ஆய்வுமேற்கொள்ளும்போதும்இந்தஆய்வில்பங்குபெறும்மருத்துவர்,
என்னுடையமருத்துவஅறிக்கைகளைபார்ப்பதற்குஎன்அனுமதிதேவைஇல்லைஎனஅறிந்துகொள்கிறேன்.
இந்தஆய்வின்மூலம்கிடைக்கும்தகவலையோ,முடிவையோபயன்படுத்திக்கொள்ளமறுக்கமாட்டேன்.

இந்தஆய்வில்பங்குகொள்ளஒப்புக்கொள்கிறேன்.இந்தஆய்வைமேற்கொள்ளும்மருத்துவஅணிக்குஉண்மையுடன்இருப்பேன்என்றுஉறுதியளிக்கிறேன்.

பங்கேற்பவரின்கையொப்பம்: சாட்சியாளரின்கையொப்பம்

இடம்: இடம்:

தேதி: தேதி :

பங்கேற்பவரின்பெயர்மற்றும்விலாசம்:

ஆய்வாளரின்கையொப்பம்:

இடம்:

தேதி:

Number	Age	Residence	Occupation	Spouse O	Parity	Contracep	Discharge	Abd pain	Itching	BM	Signs	Wetmount	DGS	Pap	Bac cult	Fung cult	PCR	Syphilis	HBV	HCV	HSV	HIV
1	21	1	1	3	P1L1	Nil	-	+	+	-	D	-	GNB/PC	inflammation	Kleb.pneumoniae	NG	Negative	NR	Negative	Negative	Positive	Negative
2	27	2	2	5	P2L2	PS	-	+	-	-	D	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
3	23	1	1	2	P1L1	Nil	+	+	-	-	R	yeast cells	GPBYC	NILM	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
4	24	1	1	2	P2L2A1	Nil	+	+	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
5	40	1	2	2	P3L3	PS	+	+	-	-	D	-	GNB	inflammation	Escherichia coli	NG	Negative	NR	Negative	Negative	Positive	Negative
6	22	1	1	4	P1L1	Nil	+	-	+	-	D,R	yeast cells	pseudo	candida	Escherichia coli	C. keyfr	Positive	NR	Positive	Negative	Positive	Positive
7	23	1	1	5	P1L1	Nil	+	+	-	-	D	-	GPB, GPC	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
8	23	2	2	2	P1L1A1	Barrier	+	-	-	-	Nil	-	GPB/GPC/PC	inflammation	Staph.aureus	NG	Negative	NR	Negative	Negative	Negative	Negative
9	21	1	1	5	nulli	Nil	-	-	+	+	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
10	25	1	1	5	P2L2	PS	-	+	-	-	Nil	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
11	20	1	2	5	A1	Nil	+	-	-	-	D	-	GPB/PC	inflammation	Kleb.pneumoniae	NG	Negative	NR	Negative	Negative	Negative	Negative
12	24	2	2	2	P1L1A1	IUCD	+	+	-	-	Nil	-	GPB	NILM	NG	NG	Positive	NR	Negative	Negative	Negative	Negative
13	25	1	1	5	P2L2	PS	-	+	-	+	Nil	-	GPB/GNB	inflammation	Escherichia coli	NG	Negative	NR	Negative	Negative	Positive	Negative
14	23	1	1	3	nulli	Nil	+	+	+	+	D,R	motile TV	-	TV	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
15	22	1	1	2	nulli	Nil	+	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
16	33	2	1	3	P2L2	PS	+	-	+	-	D	yeast cells	GPBYC	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
17	28	1	2	5	P2L2	PS	+	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
18	22	2	1	2	P1L1	IUCD	+	-	+	+	D,R	yeast cells	GPBYC	candida	NG	C. tropicalis	Negative	NR	Negative	Negative	Negative	Negative
19	24	2	1	5	P1L1	Barrier	+	-	-	+	Nil	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
20	22	1	1	2	nulli	Nil	+	-	-	-	Nil	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
21	21	1	1	4	P1L1	IUCD	+	+	-	-	Nil	-	clue cells, GPC	shift in flora	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
22	20	2	2	5	P1L1	Nil	+	-	-	-	Nil	-	GPBYC	NILM	NG	NG	Positive	NR	Negative	Negative	Positive	Negative
23	26	1	1	5	P2L2	PS	+	-	+	-	D	yeast cells	GPBYC	candida	NG	C. tropicalis	Negative	NR	Negative	Negative	Negative	Negative
24	21	1	4	5	nulli	Nil	+	-	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
25	23	2	1	4	P1L1	IUCD	-	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
26	26	1	2	2	P2L2	PS	+	-	+	-	D	-	GPBYC	NILM	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
27	23	1	1	3	P1L1	Nil	-	+	-	-	Nil	-	-	inflammation	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
28	23	1	1	5	P1L1	Nil	+	-	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Positive	Negative	Positive	Positive
29	23	2	1	3	P2L2	PS	-	+	-	-	D	-	PC	inflammation	Escherichia coli	NG	Negative	NR	Negative	Negative	Negative	Negative
30	21	1	1	3	P1L1	Nil	+	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
31	20	1	1	5	A1	Nil	+	+	-	-	D	-	GNB/PC	inflammation	Citrobacter koseri	NG	Positive	NR	Negative	Negative	Positive	Negative
32	23	1	1	3	P1L1A1	Nil	+	+	+	-	D,R	yeast cells	GPBYC	candida	NG	C. albicans	Negative	NR	Negative	Negative	Negative	Negative
33	30	1	2	2	P2L2	PS	-	+	+	-	D,nodule	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
34	20	1	1	3	P1L1	Nil	+	+	-	-	D	-	GPBYC	candida	Escherichia coli	C. keyfr	Negative	NR	Negative	Negative	Positive	Negative
35	23	2	1	5	P1L1A1	IUCD	+	-	+	+	D	yeast cells	GPBYC	candida	NG	C. albicans	Negative	NR	Negative	Negative	Negative	Negative
36	27	1	2	3	P2L2	PS	-	+	-	-	Nil	-	-	NILM	NG	NG	Negative	Reactive	Negative	Negative	Positive	Negative
37	21	1	1	4	nulli	Nil	+	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
38	25	1	1	3	P2L2	PS	-	+	-	-	Nil	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
39	40	1	1	5	P3L3	PS	+	-	-	+	D	-	GPB/GNB	inflammation	Escherichia coli	NG	Negative	NR	Negative	Negative	Negative	Negative
40	24	1	1	5	P2L2	PS	+	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
41	24	1	1	3	P2L2	PS	-	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
42	26	1	1	3	P2L2	PS	+	+	-	-	D	-	GPB	NILM	NG	NG	Positive	NR	Negative	Negative	Negative	Negative
43	21	2	1	3	nulli	Nil	+	+	+	-	D,R	yeast cells	GPBYC	candida	NG	C. albicans	Negative	NR	Negative	Negative	Positive	Negative
44	32	2	1	4	P3L3	PS	-	+	-	-	Erosion	-	GPC	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
45	38	1	1	5	P2L2	PS	+	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
46	40	1	1	3	P3L3	PS	+	-	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
47	27	1	1	4	P4L1	PS	+	+	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
48	32	1	1	2	P2L2	PS	-	+	-	-	R	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
49	36	1	1	3	P3L3	PS	+	+	-	-	D	pus cells	GPC/PC	inflammation	Streptococcal Sp.,	NG	Negative	NR	Negative	Negative	Negative	Negative
50	40	1	1	3	P3L3	PS	+	-	+	-	D,R	yeast cells	GPBYC	candida	NG	C. tropicalis	Negative	NR	Negative	Negative	Positive	Negative
51	35	1	1	4	P2L2	PS	-	+	-	-	Nil	-	-	inflammation	Kleb.pneumoniae	NG	Negative	NR	Negative	Negative	Negative	Negative
52	34	1	1	2	P2L2	PS	-	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
53	26	1	1	3	P2L2	PS	+	-	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
54	24	2	1	2	P1L1	IUCD	-	+	-	-	D	pus cells	-	inflammation	Kleb.pneumoniae	NG	Negative	NR	Negative	Negative	Negative	Negative
55	25	1	6	6	nulli	Nil	+	-	+	-	D,R	yeast cells	GPBYC/PC/GPC	candida	Staph.aureus	C. albicans	Negative	NR	Negative	Negative	Negative	Negative
56	40	1	2	2	P3L3	PS	+	+	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
57	21	1	1	4	P1L1	Nil	+	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
58	24	1	1	5	P2L2	PS	+	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative

59	26	2	3	5	P2L2A1	PS	-	+	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
60	24	1	6	5	P2L2	PS	+	-	+	-	D,R	yeast cells	GPBYC	candida	NG	C. albicans	Negative	NR	Negative	Negative	Positive	Negative
61	27	1	2	2	P2L2	PS	-	+	-	+	Nodule	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
62	21	1	1	5	P1L0	Nil	+	+	+	-	D,R	-	clue cells, GPC	shift in flora	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
63	26	2	2	4	P1L1	IUCD	+	+	-	-	Nil	-	GPBYC	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
64	30	1	1	5	P2L2	PS	+	+	-	-	Erosion	-	-	NILM	NG	NG	Positive	NR	Negative	Negative	Positive	Negative
65	22	1	1	2	nulli	Nil	+	-	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
66	22	1	1	4	P1L1	Nil	+	-	-	-	D	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
67	32	2	2	5	P1L1	IUCD	+	-	+	-	D,R	yeast cells	GPBYC	candida	NG	C. glabrata	Negative	NR	Negative	Negative	Negative	Negative
68	22	1	1	2	P1L1	Nil	+	-	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
69	24	1	5	5	nulli	Nil	+	+	+	-	D,erosions	yeast cells	clue cells, GPBYC	inflammation	Escherichia coli	C. albicans	Negative	NR	Negative	Negative	Negative	Negative
70	34	2	1	5	P2L2	PS	+	-	-	+	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
71	29	1	1	5	P2L2	PS	+	+	-	-	Nil	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
72	31	1	1	3	P2L2	PS	+	+	-	-	D	pus cells	GPB/PC	inflammation	Escherichia coli	NG	Negative	NR	Negative	Negative	Negative	Negative
73	24	2	1	4	P1L1	Barrier	-	+	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
74	43	1	1	3	P2L2	PS	+	+	-	-	Nil	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
75	25	2	1	3	P1L1A1	IUCD	+	-	-	+	D	yeast cells	GPBYC	candida	NG	C. glabrata	Positive	NR	Negative	Negative	Negative	Negative
76	33	1	1	3	P3L2	PS	+	+	-	-	D,R	motile TV	GPBYC	TV	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
77	29	1	2	2	P2L2	PS	+	-	-	+	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
78	26	1	2	2	P2L2	PS	+	-	+	-	D	pus cells	GPC	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
79	27	1	2	4	P3L2	PS	-	+	-	-	R	-	GPB,GPBYC	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
80	28	1	3	3	P2L2	PS	+	-	-	-	D	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
81	25	1	2	3	P2L2	PS	+	-	+	-	D,erosions	yeast cells	GPBYC	candida	NG	C. glabrata	Negative	NR	Negative	Negative	Positive	Negative
82	25	2	1	2	P1L1	Nil	+	-	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
83	42	1	2	3	P2L2	PS	+	-	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
84	27	1	1	3	P2L2	PS	-	+	-	+	D	-	-	NILM	NG	NG	Positive	NR	Negative	Negative	Negative	Negative
85	28	1	2	3	P2L2	PS	+	-	-	-	D	pus cells	GPBYC/PC	inflammation	Escherichia coli	NG	Negative	NR	Negative	Negative	Negative	Negative
86	28	1	1	2	P2L2	PS	+	-	-	-	R	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
87	25	2	1	3	P1L1	Nil	-	+	-	-	Nil	-	GPB	NILM	NG	NG	Negative	NR	Positive	Negative	Positive	Positive
88	26	1	1	5	P1L1	Nil	+	-	+	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
89	26	1	2	2	P2L2	PS	+	-	-	-	Erosion	-	clue cells	shift in flora	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
90	27	1	2	3	P2L2	PS	+	-	-	-	R	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
91	26	2	2	2	P1L1	IUCD	+	-	+	+	D,R	yeast cells	GPBYC	candida	NG	C. albicans	Negative	NR	Negative	Negative	Negative	Negative
92	28	1	1	4	P2L2	PS	+	-	-	-	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
93	28	1	2	2	P2L2	PS	+	-	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
94	28	1	1	3	P2L2	PS	-	+	-	+	D	-	GPBYC	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
95	25	2	3	3	P1L1	Nil	+	-	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
96	25	1	1	5	P2L2	PS	+	-	-	-	D	motile TV	-	TV	NG	NG	Negative	NR	Negative	Negative	Positive	Negative
97	26	1	2	2	P2L2	PS	-	+	-	+	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
98	26	1	1	3	P2L2	PS	+	+	-	+	D	-	PC	inflammation	Kleb.pneumoniae	NG	Positive	NR	Negative	Negative	Negative	Negative
99	30	1	1	4	P2L2	PS	+	-	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
100	36	1	1	3	P3L3	PS	+	+	+	+	D,erosions	yeast cells	GPBYC	candida	NG	C. keyfr	Negative	NR	Negative	Negative	Positive	Negative
101	28	1	2	2	P2L2	PS	+	+	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
102	29	1	1	2	P2L2	PS	-	-	+	-	Nil	-	GPB	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
103	32	1	2	2	P3L3	PS	+	-	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
104	33	2	1	4	P2L1	IUCD	+	-	+	-	D	yeast cells	GPBYC	inflammation	Escherichia coli	NG	Negative	NR	Negative	Negative	Negative	Negative
105	25	2	1	4	P1L1	IUCD	-	+	-	+	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
106	27	1	1	5	P2L2	PS	+	-	-	-	D	yeast cells	GPBYC	NILM	NG	C. albicans	Negative	NR	Negative	Negative	Negative	Negative
107	26	1	2	3	P3L2	PS	+	+	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
108	27	1	1	4	P2L2	PS	+	+	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
109	25	2	2	5	P2L1	Barrier	+	-	-	-	D	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Negative	Negative
110	29	1	1	4	P2L2	PS	-	+	+	+	Nil	-	-	NILM	NG	NG	Negative	NR	Negative	Negative	Positive	Negative