A STUDY ON THE PREVALENCE OF CHLAMYDIA TRACHOMATIS AND NEISSERIA GONORRHOEAE INFECTIONS USING POLYMERASE CHAIN REACTION

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

CHENNAI

MARCH 2009

CERTIFICATE

Certified that this dissertation entitled **"A STUDY ON THE PREVALENCE OF CHLAMYDIA TRACHOMATIS AND NEISSERIA GONORRHOEAE INFECTIONS USING POLYMERASE CHAIN REACTION"** is a bonafide work done by **DR.K.S. SRIDEVI**, Post Graduate Student in **M.D. Dermatology, Venereology and Leprosy**, Madras Medical College, Chennai-600 003, during the academic year 2006-2009.This work has not previously formed the basis for the award of any degree.

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CONTENTS

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PROFORMA

NAME:

AGE:

SEX:

FATHER/ HUSBAND/GUARDIAN:

ADDRESS:

EDUCATIONAL STATUS: Uneducated/ Up to 5th / 6-12th / College

OCCUPATION:

INCOME: <1000/1000-1999/2000-4999/>5000

MARITAL STATUS: Single/Married/Separated/Divorced.

REFFERED BY: Self /Other Department /Other General Hospital /Private hospital /NGO

PRESENTING COMPLAINTS: DURATION

GENERAL CHECKUP/SCREENING

GENITAL DISCHARGE: Colour/ Foul smelling/Consistency/Amount.

GENITAL ULCER:- Number/Recurrence/Painful

ITCHING GENITALIA

LOWER ABDOMINAL PAIN

SWELLING IN INGUINAL REGION

SKIN RASH

BURNING MICTURITION

JAUNDICE

FEVER,LOSS OF WEIGHT,LOSS OF APPETITE

ORAL LESIONS

DYSPAREUNIA

OTHER COMPLAINTS

TREATMENT TAKEN FOR PRESENT ILLNESS:

PAST HISTORY

Previous STDs & Treatment taken

Diabetic/ Hypertensive/ Surgeries/ Blood transfusion/ TB/Asthma

CONTACT HISTORY:

Partner Name /Occupation/History and Investigation Details

PERSONAL HISTORY

Smoker/Alcoholic/Tobacco chewer/Drug addiction/Tatooing

SEXUAL HISTORY

Sexual orientation: Heterosexual/ Homosexual/ Bisexual

Recent exposure with dates-MC/PMC/EMC/LC

MENSTRUAL HISTORY

LMP Cycle: Regular/ Irregular

OBSTETRIC HISTORY Total no. of children/ Last Child Birth

No. of children /Age/Mode of delivery/ Child health

H/o Abortion- If yes - 1) Induced/ Spontaneous 2) Duration of Gestation

H/o Ectopic Pregnancy/ Infertility/ Sterilization

GENERAL EXAMINATION:-

Pallor/Icterus/Cyanosis/Clubbing/Lymphadenopathy/Pedal edema

Pulse rate/BP

SYSTEMIC EXAMINATION:-

CVS

RS

P/A

CNS

GENITAL EXAMINATION:-

FEMALES

Inguinal nodes

External urethral meatus

Speculum Examination:-Cervix-Healthy/erosion/ulcer/growth

Genital Examination: Soddening of vulva

Ulcer - single / multiple , painful/painless,

soft/indurated, bleeds on touch/manipulation

Wart – site / number

Discharge - Scanty/ moderate/ Profuse

Mucoid/ mucopurulent /purulent

Homogenous/floccular/curdy white

Foul-Smelling.

Skin&Mucous membrane

Bones&Joints

MALES

Circumscribed/Uncircumscribed

Genital ulcer-- single / multiple

painful/painless (tenderness) ,soft/indurated

bleeds on touch/manipulation

Urethral discharge/Subprepucial discharge

Inguinal nodes

Skin&Mucous membrane

Bones&Joints

INVESTIGATION

- Blood VDRL
- TPHA
- ELISA for HIV
- Urine :Albumin/ Sugar/ Deposits
- Culture for GC

  Urine(males) ,Endocervix(females)
- Chlamydial PCR – Urine(both sexes)

  Urethral (Males)

  Endocervical(females)
- Gonococcal PCR – Urine(both sexes)

  Urethral (Males)

  Endocervical(females)
- <u>Genital Discharge</u>
  - Wet film for TV
  - KOH for Candidiasis

- Gram's Stain
    - Urethral smear(males)-Gonococci
    - Vaginal &Endocervical Smear- clue cells / candida/ lactobacilli/ Gonococci.

- For Genital Ulcers
    - DF for TP
    - Leishman's stain for DB
    - Gram's stain for DUC
- USG for Abdomen
- Urine culture & sensitivity
- Blood for HbsAg and AntiHCV

Consent

I am willing for clinical examination and PCR testing for Chlamydial and Gonococcal antigen detection. The procedures have been explained to me.

Date: Signature:

Name:

INTRODUCTION

Sexually transmitted infections (STIs) are a major global cause of acute illness, infertility, long term morbidity and mortality, with severe medical and psychological consequences of millions of men, women and infants[1]. STIs most commonly affect people aged between 15 to 44 years.

In 2006, 1,030,911 Chlamydial infections and 3,58,366 Gonococcal infections were reported to CDC from 50 states and the districts of Columbia in United states[2,3]. Chlamydia trachomatis and Neisseria gonorrhoeae remain the two most preventable causes of human infertility[4]. Untreated Gonococcal and Chlamydial infections in women will result in pelvic inflammatory disease in upto 40% of cases. One in four of these will result in infertility [5].

Demographic risk factors for the acquistion of gonorrhoeae include migration of population, unemployment, lack of education, poverty, early onset of sexual activity,unmarried marital status,past history of gonorrhoeae,illicit drug use and now commercial sex have been operative universally, but more severely in certain societies and countries.

Among several risk factors for chlamydial infection age is the most important one. Other risk factors include previous chlamydial infection, recent change in partner, a symptomatic partner, failure to use barrier contraceptives, low socioeconomic status,use of oral contraceptives and the coinfection with

gonorrhoeae [6,7].The highest infection rates are found in sexually active adolescents where approximately 15% of girls and 5-10% of boys are infected [6,7].

Since publications of CDC's 1993 guidelines NAATs have been introduced as critical new tools to diagnose and treat Chlamydia trachomatis and Neiserria gonorrhoeae infections [8].

Nucleic acid amplification tests (NAATs) have enabled the epidemiology of chlamydia to become clearer even in difficult to reach population.The recent development of NAATs for gonorrhoeae have also enabled the epidemiology of gonorrhoeae to become clearer, though culture is still the most commonly used diagnostic method [9-11].

The Amplicor Chlamydia trachomatis / Neisseria gonorrhoeae (CT /NG) test is a qualitative in vitro test for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae in urine from both sexes,in endocervical swab specimens from females and in urethral swab specimens from males. The test utilizes Polymerase Chain Reaction (PCR) nucleic acid amplification and nucleic acid hybridization for the detection of C.trachomatis and N.gonorrhoeae in urogenital specimens.

REVIEW OF LITERATURE

Sexually transmitted infections (STIs) are a major cause of morbidity in the developed as well as developing countries which also result in lot of psychological morbidity in the young sexually active adults. The World Bank estimates that STIs, excluding AIDS, are the second leading cause of healthy life lost amongst women aged between 15 and 44 in the developing world [12].

GONOCOCCAL INFECTIONS

Gonorrhoeae is a common and universally encountered sexually transmitted bacterial disease, caused by a gram negative diplococcus, Neisseria gonorrhoeae.

Morphology of Neisseria gonorrhoeae

Gonococci are gram negative, aerobic, nonmotile, nonsporing cocci typically arranged in pairs (diplococci) with adjacent sides concave, typically reniform or pear shaped. In urethral discharges, they are generally present intracellularly within polymorphonuclear leucocytes.

Prevalence of Gonorrhoeae:

Gonorrhoeae is a well recognized public health problem. It is the second most common bacterial STI in the world next to Chlamydia trachomatis infection.WHO estimates that gonorrhoeae accounted for 18% of the new cases

of curable STIs Worldwide in 1999 with 62 million new cases amongst adults aged 15 to 49 years [1].

Numerous surveys conducted in recent years have shown that gonorrhoeae is the most common cause of male urethritis accounting for approximately 53-80% of the all STD cases.In India also, gonorrhoeae is a major public health problem with majority of the reported cases occurring in the 20-24 years age group [13-16].

In India incidence of gonorrhoeae vary from 3% to 19% among the STD clinic attendees from different regions [14-19].In a survey of women attending an STD clinic in Mumbai in 1996, 9.7% were positive for gonococcal infections [20].The apparent ratio of male to female cases is 10:1, with 80-90% men acquiring infection from commercial sex workers [21].

Genital gonorrhoeae may be asymptomatic in both men and women, but it is more frequently silent in women. 30% to 80% of female cases of gonorrhoeae are asymptomatic in comparison with less than 5% of infected men [22].

Clinical features

The incubation period ranges from 1 to 14 days but majority of men develop symptoms within 2 to 5 days [23]. Neisseria gonorrhoeae has a

predilection for columnar and cuboidal epithelium. Asymptomatic infection occurs at urethra,endocervix, rectum and pharynx.

Acute Gonorrhoeae in men presents as acute anterior urethritis.If prompt treatment is not initiated, posterior urethritis may ensue in 10-14 days.Acute gonococcal infection may involve the glands of Tyson, paraurethral, periurethral and Littre's glands and present as acute tysonitis, acute littritis, inflammation of paraurethral and periurethral glands and gonococcal balanitis.Acute cowperitis, prostatitis, seminal vesiculitis and epididymitis are the other acute complications in males especially when the posterior urethra is affected.The patient may develop urethral strictures, fistulae leading to watering can perenium, chronic littritis, cowperitis, prostatitis, seminal vesiculitis or epididymitis.

Uncomplicated gonorrhoeae in women may remain asymptomatic in about 50 percent cases that may act as reservoir of infection. Acute gonococcal infection in females presents as endocervicitis, acute skenitis, acute bartholinitis, acute gonococcal vulvitis, gonococcal cystitis and trigonitis.

Pelvic inflammatory disease (PID) is the most common local complication accounting for 10 to 20 percent of cases with acute gonococcal infection [24].Complications include salpingitis, pyosalpinx, tubo-ovarian abscess, pelvic abscess, pelvic peritonitis. PID is a serious health problem

resulting in tubal infertility, ectopic pregnancies or chronic pelvic pain[25].Organization of pus in the pelvis leads to adhesion between pelvic organs and intestines termed as frozen pelvis. Chronic urethritis, skenitis, bartholinitis and proctitis are the other complications in women.

Gonococcal proctitis in males usually results from anal coitus in passive homosexuals and rectum is a frequent site of infection in 40 percent of homosexual men [26].In women, rectal mucosa is infected in 35% to 50% of cases with gonococcal cervicitis.Pharyngeal gonorrhoeae is transmitted by orogenital contact and is more efficiently acquired by fellatio than by cunnilingus [27]. Gonococcal conjunctivitis is a rare entity in adults and often seen in patients with concomitant anogenital gonorrhoeae as a consquence of direct contamination by fingers or towels.

In Disseminated gonococcal infection(DGI) the characteristic clinical findings include suppurative arthritis and skin lesions. DGI is more common in females with a male to female ratio of 1:4 [28]. Risk factors for DGI include pregnancy, premenstrual period, pharyngeal gonorroheae [29] and complement deficiency. Most of the cases are caused by AHU auxotype(AHU-).

During pregnancy, there is a high risk of developing salpingitis and PID. Gonococcal chorioamnionitis may occur which leads to septic abortions and also increases the risk of premature rupture of membranes, preterm births,

prematurity, perinatal mortality or low birth weight [30].In Neonatal gonorrhoeae, the most frequent manifestation is Opthalmia neonatarum.

Coinfection with C.trachomatis

Genital infection with Chlamydia trachomatis commonly accompanies gonococcal infection in 10-20% of men and 20-30% of women [31].

A gene present on the gonococcal chromosome called the sac-4 gene has been postulated to increase the risk of developing mixed gonococcal and chlamydial infection. This gene confers stable complement factor c1q dependent serum resistance. The prevalence of the gene was 69.5% in gonococcal isolates from patients with co-existing chlamydial infection compared with 57.9% from those without chlamydia [31].

LABORATORY DIAGNOSIS

The sites for specimen collection in females include the endocervix, rectum, urethra and pharynx. In heterosexual males urethra and pharynx are the common sites affected. Specimens should be collected with dacron, rayon or polyethylene terephthalate (PET) swabs or sterile platinum loop [32]. The specimen is transported in Amie's or modified Stuart's medium.

Direct microscopy of stained smears

Gonococci are readily demonstrated in Gram stained smears as pink coloured intracellular diplococci present within polymorphonuclear leucocytes.

Culture

Culture is considered the 'gold standard' for the diagnosis of gonorrhoeae. Gonococci are fastidious organisms requiring enriched media for growth. They are aerobic and capnophilic. Optimum growth occurs at 35-36°C & pH between 7.2 and 7.6.

Gonococci grow on enriched, selective media like Modified Thayer-Martin Media (MTM), Martin Lewis, New York City,Chacko-Nair Medium and GC-Lect-Medium[33-34]. Vancomycin and colistin inhibits gram +ve and gram –ve bacteria including saprophytic neisseriae species and nystatin, amphotericin-B are added to inhibit yeasts and moulds. Sensitivity of culture is reported between 80% and 95% with false negative results attributed to poor specimen storage, transport problems and inhibiting of growth by the components of selective media [35].

Gonococci form small, round, translucent, soft emulsifiable and convex colonies with fine granular surface and lobate crenated margins. The colonies types recognizable are upto T1-T4.

Biochemical identification: In Oxidase test, a loopful of freshly prepared aqueous tetramethyl -p-phenylene diamine Hcl is poured over the colonies. Gonococci colonies turn pink within 10 seconds & rapidly deepening to purple. Gonococci is catalase positive. It ferments glucose, but not maltose,sucrose,fructose or lactose producing acid but no gas.

Direct Antigen Detection in Clinical Specimens

Enzyme Immunoassay

Gonozyme test is a solid-phase enzyme immunoassay for the direct detection of gonococcal antigen in male urethral and female endocervical specimens [36]. Antibiotic sensitivity determination cannot be performed by this system.

Molecular techniques

1).Nucleic acid probes: A commercially available probe called 'PACE' (Probe assay chemiluminescence enhanced) has been developed for direct detection of gonococci in cervical and urethral specimens [37].

2)Hybrid Capture II Test (HC II GC Test).

3)Nucleic Acid Amplification Test (NAAT)

These tests enable the use of noninvasive specimens such as first void urine and self obtainable vaginal swabs for diagnostic testing and for screening of asymptomatic low-prevalence and hard to access populations.

a)Polymerase chain reaction

This test amplifies target sequences on the 2.6 MDa (14.2kb) cryptic plasmid that is also represented on the gonococcal chromosome [38]. Alternately, amplification of the target sequence within gonococcal 16S rRNA gene is also performed. Recently, Roche Molecular systems have developed a multiplex PCR-based test for N-gonorrhoeae and C.trachomatis that allows simultaneous amplification of both the targets [39]. It is available in two formats. The fully automated COBAS amplicor CT/NG and the semiautomated AMPLICOR CT/NG microwell plate format tests. The sensitivity from endocervical swab specimens is 92.4% and from female urine samples is 64.8%. The corresponding specificities are 99.5% and 99.8% respectively. In symptomatic men, sensitivity is 94.1% for urine and 98.1% for urethral swabs and in asymptomatic men 42.3% and 73.1% respectively. The specificity is 98.9% for urethral swab specimens and 99.9% for urine samples in men.

b)Ligase chain reaction

It is a rapid, highly sensitive non-culture method for gonococcal detection[40]. This technique exponentially amplifies targeted DNA sequences especially within twc cpacity genes(Opa-1 gene).

4)**DNA Hybridization Test** This test utilize the 2.6 MDa gonococcal cryptic plasmid as radiolabelled probe [41]. It can detect as few as 100 colony forming gonococcal units and upto 0.1 pg of purified gonococcal plasmid DNA.

Serological tests Gonococcal antibodies such as pilin, P.I. or P.II. antibodies can be detected. These tests include complement –fixation test, latex agglutination test, indirect immunofluorescence, radioimmuno-assay, indirect haemagglutination test, ELISA and western blotting.

Treatment

The recommended treatment for uncomplicated gonococcal infection, is Inj. Ceftriaxone 250mg IM as a single dose (or) cefixime 400mg orally single dose.The treatment of gonorrhoeae should be combined with Azithromycin 1g orally single dose or Doxycycline 100mg orally twice a day for 7 days for concomitant C. trachomatis infection [6].

CHLAMYDIA TRACHOMATIS INFECTIONS

Chalmydia trachomatis is considered the most common sexually transmitted bacterial pathogen [42].

Epidemiology

WHO estimates that 92 million new cases of Chlamydia occurred worldwide in 1999 amongst those aged 15 to 49 years [1].It is estimated that 85%

women and 40% of men are asymptomatic and therefore only detectable with screening programmes[7]. There are relatively few studies from India, but those that do exist found relatively high prevalence in selected populations.

Morphology:

Chlamydia trachomatis are small obligate intracellular bacteria and they contain DNA, RNA and ribosomes and make their own proteins and nucleic acids. They are nonmotile and are gram negative. The human C. trachomatis pathogens can be divided into atleast 15 serovars by microimmunofluorescent (Micro IF) test [43]. A,B, Ba, C serovars are associated with hyperendemic blinding trachoma. The D to K Serovars commonly cause nongonococcal urethritis, conjunctivitis and infantile pneumonia.The L1 to L3 serovars represent the LGV biovar. Species, serogroup and serovar specific antigens can be found on the MOMP membrane [44].

Clinical features **The most common manifestation is nongonococcal urethrtits (NGU) in men [7]. NGU is diagnosed by demonstrating a 'significant' number of PMNs in first -catch urine or a smear prepared from a urethral swab. C. trachomatis is responsible for 70% to 90% of Postgonococcal urethritis [45]. Because of the high (>20%) double infection rate, CDC and WHO recommended that all case of gonorrhoeae be treated presumptively for Chlamydial infection[46]. Ascending Chlamydial infections can result in epididymitis.**

In the female, the most commonly affected site is the cervix, where the organism can cause a mucopurulent endocervicitis (MPC). Women with gonorrhoeae often have chlamydial infection. Double infection rates may be

twice as high (35%-45%) as those observed in men. It is even more important to treat women with gonorrhoea for chlamydial infection as it reduces subsequent development of salpingitis [46]. Chalmydia infection has been associated with sterile pyuria in young women.

10-40% of women with untreatable chlamydial infection develop symptomatic PID. Post infection tubal damage is responsible for 30-40% cases of female infertility. Furthermore women who have had PID are 6-10 times more likely to develop on ectopic pregnancy. C. trachomatis is also associated with the Fitz–Hugh Curtis Syndrome(Perihepatitis) a complication of salpingitis.Chalmydia positive women may develop postpartum endometritis after vaginal delivery. Some studies found an association with foetal wastage and prematurity [47].Other manifestations include bartholinitis, arthritis and dermatitis .

Laboratory diagnosis

Direct Microscopy. For resuspension of the centrifuged sediment of a 10 to 15 ml of first catch urine,the usual criteria for diagnosis is 15 or more PMNs per X 400 high -power field and for a smear obtained by urethral swabbing the cut off is 5 or more PMNs per X 1000 field [7]. In females, >30 PMN leucocytes

per OIF or gram stained smears of cervical mucus is suggestive of chalmydial cervicitis. Other stains include Giemsa and Iodine stains**.**

Tissue culture The gold standard in the diagnosis of genital chlamydial infection is tissue culture. Irradiated or cycloheximide treated McCoy cells, BHK-21, Hela –229 have been used successfully for the isolation of C. trachomatis [48].Confirmation of the isolates is done either by Giemsa or Iodine stains or by the use of fluorescein labelled monoclonal antibodies.

Antigen detection 1) Direct immunofluorescence assay (DFA)

This is a rapid diagnostic test carried out on clinical specimens using monoclonal fluorescein labelled anti-chalmydial antibodies against the species specific epitope of major outer membrane protein (MOMP) or genus specific lipopolycaccharide (LPS)[49].

2) Enzyme –linked immunosorbent assay (ELISA) These products detect chlamydial LPS, which is more soluble than the MOMP. The tests use either monoclonal or polyclonal antibodies to LPS and thus theoretically could detect all chalmydia.

Rapid tests

There are three commercially available rapid assays,which are quite expensive but give results within 30 minutes and thereafter are useful in field conditions. They are as follows Sure cell Chalmydia test,Clear view Chalmydia test ,The test pack Chalmydia test [50].

Detection of DNA in clinical specimens

i)DNA probes Probe assay Chemiluminescence enhanced (PACE) test utilizes a nonisotopic DNA probe (a single stranded DNA labelled with acridinium ester) for the detection of specific rRNA of C-trachomatis in endocervical and urethral specimens [51].

ii) Polymerase chain reaction(PCR)

Plasmid DNA amplification has become the most popular approach in PCR of C. trachomatis. A variety of amplification targets have been used for PCR including the common cryptic plasmid, which was found to be the best[52]. A number of studies comparing PCR [52] with the other diagnostic methods showed PCR as more sensitive than culture (99% vs 78%) and the specificity was above 99%.

The results of a recent study indicate that Amplicor CT/NG multiplex PCR test performed on urine in men provides a highly sensitive, specific and

robust method for the diagnosis of both C.trachomatis and N.gonorrhoeae for the early detection of both symptomatic and asymptomatic individuals[53]. Multiplex PCR developed by Mahoney et al was 100% sensitive and specific for C. trachomatis [54].Depending on the prevalence of infection, pooling of the first catch urine samples for PCR testing is more economical than testing individual samples [55].

iii)Ligase chain reaction The sensitivity of LCR performed on endocervical specimens has ranged from 87% to 97% and in female FVU, the sensitivity was equal to cervical culture [55].For FVU in men, the test has a sensitivity of approximately 90-96% in detection of chlamydial urethritis [56].

iv)Other tests a)Gen Probe Amplified CT (AMP CT) assay uses the transcription mediated amplification and hybridisation technique to detect C. trachomatis rRNA in endocervical and urinary specimens [58]**.** b) Q-beta replicase amplified hybridation assay (Gen Trak, Inc)[59].

Serology

Serological assays may be useful in the detection of C. trachomatis infections of the genital tract. However 45-65% of patients may have antibodies resulting from a past infection and the traditional approach for the detection of four – fold rise in titre does not seem to be applicable.Elevated titres are detected by

EIA or complement fixation (CF). The detection of IgM antibodies may also be helpful in establishing acute Chlamydial infections of the genital tract.The Calbiotech Inc, Chlamydia trachomatis IgM ELISA test system detects IgM class antibodies to C.trachomatis human serum or plasma [60].

Treatment For lower genital tract infections, Azithromyin 1g orally single dose or doxycyline 100mg orally bid X 1 week are the treatment of choice[6].

Gonococcal and Chlamydial Infections in HIV- Infected Patients

Classical STIs, both ulcerative and non-ulcerative, could facilitate HIV-1 transmission by increasing either the infectiousness of the index case, the susceptibility of the partner, or both [61-62].Wasserheit has called the relationship between HIV and STIs as epidemiological synergy[62].

Ho JL, et al showed that chlamydia trachomatis increase the replication of HIV-1, probably through the generation of reactive oxygen products and cytokines secreted by granulocytes [63]. Moss et al detected HIV-1 RNA in the urethra more frequently in patients with coexistent gonococcal urethritis than in controls [64].

POLYMERASE CHAIN REACTION (PCR)

PCR, the Polymerase chain reaction was discovered by Kary Mullis in 1983[65]. Thermostable Taq DNA Polymerase, the enzyme used in PCR was chosen as the molecule of the year 1989.

PRINCIPLE OF PCR [66]

The purpose of a PCR is to make a huge number of copies of a gene. It is a Nucleic acid amplification test . In PCR there are three major steps which are repeated for 30 or 40 cycles. This is done on a automated thermal cycler, which can heat and cool the tubes with the reaction mixture in a very short time.

1. Denaturation at 94°C - During the denaturation, the double strand melts open to single strand DNA.

2. Annealing at 54°C-when the temperature is cooled to 54°C, the oligonucleotide primers which are added to the mixture designate the boundaries of the DNA strand being duplicated.

3. Extension at 72°C- This is the ideal temperature for the polymerase enzyme. The thermostable Taq DNA polymerase enzyme in presence of all the four essential deoxy nucleoside triphosphates including deoxyadenosine, deoxyguanosine, deoxycytidine, dexoyuridine (in place of deoxy thymidine) triphosphates, extends the annealed primers along the target template. In a thermal cylcer, this process is automatically repeated 30-40 times for 2 to 4 hours.

The thermostable enzyme Taq DNA Polymerase was isolated from Thermus aquaticus growing in hot springs .This enzyme acts best at 72°C and the denaturation temperature of 90°C does not destroy its enzymatic activity. Other thermostable enzymes are Pflu DNA polymerase from pyrococcus furiosus and Vent polymerase from Thermococcus litoralis.

DIFFERENT SCHEMES OF PCR[67]

1. **Reverse transcriptase PCR(RT-PCR**)-The formation of complementary or copy DNA from RNA is the first step of a usually two step process of RT-PCR.The second major step is the amplification of c-DNA sequence using primers specific for the DNA sequence [67].
2. **Multiplex PCR:** It is used for simultaneous detection of two or more organisms. AMPLICOR CT/NG PCR is multiplex PCR which simultaneously detects gonococcal and chlamydial infections[69]. Multiplex PCR has been developed for simultaneous detection of Treponema pallidum, Haemophilus ducreyi and herpes simplex virus types 1 and 2 for the diagnosis of genital ulcers [68].
3. **Real time PCR-**It is characterized by the point in time during cycling when amplification of the PCR product of interest is first detected rather

than the amount of the PCR product of interest which is accumulated at the end-point after PCR which contained a large number of cycles [67].

4. **Inverse PCR** – functions to clone sequences flanking a known sequences. Flanking DNA sequences are digested and ligated to generate cicular DNA.PCR primers pointing away from the known sequences are then employed to amplify the flanking sequences [67].

5. **Anchored PCR** It will utilize only one primer instead of two primers. In this technique, only one strand will be copied first, after which Poly –G tail will be attached at the end of this new strand.This new strand then becomes template using anchor primer with Poly-C sequence for the daughter strand . In the next cycle, both original and anchored primer will be used for amplification [67].
6. **Nested PCR** It requires two sets of primers which are used to amplify a specific DNA fragment using two separate runs of PCR.The second pair of primers function to amplify a smaller specific DNA fragment located within the first PCR product[67].
7. **Long PCR** is a PCR in which extended or longer than standard PCR, measuring over 5 kilobases (frequently over 10kb)[67].

8. **Colony PCR**- screening of bacterial (E-Coli) or yeast clones for correct ligation or plasmid products done [67].
9. **Hot start PCR** –It allows the inhibition of polymerase activity during PCR reaction preparation thereby reduces nonspecific amplification and increases PCR product target yield [67].
10. **OTHER TYPES-**Amplified Fragment Length Polymorphism PCR (AFLP –PCR), Alu PCR, Asymmetric PCR, Optimizing PCR, Touch down PCR are the other types [67].

Allele specific PCR,Assembly PCR,Helicase dependent amplification,Intersequence-specific PCR(ISSR), Insitu PCR, Ligation mediated PCR,Methylation Specific PCR(MSP),Miniprimer PCR,Multiplex-ligation dependent Probe Amplification(MLPA),Overlap-extension PCR,Quantitative PCR(Q-PCR),Solid phase PCR, TAIL-PCR(thermal asymmetric interlaced PCR),PAN-AC,Universal fast walking are the other variants [65].

THE AMPLICOR CHLAMYDIA TRACHOMATIS / NEISSERIA GONORRHOEAE (CT /NG) PCR TEST [69]

The AMPLICOR CT /NG test is a qualitative in vitro test for the detection of Chlamydia trachomatis and / or Neisseria gonorrhoeae in urine from both sexes,in endocervical swab specimens from females, and in urethral

swab specimens from males.The test utilizes Polymerase chain reaction (PCR) nucleic acid amplification and nucleic acid hybridization for the detection of C. trachomatis and for Neisseria gonorrhoeae in urogenital specimens.

It consist of the following kits-Specimen preparation kit,amplification kit,detection kit for C.trachomatis, Neisseria gonorrhoeae and internal control , STD swab specimen collection and transport kit.

PRINCIPLES OF THE PROCEDURE

The AMPLICOR CT /NG Test is based on four major processes.

a) Specimen Preparation.
b) PCR amplification of target DNA using biotinylated primers [70].
c) Hybridisation of the amplified products to oligonuculeotide probes specific to targets.
d) Detection of the probe -bound amplified products by color formation.

The AMPLICOR CT /NG Test is a multiplex assay that permits the simultaneous amplification of C. trachomatis target DNA, N. gonorrhoeae target DNA and Internal Control (IC) DNA. The detection of CT /NG IC must be performed when testing swab specimens collected and transported with the AMPLICOR STD swab specimen collection and transport kit.

SPECIMEN PREPARATION

The only acceptable specimens are

a) Urine specimens (male and female) transported in clean polypropylene containers without preservatives.

b) Female endocervical and male urethral swab specimens collected and transported using the AMPLICOR STD Swab specimen collection and transport kit.

A. URINE SPECIMENS

Patient must not have urinated during the previous 2 hours.

1. Collect 10-15ml of first catch urine into a clean polypropylene container without preservatives.
2. Urine specimens are stable for 24 hours at room temperature. Urine specimens that will not be processed within 24 hours of collection can be stored at 2-8°C but must be processed within 7 days of collection. Urine specimens that cannot be processed within 7 days of collection can be stored at -20° C or colder for upto 2 months.

B. SWAB SPECIMENS

Swabs are collected in AMPLICOR Specimen transport medium (STM).

1. Remove mucus from the ectocervix and insert the large swab into the endocervical canal ,rotate for 3-5 seconds and withdraw, avoiding contact with vaginal surface.
2. Place the swab in the AMPLICOR STM tube,shake vigorously the swab in the liquid for 15 seconds.Remove the swab and excess mucus in it and discard.
3. Label the transport tubes approximately. Swab specimens are stable at room temperature for 10 days.

SPECIMEN PREPARATION: Epithelial cells, leucocytes and associated C. trachomatis and N.gonorrhoea cells, collected on swabs or pelleted from urine, are treated with a detergent solution to release both the Chlamydial DNA contained in Chlamydial reticulate bodes and the Neisserial DNA. A second detergent solution is then added to prepare the lysed specimen for amplification.

URINE SPECIMENS

Using a Sterile Pipette tip, add 500µL of CT/NG urine wash to the appropriate number of 2 ml Polypropylene tubes.Vortex urine thoroughly (3-10 seconds)Using a pipette, with an aerosol barrier tip, add 500µL of well mixed

specimen to the appropriate tube containing CT/NG urine wash and mix well by vortexing.Incubate at 37°C for 15 minutes. Centrifuge at >12,500 X g for 5 minutes.Pour off specimen and blot each tube on a separate sheet of absorbent paper.

Add 250 µL of CT/NG LYS to each tube. Recap tubes and mix well by vortexing. Incubate tubes for 15 minutes at room temperature.Add 250 µL CT/NG DIL to each tube. Recap tubes and mix well by vortexing.Centrifuge tubes for 10 minutes at >12,500xg .Transfer 50 µL of the supernatant to the appropriate tube containing working master mix.

REAGENT PREPARATION

Prepare working master mix (MMX) by adding 100 µL of CT/NG IC to one vial of CT/NG MMX. Add 50 µL of working master mix into each reaction tube. CT/NG mastermix consists of Taq DNA Polymerase enzyme, deoxyribonucleotides- d-UTP, dATP, dCTP, dGTP, Amperase (Uracil - N-glycosylase) enzyme,biotinylated CP24 and CP27 primers, sodium azide, Tris –Hcl buffer, EDTA, 100mM potassium chloride and glycerol.

C. CONTROL PREPARATION

CT (+) C serves as the positive control for the CT MWP and the negative control for the NG MWP. The tube, labelled NG (+)C serves as the positive control for the NG MWP and the negative control for the CT MWP. Therefore, both the CT and NG controls must be prepared even if specimens will be tested for CT or NG only.

PCR AMPLIFICATION

Target Amplification-The AMPLICOR CT/NG test uses the primers cryptic plasmids CP24 and CP27 to define a DNA sequence of approximately 207 nucleotides within the cryptic plasmid of C. trachomatis and for N.gonorrhoeae uses the primers SSO1 and SSO2 to define a sequence of 201 nucleotides within the putative cytosine DNA methyltransferase gene [71]. The processed specimens are added to the amplification mixture in reaction tubes in which PCR takes place. The thermal cycler heats the reaction mixture in the tube to denature the double standard DNA helix and expose the primer target sequences. As the mixture cools, the biotinylated primers CP24 and CP27 anneal to the C. trachomatis and internal control target DNA, and the biotinylated primers SSO1 and SSO2 anneal to the N. gonorrhoeae target DNA.

The thermostable DNA Polymerase, Taq Polymerase, in the presence of excess deoxynucleoside triphosphate (dNTPs), including deoxyadenosine,

deoxyguanosine, deoxycytidine (in place of deoxy thymidine) triphosphates, extends the annealed primers along the target templates to produce a DNA sequence termed as amplicon. AMPLICON can be distinguished from native DNA because it contains dUTP. This process is repeated for a number of cycles, each cycles effectively doubling the amount of amplicon.

INTERNAL CONTROL AMPLIFICATION:

The CT/NG Internal control has been added to the AMPLICOR CT/NG test to identify processed specimen containing substances that may interfere with PCR amplification.

SELECTIVE AMPLIFICATION

Selective Amplification of target DNA from the clinical specimen is achieved in the AMPLICOR CT/NG test by the use of AMPErase (uracil-N-glycosylase) enzyme[72] and deoxyuridine triphosphate (dUTP). AMPErase enzyme recognizes and catalyses the destruction of DNA strands containing deoxyuridine, but not strains of target DNA containing deoxythymidine. Amperase enzyme below 55°C at the start of the test degrades any prior contaminating amplicon at C1 position of deoxyuridine.

Following amplification, any residual enzyme is denatured by the addition of the denaturation solution, thereby, preventing the degradation of any target amplicon.

AMPLIFICATION AND DETECTION

Place the Tray /Retainer Assembly into the GeneAmp 9700 thermal cycler sample block.Program the Applied Biosystems Gene Amp PCR System 9700 as follows

Hold Program :2 min50°C ,Hold Program :5 min 94°C. Cycle Program (36 Cycles) **Denaturation**-20 Sec 93°C; **Annealing** -60 Sec 61°C; **Extension** - 40 Sec 71°C; Hold Program-5 min 72°C;Hold Program-72°C Forever.

Start the METHOD Programme. The Program runs approximately 2 hours. Specimens may be removed at any time during the final HOLD program, but must be removed within 24 hours.Immediately add 100µL of DN and incubate for 10 minutes at room temperature. Store denature AMPLICON at room temperature for 2 hours, but after two hours,store at 2-8°C for upto one week.

HYBRIDISATION REACTION

Add denatured AMPLICON to separate Microwell Plate (MWP) containing oligonucleotide probe (100 µL of CT/NG HYB) specific for the CT, NG or IC targets. The biotin labeled amplicon is captured by the probe - coated MWP. Incubate for an hour at 37°C

DETECTION REACTION

Following the hybridization reaction, the MWP is washed to remove any unbound material and an Avidin – horseradish peroxidase conjugate (AV-HRP) (100µL) is added to each well of the MWP. The Avidin –horseradish peroxidase conjugate binds to the biotin – labelled amplicon hybridized by the plate -bound oligonucleotide probes. The MWP is washed again to remove unbound conjugate and a substrate solution containing 3,3´5,5´ tetramethylbenzidine (TMB) is added to the wells. In the presence of hydrogen peroxide, the bound horseradish peroxidase catalyzes the oxidation of TMB to form a coloured complex.

The reaction is stopped by addition of a weak acid, (100µL STOP) and the absorbance at 450 nm is measured using an automated microwell plate reader. Within hour of adding the STOP, record the absorbance value for each patient specimen and control tested.

RESULTS

A.CT RESULT: Interpretation of Results without Internal Control Detection

A_{450}	Interpretation
<0.2	C. trachomatis DNA not detected

≥ 0.8	C. trachomatis DNA detected
≥0.2- <0.8	Equivocal.

Interpretation of Results –With Internal Control Detection

CT Result A_{450}	IC Result A_{450}	Interpretation
<0.2	≥0.2	C.trachomatis not detected
<0.2	<0.2	Inhibitory specimen

For valid run, Specimen with A_{450} >0.8 are interpreted as positive for C.trachomatis regardless of the IC result.

B. NG Result : Interpretation of Results without Internal Control detection

A_{450}	Interpretation
<0.2	N.gonorrhoeae DNA not detected
≥0.2	N.gonorrhoeae DNA detected.

Interpretation of Results –With Internal Control Detection

CT Result	IC Result	Interpretation

A_{450}	A_{450}	
<0.2	≥0.2	N.gonorrhoeae DNA not detected
<0.2	<0.2	Inhibitary Specimen
≥0.2	Any	N.gonorrhoeae DNA detected

AIMS AND OBJECTIVES

1. To study the prevalence of Chlamydia trachomatis and Neisseria gonorrhoeae infections in patients attending the Out-Patient Department of Institute of Venereology, using AMPLICOR CT/NG Polymerase chain reaction.
2. To study the prevalence of Chlamydia trachomatis and Neisseria gonorrhoeae infections among HIV positive patients in the study group.
3. To study about the age distribution, socioeconomic background, educational level and marital status among the patients in the study group.
4. To compare the sensitivity and specificity of AMPLICOR CT/NG Polymerase chain reaction with Culture for Gonococci and IgM ELISA test for Chlamydia trachomatis.

MATERIALS AND METHODS

STUDY DESIGN:

Prospective observational study.

SAMPLE:

The study population comprised of all male and female patients attending the out patient department(OPD) of Institute of Venereology, Government General Hospital, Chennai -3 from March 1,2007 to February 29, 2008.

INCLUSION CRITERIA

1. All patients attending the OPD irrespective of symptoms.
2. No antibiotics taken four weeks prior to examination.
3. Non-Pregnant females.

During the study period 280 males and 270 females, a total of 550 patients were enrolled.

METHODS:

The study patients were interviewed regarding their age, educational status, occupation,marital status, presenting complaints, sexual history, past history of venereal diseases and treatment taken.

All the patients underwent a complete physical examination and genital examination. All these patients were clinically analysed for the genital manifestation and supported by laboratory diagnosis.

All patients were screened for Chlamydia trachomatis and Neisseria gonorrhoeae infections using Roche AMPLICOR CT/NG Polymerase chain reaction.

For AMPLICOR CT /NG PCR Specimens collected were 1)First catch 30ml of urine from both sexes,2)Endocervical swab from females, 3)Urethral swab from males. In addition, following investigations were done for all patients.

1)Blood for VDRL and TPHA.

2)ELISA for HIV and CD_4 cell count in HIV positive patients.

3)Urine -Albumin, sugar, deposits.

4)Culture for Gonococci –Urine,urethral swab (males), Endocervical swab(females).

5)IgM ELISA for Chlamydia trachomatis.

6)Screening for HBsAg and Anti HCV antibodies.

7)Ultrasound abdomen for females.

In cases of genital discharge following tests were done.

1)Wet film for Trichomonas Vaginalis and clue cells (females).

2)10% KOH Preparation for Candida albicans.

3)Gram's stain of vaginal smear, endocervical smear to identify Neisseria gonorrhoeae, Lactobacillus, clue cells, candidal hyphae and

polymorphonuclear leucocytes. Gram's stain of male urethral smear for gonococci,candidal hyphae and polymorphonuclear leucocytes.

In addition, for females pH of the genital discharge,Whiff test by adding 10% KOH to the genital discharge were done.In case of genital ulcers following tests were done.

1) Dark field examination for treponema pallidum.
2) Gram stain for Haemophilus ducreyi and candida.
3) Tissue smear and Leishman's stain for Donovan bodies.
4) Tzanck test for multinucleated giant epithelial cells.
5) Ziehl-Neelsen staining for Mycobacterium tuberculosis.
6) Wet mount for amoebic infestations.

In case of genital growth, Histo-pathological examination of biopsy specimen was done for appropriate cases. Urine culture and sensitivity was done for needed patients.Liver function tests, complete blood count, renal function test,random blood sugar, X-ray chest, ECG, Sputum examination for AFB, Mantoux test, were also done for needed patients.In needed symptomatic patients opinion from concerned specialists such as Dermatology, Obstetrics & Gynaecology, Dental, Opthalmology, Chest Clinic, Cardiology, Neurology, Nephrology, Urology and gastroenterology were obtained.Patients were

offered standard treatment according to clinical condition and prophylaxis for opportunistic infections.

RESULTS

Total number of patients enrolled in the study group - 550 .

Total number of Males -280.

Total number of Females -270.

Table-1: Prevalence of Chlamydia trachomatis and Neisseria gonorrhoeae infections in the study group

	Males (n-280)		Females (n-270)	
Neisseria gonorrhoeae infections	Number	Percentage%	Number	Percentage %
	41	14.64	36	13.34
Chlamydia trachomatis infections	10	3.57	10	3.70

Prevalence of Gonococcal infections(Males –41, Females –36) was high compared to Chlamydia trachomatis infections (Males-10, Females –10) among 280 men and 270 women tested.

In gonococcal infections men (14.64%), were more commonly infected than women (13.34%). In Chlamydial infections females (3.70%) were more commonly infected than males (3.57%).

TABLE- 2: Prevalence of Chlamydia trachomatis and Neisseria gonorrhoeae infections among HIV positive patients

In the study group HIV was positive in Males –100 and Females –100

	HIV Positive Males N-100		HIV Positive Females N-100	
	Number	Percentage %	Number	Percentage %
Neisserria gonorrhoeae infections	14	14	7	7
Chlamydia trachomatis Infections	3	3	1	1

In HIV positive patients also, prevalence of gonococcal infections (m-14%, f-7%) was high compared to chlamydia trachomatis (m-3%, f-1%) infections. Prevalence of both gonococcal and chlamydial infections was high in HIV positive males(NG-14%,CT-3%) compared to HIV positive females.(NG-7%,CT-1%).

Table -3: Number of positivity in urine and urethral swab specimens of affected males

Neisseria gonorrhoea Infections n-41			Chlamydia trachomatis infections n-10			Both NG and CT n-4		
Urine	Swab	Both	Urine	Swab	Both	Urine	Swab	Both
16	13	12	7	2	1	3	-	1

In Gonococcal and Chlamydial infections, urine specimens showed more positivity than urethral swab specimens.

Table -4 : Number of positivity in urine and endocervical swab specimens of affected females

Neisseria gonorrhoea infections n-36			Chlamydia trachomatis infections n-10			Both NG and CT n-7		
Urine	Swab	Both	Urine	Swab	Both	Urine	Swab	Both
15	13	8	3	6	1	2	5	-

In Gonococcal infections urine specimens showed more positivity than endocervical swab specimens. In Chlamydial infections & concomitant infections of both CT and NG, endocervical swab specimens showed more positivity than urine specimens.

Table-5a :Asymptomatic Cases in Chlamydial Infection

Total Positive M-10, F-10

Asymptomatic patients	HIV (M-3 ,F-1)		NON HIV (M-7, F-9)	
	Number	Percentage %	Number	Percentage %
Males -2 (20%)	2	66.67	-	-
Females –4 (40%)	1	100	3	33.34

Table –5b: Asymptomatic cases in Gonococcal Infection

Total Positive M-41, F-36

Asymptomatic patients	HIV (M-14 ,F-7)		NON HIV (M-27, F-29)	
	Number	**Percentage %**	Number	Percentage %
Males –12(29.26%)	8	57.14	4	14.8
Females – 10(27.78%)	4	57.14	6	20.68

Asymptomatic Gonococcal infection was common in males (29.26%) than females (27.78%). Asymptomatic Chlamydial infection was more common in

females (40%) than males (20%).In males, asymptomatic gonococcal and chlamydial infections were common in HIV positive patients (NG-57.14%, CT-66.67%) compared to HIV negative patients (NG-14.8% CT-0%).

In females also, asymptomatic gonococcal and chlamydial infections was common in HIV positive patients (NG-57.14%, CT-100%) compared to HIV negative patients (NG –20.68%, CT-33.34%).

TABLE-6: Evaluation of AMPLICOR CT/NG PCR Gonococcal infections in affected males with reference to culture.

PCR	CULTURE FOR GONOCOCCI		TOTAL
	Positive	Negative	
Positive	19	22	41
Negative	0	239	239
	19	261	280

In gonococcal infection positive males, AMPLICOR CT /NG PCR had sensitivity ,specificity,positive predictive and negative predictive values of 100%,92%,46%,100% respectively.

TABLE-7: Evaluation of AMPLICOR CT /NG PCR with reference to culture for gonococcal infections in affected females.

PCR	CULTURE FOR GONOCOCCI	TOTAL

	Positive	Negative	
Positive	16	20	36
Negative	0	234	234
	16	254	270

In gonococcal infection positive females, AMPLICOR CT /NG PCR had sensitivity, specificity, positive predictive value and negative predictive values of 100%, 92%,44% and 100% respectively.

TABLE-8 : Evaluation of AMPLICOR CT /NG PCR with reference to IgM ELISA for Chlamydia trachomatis infection positive males

PCR	IgM ELISA for C.trachomatis		TOTAL
	Positive	Negative	
Positive	7	3	10
Negative	-	270	270
	7	273	280

In Chlamydia trachomatis infection positive males, AMPLICOR CT/NG PCR had sensitivity specificity, positive predictive value, and negative predictive value of 100%, 99%, 70% and 100% respectively.

Table-9:Evaluation of AMPLICOR CT /NG PCR with reference to IgM ELISA for Chlamydia trachomatis infection positive females.

PCR	IgM ELISA for Chlamydia		TOTAL
	Positive	Negative	

Positive	5	5	10
Negative	0	260	260
	5	265	270

In Chlamydia trachomatis infection positive females, AMPLICOR CT/NG PCR had sensitivity, specificity, positive predictive value and negative predictive value of 100%, 98%, 50% and 100% respectively.

TABLE-10a: Age Distribution of Chlamydia trachomatis and Neisseria gonorrhoeae infections in affected males.

AGE	Gonococcal infections (n-41)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage %
16-19	-	-	1	10
20-29	18	43.90	5	50
30-39	12	29.26	3	30
40-49	11	26.82	1	10
More than 50	-	-	-	-

Majority of Gonococcal (43.90%) and Chlamydia trachomatis (50%)infections positive patients belonged to 20-29 years age group.

TABLE-10b: Age distribution of Chlamydia trachomatis and Neisseria gonorrhoeae infections in affected females.

AGE	Gonococcal infections (n-36)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
16-19	-	-	-	-
20-29	17	47.23	6	60%
30-39	14	38.89	3	30%
40-49	4	11.12	1	10%
More than 50	1	2.78	-	-

Majority of gonococcal (47.23%) and Chlamydial (60%) infections positive females belonged to 20-29 years age group.

TABLE -11a: Socio economic status of affected males

Monthly Income (in rupees)	Gonococcal infections (n-41)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
<1000	34	82.92	9	90%
1000-1999	5	12.19	1	10%
2000-4999	1	2.43	-	-
>5000	1	2.43	-	-

Majority of affected males belonged to lower socioeconomic status (NG-82.92%, CT-90%) i.e. less than Rs.1000 per month.

TABLE- 11b: Socio –Economic Status of affected Females

Monthly Income (in rupees)	Gonococcal infections (n-36)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
<1000	28	77.78	8	80
1000-1999	6	16.67	2	20
2000-4999	1	2.78		
>5000	1	2.78		

Majority of affected females belonged to lower socioeconomic status (NG-77.78%,CT-80%) i.e. less than Rs.1000 per month.

TABLE-12a: Educational Status among affected males

Educational Status	Gonococcal infections (n-41)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
Uneducated	8	19.51	1	10
1-5th	12	29.26	3	30
6-10th	19	46.34	4	40
11-12th	2	4.87	2	20
College	-	-	-	-

Majority of affected males had High School education (NG-46.34%, CT-40%).

TABLE-12b :Educational Status among affected females

Monthly Income (in rupees)	Gonococcal infections (n-36)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
Uneducated	8	22.23	2	20
1-5th	10	27.78	3	30
6-10th	17	47.23	3	30
11-12th	1	2.78	2	20
College	-	-	-	-

Majority of affected females had high school education (NG-47.23%, CT-30%).

TABLE- 13a: Marital Status among affected Males

Marital Status	Gonococcal infections (n-41)		Chlamydial infections (n-10)	
	Number	Percentage %	Number	Percentage %
Married	22	53.65	7	70%
Single	19	46.34	3	30%
Divorced /Separated	-	-	-	

Widow	-	-	-	-

Majority of affected males in the study group were married (NG-53.65%, CT-70%).

TABLE- 13b : Marital Status among affected females

Marital Status	Gonococcal infections (n-36)		Chlamydial infections (n-10)	
	Number	Percentage %	Number	Percentage %
Married	30	83.34	8	80
Single	1	2.78	2	20
Divorced /Separated	2	5.56	-	
Widow	3	8.34	-	-

Majority of affected females in the study group were married (NG-83.34%, CT-80%).

TABLE- 14a: Presenting Complaints of the affected males

Presenting Complaints	Gonococcal infections (n-41)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%

Screening	12	29.26	2	20
Burning Micturition	22	53.65	8	80
Genital Discharge	9	21.95	2	20
Genital Ulcer	3	7.31	3	30
Growth Genitalia	2	4.87	-	-
Skin rash	3	7.31	-	-
Loss of appetite	3	7.31	1	10
Genital itching	1	2.43	-	-
Sexual dysfunction	1	2.43	1	10

Burning micturition was the main complaint in affected males (NG-53.65%, CT-80%) followed by genital discharge (NG-21.95%,CT –20%) and genital ulcer (NG-7.31%, CT-30%) .

29.26% of gonococcal infection positive males and 20% of chlamydia trachomatis infection positive males came for screening.

Table -14b: Presenting Complaints of the affected females

Presenting Complaints	Gonococcal infections (n-36)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
Screening	10	27.78	4	40
Genital Discharge	18	50	5	50
Burning Micturition	7	19.45	4	40
Growth genitalia	2	5.56	1	10
Genital Ulcer	3	8.34	-	-
Genital Itching	8	22.23	2	20
Lower Abd Pain	3	8.34	1	10
Skin rash	1	2.77	-	-
Loss of appetite	2	5.56	-	-

Majority of the affected females visited for Genital discharge (NG-50%, CT-50%) followed by burning micturition (NG –19.45%, CT-40%). Genital Itching (NG-22.23%, CT-20%), lower abdominal pain (NG-8.34%,CT-10%) were the other complaints.40% of chlamydial infection positive patients and 27.78% of gonorrhoeae positive patients came for screening.

Table-15a: Clinical Signs in the affected males

Clinical Signs	Gonococcal infections (n-41)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
Urethral discharge	6	14.63	1	10
Genital Ulcer	4	9.75	3	30
Genital wart	3	7.31	1	10
Phimosis	2	4.87	-	-
Vitiligo	2	4.87	-	-
Balanoposthitis	1	2.43	-	-
Pearly Penile papules	1	2.43	-	-
Dermatophytosis	1	2.43	-	-
Folliculitis	-		1	10
Skin rash	1	2.43	-	-
No abnormal findings	24	58.53	4	40

In gonorrhoeae positive males urethral discharge (14.63%) was the most common clinical sign followed by genital ulcer (9.75%) and genital wart (7.31)%.

In Chlamydia trachomatis infection positive males genital ulcer (30%) was the most common clinical sign followed by urethral discharge (10%), genital wart (10%) and folliculitis (10%).

58.53% of N.gonorrhoeae infection positive and 40% Chlamydial infection positive patients had no abnormal clinical findings.

Table- 15b: Clinical signs in the affected females

Clinical Signs	Gonococcal infections (n-36)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
Cervical Erosions	21	58.34	7	70
Cervical Hypertrophy	6	16.67	1	10
Soddening of vulva	4	11.12	1	10
Cervix healthy	9	25	2	20
Genital ulcer	1	2.78	-	-
Genital wart	1	2.78	1	10
Mollusum contagiosum	1	2.78	-	-
Bartholin cyst	1	2.78	1	10
Folliculitis	1	2.78	-	-

In gonococcal infection positive female patients, 58.34% had cervical erosions, followed by cervical hypertrophy (16.67%), soddening of vulva (11.12%), 25% of them had healthy cervix. In Chlamydial infection positive females also, cervical erosion (70%) was the most common clinical sign followed by cervical hypertrophy (10%), soddening of vulva (10%), genital wart (10%), Bartholin cyst (10%) and 20% had healthy cervix.

Table -16a: Other associated STDs among Neisseria gonorrhoeae and Chlamydia trachomatis infection positive men

Other Associated STDs	Gonococcal infections (n-41)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
Chlamydial infection	3	7.31	-	-
Gonorrhoeae	-	-	3	30
Candidiasis	1	2.43	-	-
Genital wart	3	7.31	1	10
Molluscum Contagiosum	-	-	-	-
Herpes genitalis	3	7.31	3	30
Syphilis (ELS-1) LLS-1)	2	4.87	-	-
HBSAg	1	2.43	-	-
Trichomonas Vaginalis	-	-	-	-
More than two STDs	-	-	-	-

In Gonorrhoeae positive males, most common associated STIs were Chlamydia tracthomatis infection (7.31%), Genital wart (7.31%) and Herpes genitalis (7.31%).

In Chlamydia trachomatis infection positive females, most common associated STIs were Gonorrrhoea (30%), and Herpes genitalis (30%).

Table - 16b: Other associated STDs among Neisseria gonorrhoeae and Chlamydia trachomatis infections positive women

Other Associated STDs	Gonococcal infections (n-36)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
Chlamydial infection	7	19.45		-
Gonorrhoeae	-	-	7	70
Candidiasis	9	25	1	10
Genital Wart	1	2.78	1	10
Molluscum Contagiosum	1	2.78	-	-
Herpes genitalis	1	2.78	-	-
Syphilis	-	-	-	-
HBs Ag	-	-	-	-
Trichomonas Vaginalis	4	11.12	3	30
Bacterial Vaginosis	15	41.67	4	40
More than two STDs	8	22.23	3	30

In gonococcal infection positive women, Bacterial vaginosis (41.67%) was the commonest STI followed by candidiasis (25%) and Chlamydial infections (19.45%).

In Chlamydial infection positive women, N.gonorrhoea (70%) was the commonest STI followed by Bacterial vaginosis (40%) and trichomoniasis (30%).

Table -17: Nature of Genital Discharge among affected females

Nature of Discharge	Gonococcal infections (n-36)		Chlamydial infections (n-10)	
	Number	Percentage%	Number	Percentage%
Mucopurulent	18	50	4	40
Mucoid	11	30.56	3	30
Curdy white	3	8.34	-	-
Frothy	4	11.12	3	30

Majority of the affected women had mucopurulent vaginal discharge (NG-50%, CT-40%).

TABLE-18a:CD_4 COUNT – In HIV Positive males

CD_4 Count	Gonococcal infections (n-14)		Chlamydial infections (n-3)	
	Number	Percentage%	Number	Percentage%
Less than 300	4	28.57	1	33.34
More than 300	10	71.42	2	66.67

71.42% of gonococcal infection positive males and 66.67% Chlamydial infection positive males had CD_4 count of more than 300.

Table 18b : CD_4 count in HIV Positive females

CD_4 Count	Gonococcal infections (n-7)		Chlamydial infections (n-1)	
	Number	Percentage%	Number	Percentage%
Less than 300	2	28.57	-	-

More than 300	5	71.42	1	100%

71.42% of Gonorrhoea positive females and 100% of Chlamydia trachomatis infection positive females had CD4 count of more than 300.

DISCUSSION

During the study period, 550 patients were enrolled for Multiplex Amplicor CT/NG PCR study. Out of 550 patients 280 were males and 270 were females.

14.64% of males and 13.34% of females were positive for gonococcal infection. 3.57% of males and 3.70% of females were positive for Chlamydial infections. This was comparable with the study done by William C et al in which 3.67% males and 4.74% of females were positive for C. trachomatis infections[73].

Gonococcal infections (males –14.64%, females –13.34%) were more common than Chlamydial infections (males –3.57% females 3.70%).

In gonococcal infections men(14.64%), were more commonly infected than women (13.34%). In Chlamydial infections females (3.70%) were more commonly infected than males (3.57%). This result correlates with William C et al study in which 4.74% of females were positive for Chlamydial infection compared to males of 3.67% [73].

In the study 100 HIV positive males and 100 positive females were enrolled. In HIV positive patients also, prevalence of gonococcal infection(M-14%,F-7%) was high compared to chlamydia trachomatis (M-3%, F-1%) infections. Prevalence of both gonococcal and chlamydial infection was high in HIV positive males (NG-14%, CT-3%), compared to HIV positive females (NG-7%, CT-1%).

Urine PCR (m-16,f-15)showed more positivity than male urethral (13)and female endocervical swab PCR(13) in gonococcal infection positive patients. In Chlamydial infection positive males urine PCR(7) showed more positivity than urethral swab PCR(2), but in females endocervical swab PCR(6) showed more positivity than urine PCR(3).

Asymptomatic gonococcal infection was common in affected males (29.26%) when compared to affected females (27.78%). Asymptomatic Chlamydial infection was common in affected females (40%) than affected males (20%).Asymptomatic gonococcal (M-57.14%, F-57.14%) and chlamydial infections (M-66.67%, F-100%) were common in HIV positive patients compared to HIV negative patients. In HIV positive patients, asymptomatic gonococcal and chlamydial infections were common in females (NG-57.14%, CT-100%) than males (NG-57.14%, CT-66.67%).

In gonococcal infection positive males, AMPLICOR CT/NG PCR had sensitivity, specificity, positive predictive value and negative predictive value of 100%, 92%, 46% and 100% respectively. In gonococcal infection

positive females, AMPLICOR CT/NG PCR had sensitivity, specificity, positive predictive value and negative predictive value of 100%, 92%, 44% and 100% respectively compared with N.gonorrhoeae Culture.

In Chlamydia trachomatis infection positive males, AMPLICOR CT /NG PCR had sensitivity, specificity, positive predictive value and negative predictive value of 100%, 99%, 70% ad 100% respectively compared with IgM ELISA. In C. trachomatis infection positive females, the sensitivity, specificity, positive predictive value, and negative predictive value of AMPLICOR CT/NG PCR were 100%, 98%, 50% and 100% respectively.

Majority of Gonococcal (43.90%) and Chlamydial (50%) infection positive males belonged to 20-29 years age group, similarly in affected females majority belonged to 20-29 years age group (NG-47.23%, CT- 60%) which was similar to many studies [1, 9, 19-22] in India and worldwide. Majority of affected males belonged to lower socioeconomic status (NG-82.92%,CT-90%). In affected females also, majority belonged to lower socioeconomic status (NG-77.78%, CT-80%) .

Majority of affected males had high school education (NG-46.34%, CT-40%). Similarly, majority of affected females had high school education (NG-47.23%, CT-30%). 53.65% of gonococcal and 70% of Chlamydial

infection affected males were married. Majority of the affected females in the study group were married (NG-83.34%, CT-80%).

Burning micturition was the main complaint in affected males (NG-53.65%,CT-80%) followed by genital discharge (NG-21.95%, CT-20%) and genital ulcer (NG-7.31%, CT-30%). 29.26% of gonococcal and 20% of Chlamydial infection affected male patients came for screening. In affected females, genital discharge (NG-50%, CT –50%) was the main complaint followed by burning micturition (NG-19.45%, CT-40%) and genital itching (NG-22.23%,CT-20%). 40% of Chlamydial and 27.78% gonococcal infection positive female patients came for screening.

In gonococcal infection positive males urethral discharge (14.63%) was the most common clinical sign followed by genital ulcer (9.75%). In Chlamydial infection affected males genital ulcer (30%) was the most common clinical sign followed by urethral discharge (10%).

In affected females, cervical erosion was the main clinical finding(NG-58.34%, CT-70%). 50% of gonococcal and 40% of Chlamydial infection positive females had mucopurulent vaginal discharge.

In gonococcal infection positive males, most common associated STIs were C. trachomatis infection (7.31%), genital wart (7.31%) and Herpes

genitalis (7.31%). In Chlamydia trachomatis infection affected males, most common associated STIs were N. gonorrhoeae infection (30%) and Herpes genitalis(30%).

In gonococcal infection positive women, Bacterial vaginosis (41.67%) was the commonest associated STI followed by candidiasis (25%) and C. trachomatis infection (19.45%). In C. trachomatis infection positive women, gonococcal infection (70%) was the commonest associated STI followed by bacterial vaginosis (40%) and Trichomoniasis (30%).

Gonococcal infection was detected in 30% of men and 70% of females with C. trachomatis infection which was similar to study by Philip DJ. et al[31].

Majority of HIV positive males had CD4 count of more than 300 (NG-71.42%, CT-66.67%). In the HIV positive females also, majority had CD4 count of more than 300 (NG-71.42%, CT –100%).

CONCLUSION

1. Gonococcal infections (males –14.64%, females –13.34%) was more common than Chlamydia trachomatis (males-3.57%, females –3.70%) infections. Gonococcal infection was more common in men (14.64%) compared to women (13.34%). Chlamydial infection was more common in women (3.70%) when compared to men (3.57%).

2. In HIV positive patients also, gonococcal infections (M-14%, F-7%),were more common than chlamydial infections (M-3%, F-11%).

3. AMPLICOR CT /NG PCR for urine specimens (M-16, F-15) was highly sensitive than male urethral (13) and female endocervical swab (13) specimens in detecting gonococcal infections. In Chlamydial infections, urine PCR (7) was highly sensitive than urethral swab PCR (2) in males

but in females, endocervical swab PCR(6) was more sensitive than urine PCR (3).

4. Asymptomatic gonococcal infection was common in affected males (29.26%) when compared to affected females (27.78%). Asymptomatic Chalmydial infection was common in affected females (40%) compared to affected males(20%).Asymptomatic gonococcal (M-57.14%, F-57.14%) and chlamydial infections (M-66.67%, F-100%) were common in HIV positive patients compared to HIV negative patients.

5. AMPLICOR CT/NG PCR had 100% sensitivity in detecting gonococcal infections compared with gonococcal culture and also had 100% sensitivity in detecting chlamydial infections compared with IgM ELISA for C. trachomatis.

6. Majority of gonococoal (M-43.90%, F-47.23%) and Chlamydial (M-50%, F-60%) infection positive patients belonged to 20-29 years age group. Majority of the affected patients belonged to lower socioeconomic status.

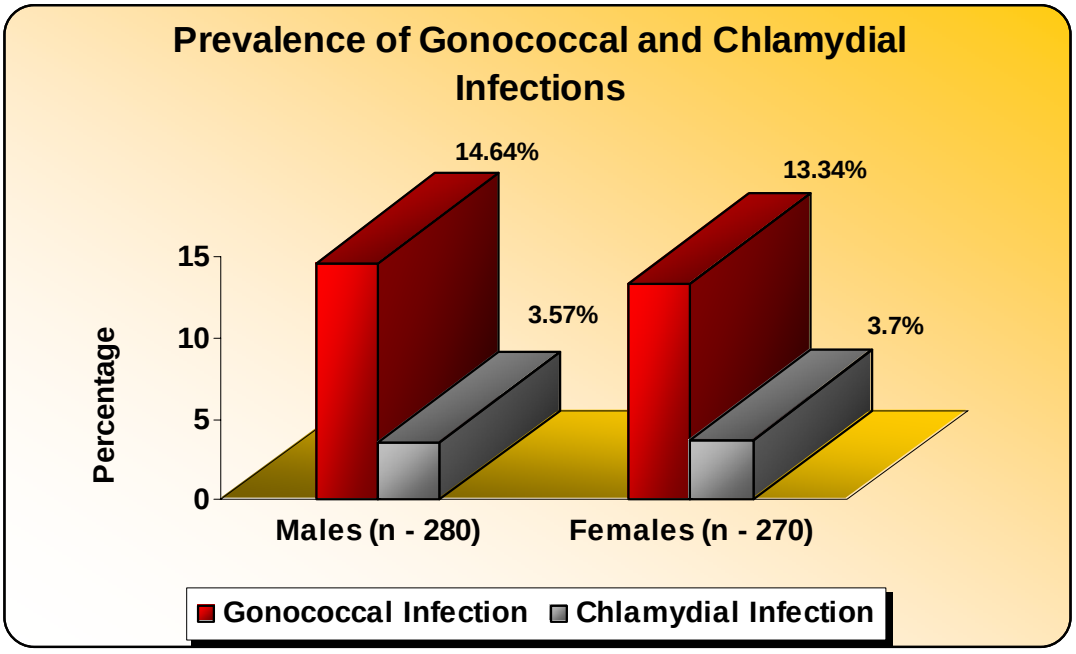
7. Burning micturition (NG-53.65%, CT-80%) was the main complaint in affected males and genital discharge (NG-50%,CT-50%) was the main complaint in affected females. In gonococcal infection positive males,

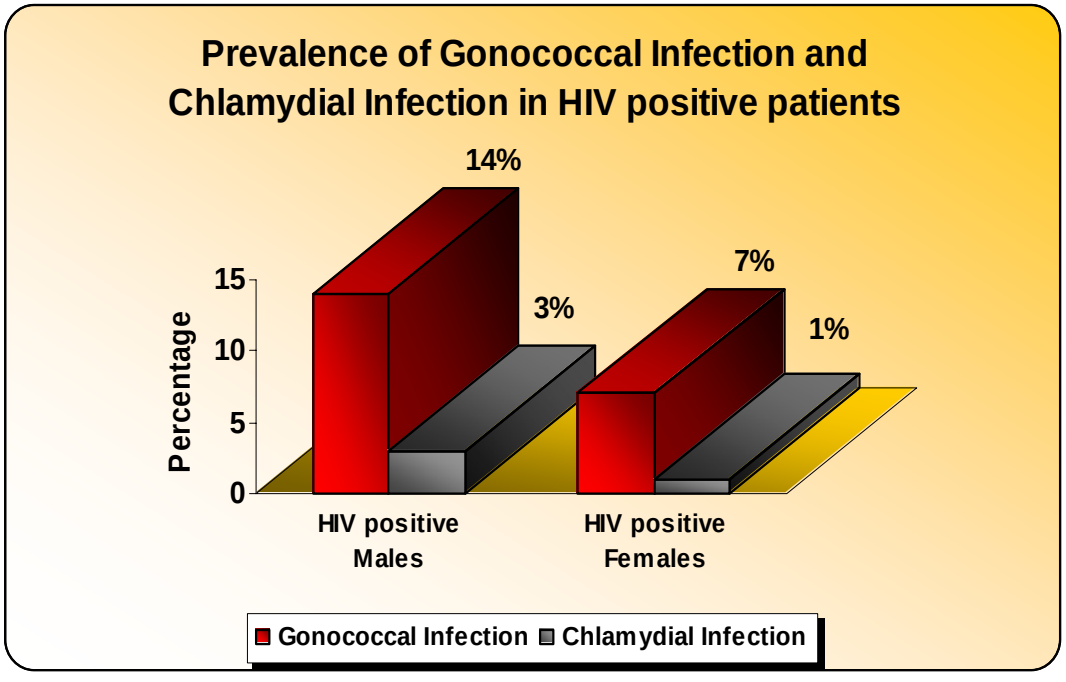
urethral discharge (14.63%) was the common clinical finding and in chlamydial infection positive males genital ulcer (30%) was the common clinical finding .Cervical erosion (NG-58.34%, CT-70%) was the common clinical finding in affected females.

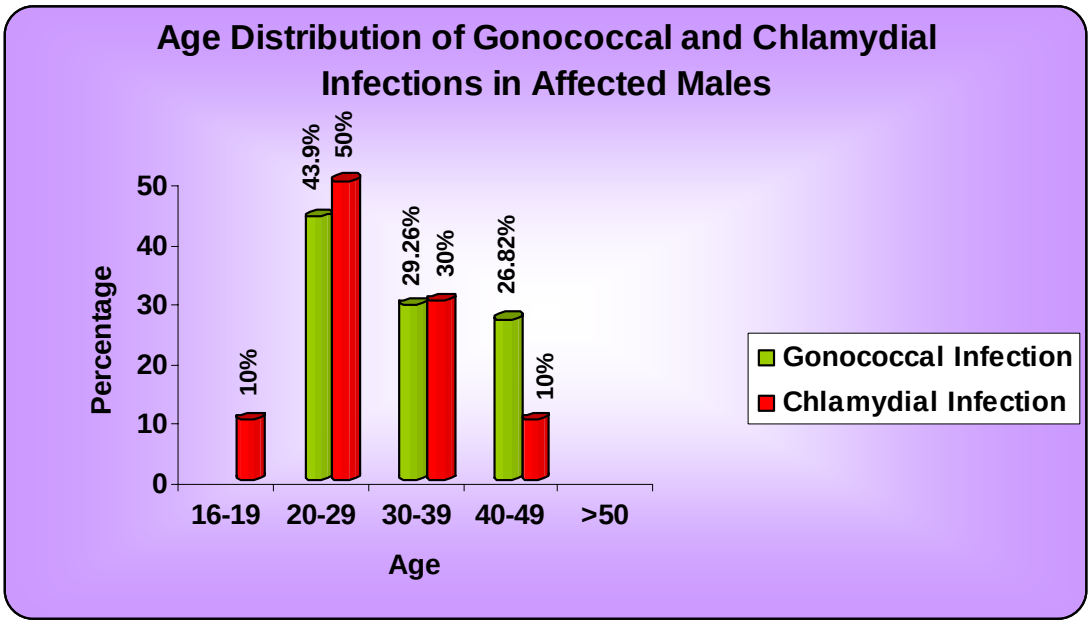
8. In Chlamydial infection positive patients, gonococcal infection was the most commonest associated STI (M-30%, F-70%). In gonococcal infection positive males, C. trachomatis (7.31%) was the commonest associated STI and in females, Bacterial vaginosis (41.67%) was the commonest associated STI.

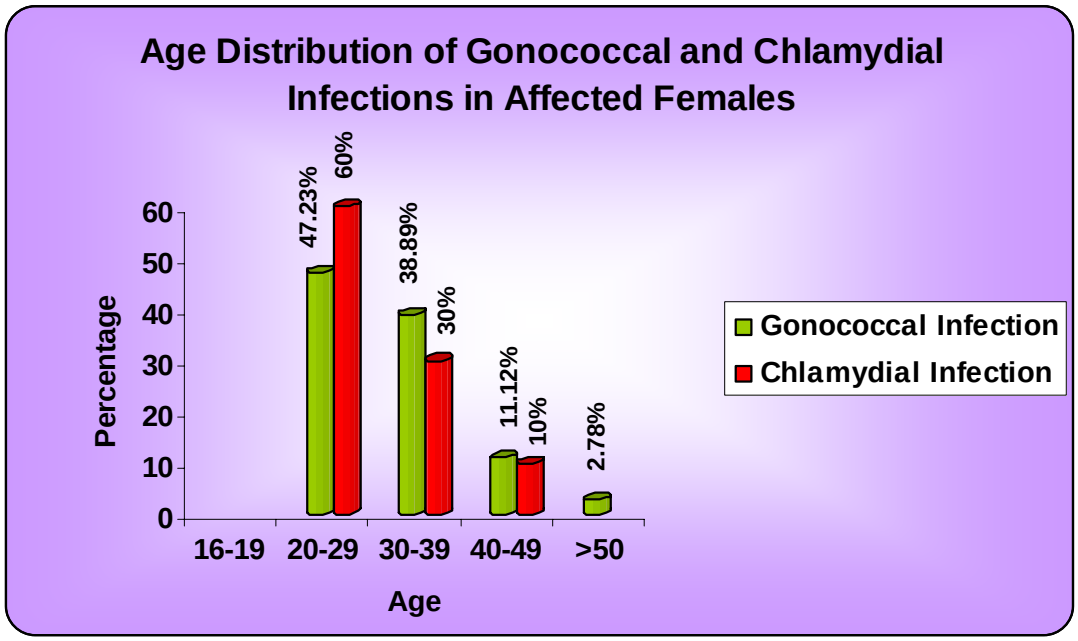
AMPLICOR CT/NG PCR had 100% sensitivity in detecting gonococcal and chlamydial infections. PCR screening will diagnose asymptomatic cases of both gonococcal and chlamydial infections.

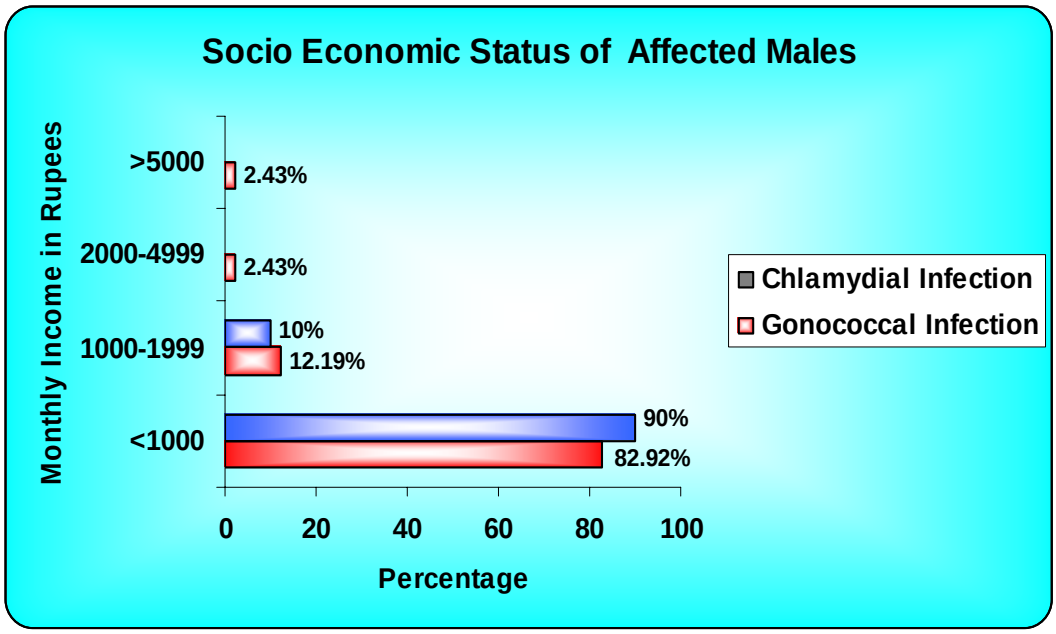
Since coinfection rate of gonococcal and chlamydial infection were high, all cases of gonorrhoeae should be treated presumptively for chlamydial infection as recommended by CDC and WHO.

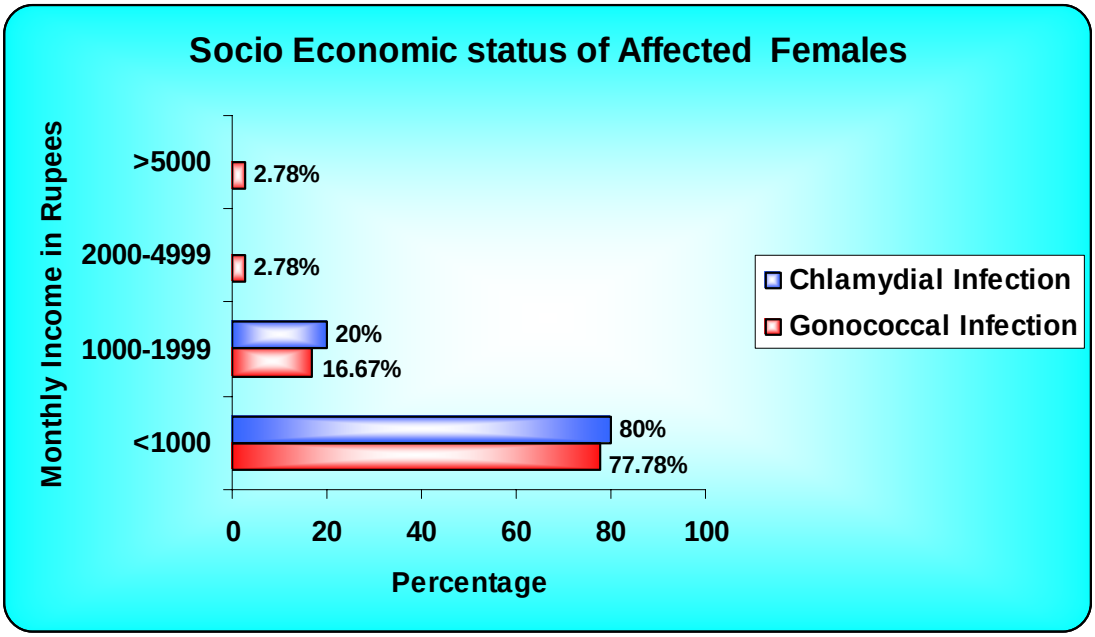

Prevalence of Gonococcal and Chlamydial Infections
14.64%
13.34%
3.57%
3.7%
15
10
5
0
Percentage
Males (n - 280)
Females (n - 270)
Gonococcal Infection
Chlamydial Infection

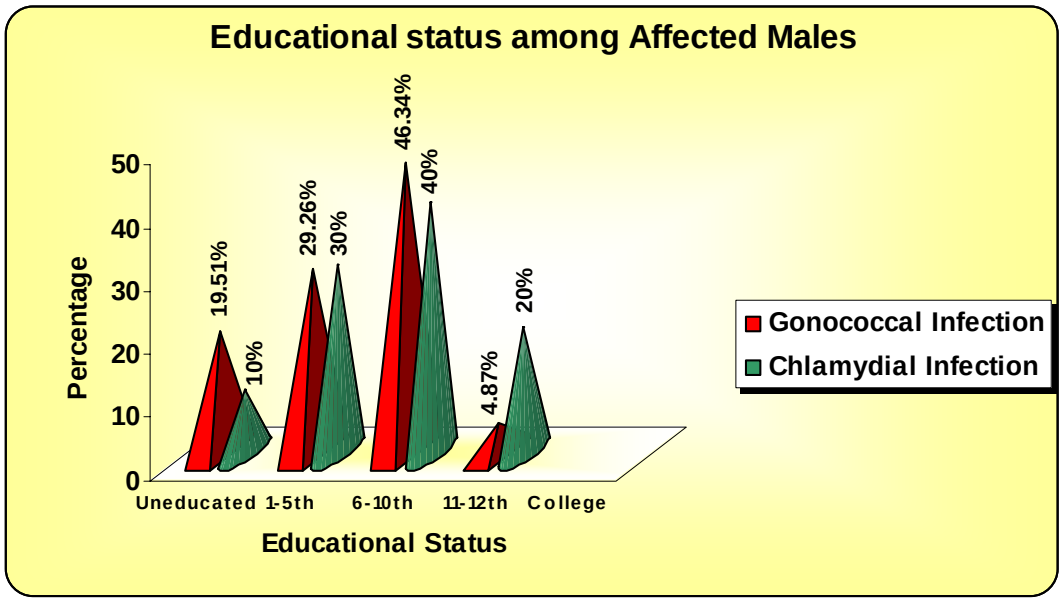

Prevalence of Gonococcal Infection and Chlamydial Infection in HIV positive patients
14%
7%
3%
1%
15
10
5
0
Percentage
HIV positive Males
HIV positive Females
Gonococcal Infection
Chlamydial Infection

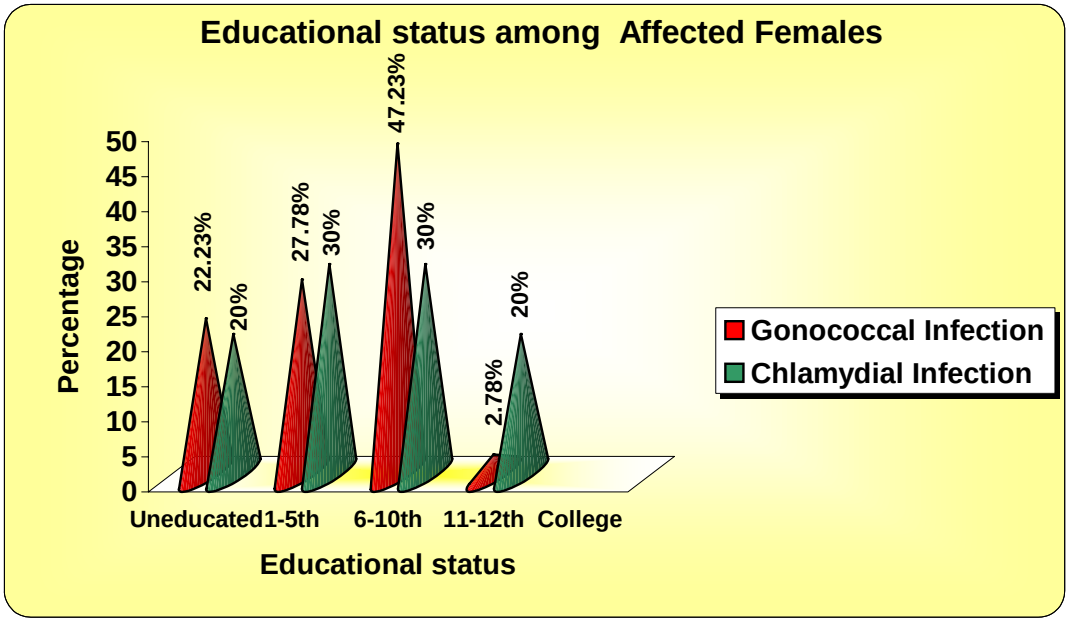

Age Distribution of Gonococcal and Chlamydial Infections in Affected Males
Percentage
50
40
30
20
10
0
10%
43.9%
50%
29.26%
30%
26.82%
10%
16-19
20-29
30-39
40-49
>50
Age
Gonococcal Infection
Chlamydial Infection

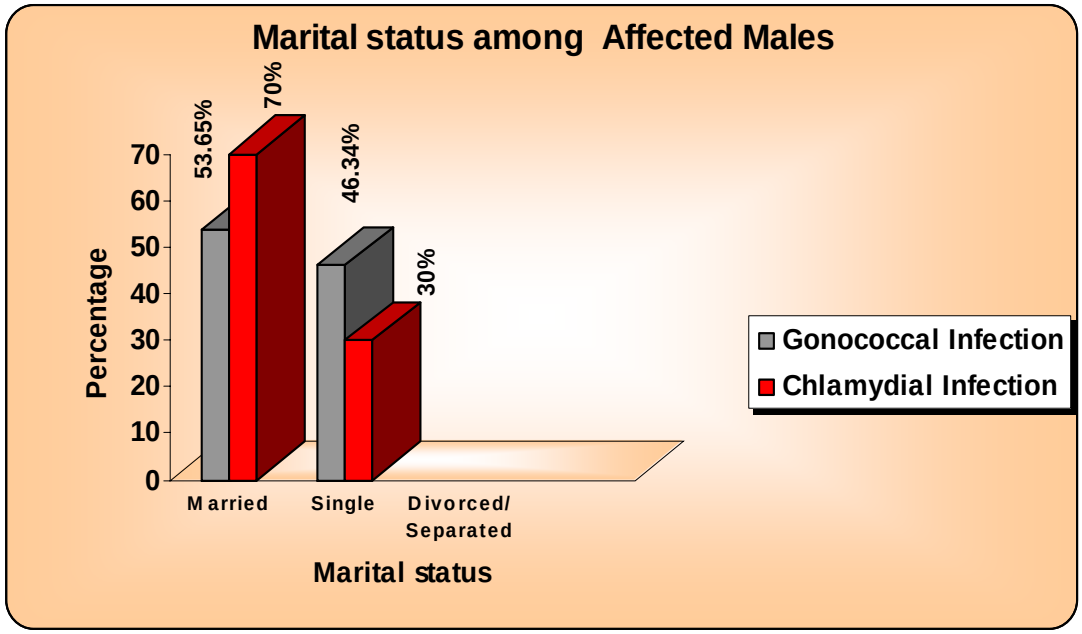

Age Distribution of Gonococcal and Chlamydial Infections in Affected Females
Percentage
60
50
40
30
20
10
0
47.23%
60%
38.89%
30%
11.12%
10%
2.78%
16-19
20-29
30-39
40-49
>50
Age
Gonococcal Infection
Chlamydial Infection

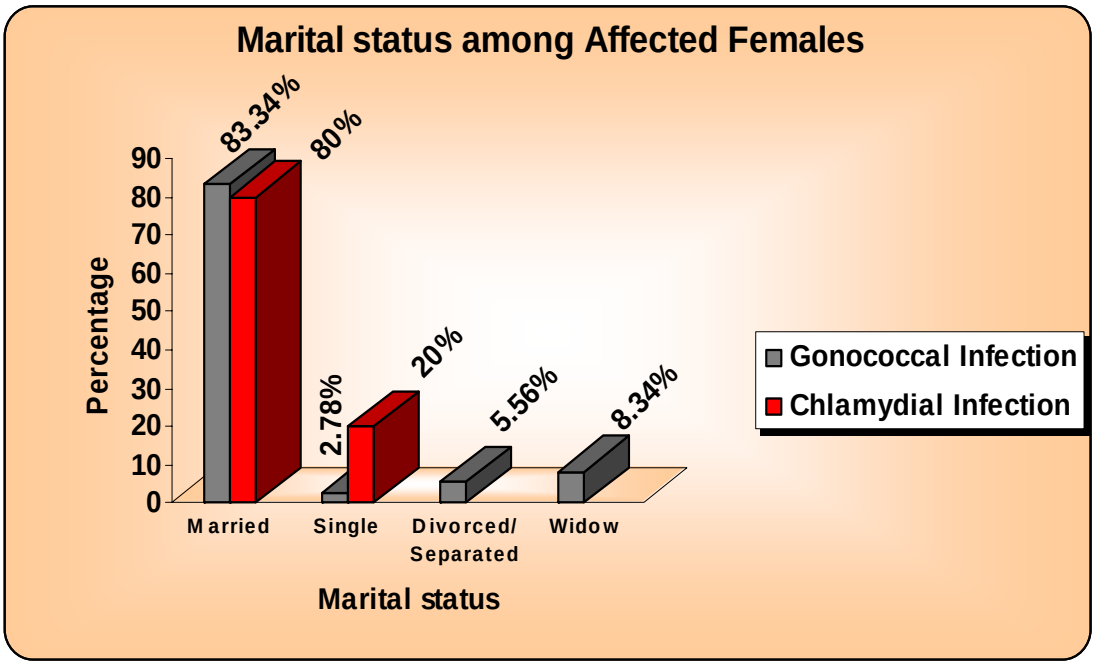

Socio Economic Status of Affected Males
Monthly Income in Rupees
>5000
2000-4999
1000-1999
<1000
2.43%
2.43%
10%
12.19%
90%
82.92%
0
20
40
60
80
100
Percentage
Chlamydial Infection
Gonococcal Infection

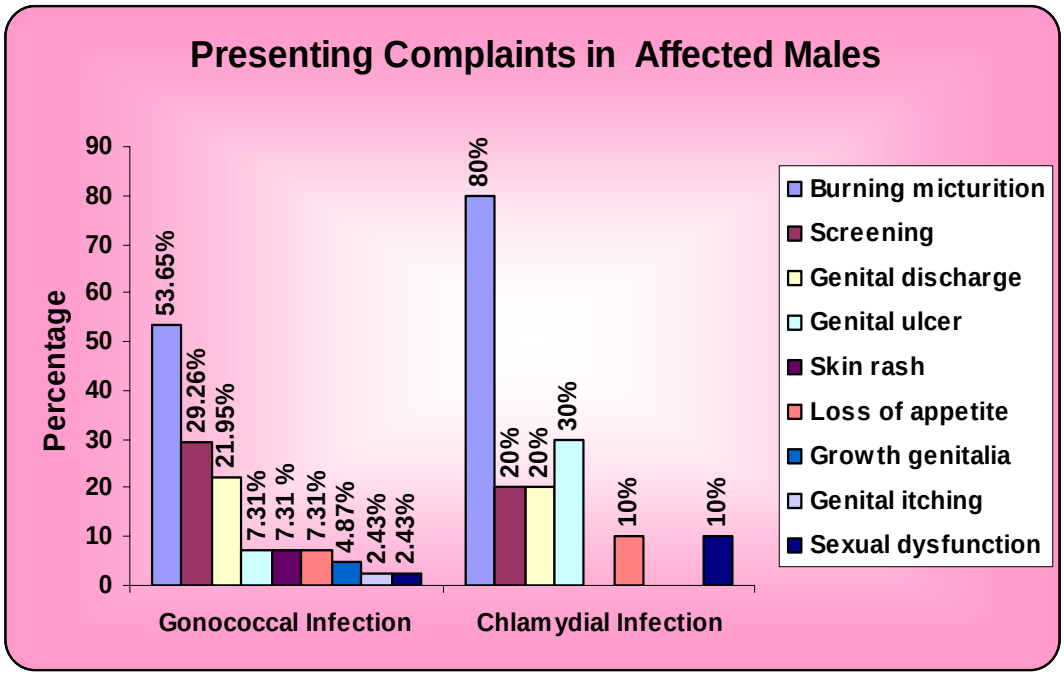

Socio Economic status of Affected Females
Monthly Income in Rupees
>5000
2000-4999
1000-1999
<1000
2.78%
2.78%
20%
16.67%
80%
77.78%
0
20
40
60
80
100
Percentage
Chlamydial Infection
Gonococcal Infection

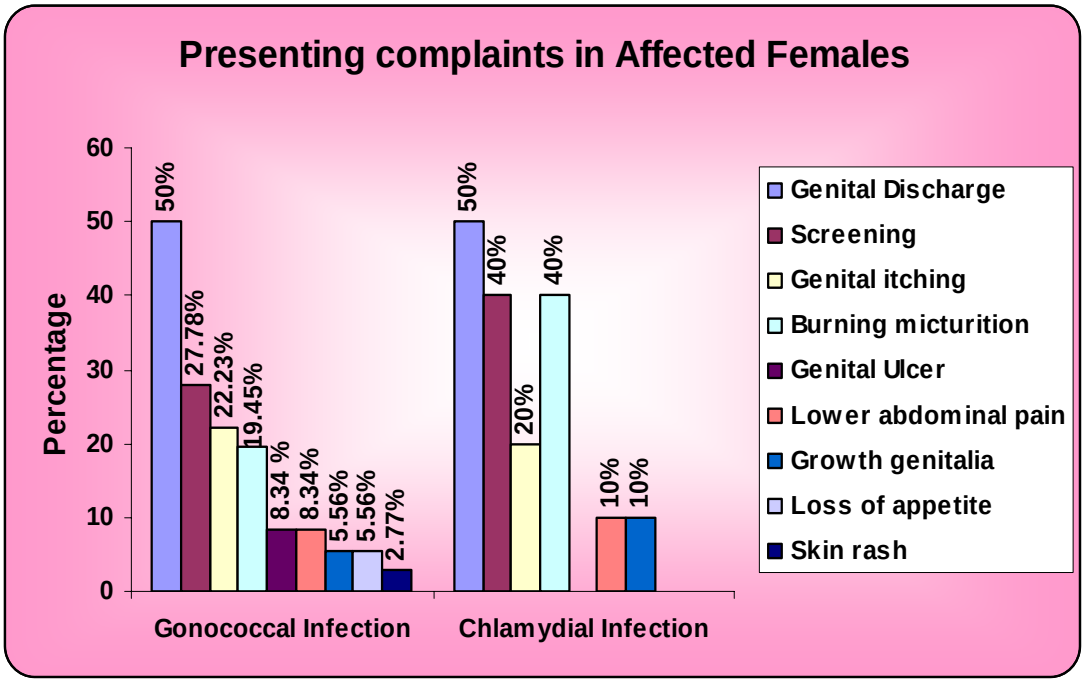

Educational status among Affected Males
Percentage
50
40
30
20
10
0
19.51%
10%
29.26%
30%
46.34%
40%
4.87%
20%
Uneducated
1-5th
6-10th
11-12th
College
Educational Status
Gonococcal Infection
Chlamydial Infection

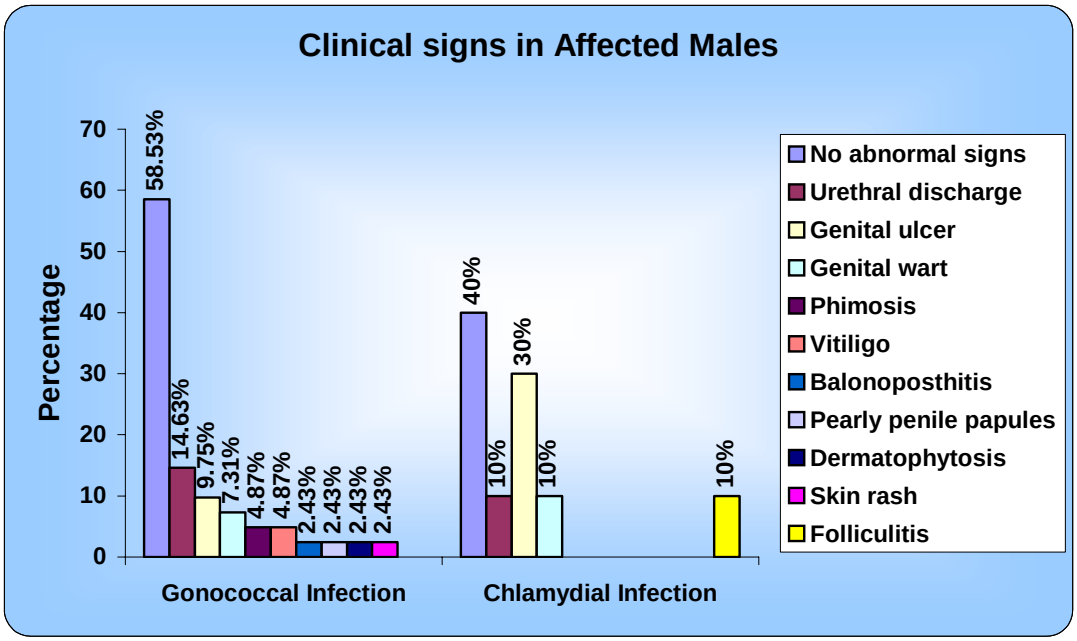

Educational status among Affected Females
Percentage
50
45
40
35
30
25
20
15
10
5
0
22.23%
20%
27.78%
30%
47.23%
30%
2.78%
20%
Uneducated
1-5th
6-10th
11-12th
College
Educational status
Gonococcal Infection
Chlamydial Infection

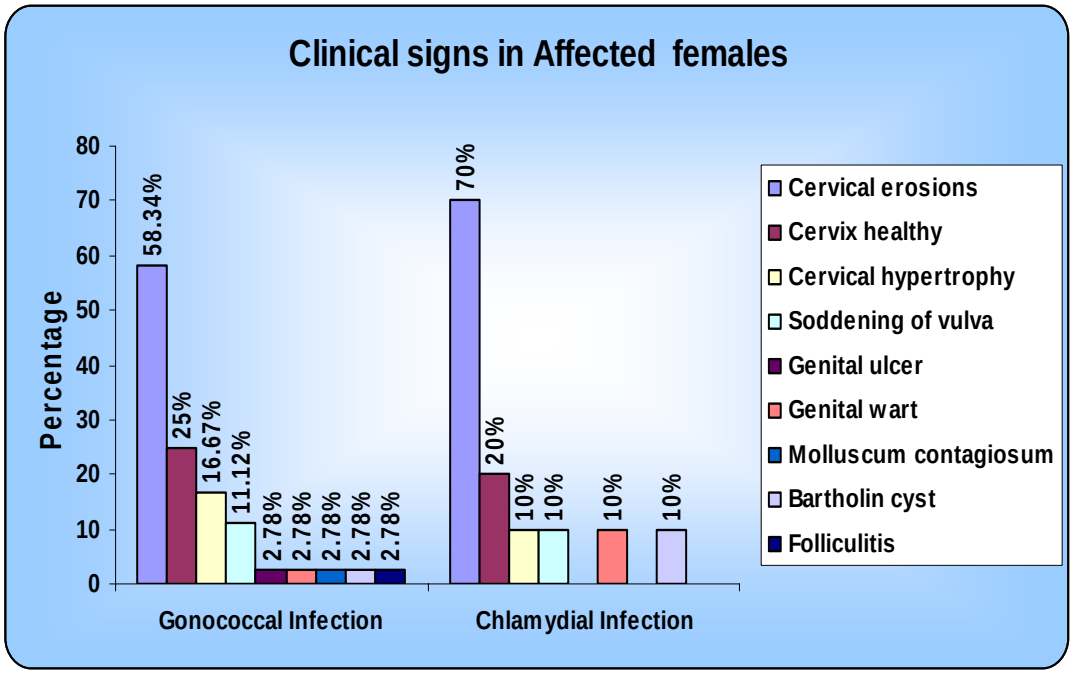

Marital status among Affected Males
53.65%
70%
46.34%
30%
70
60
50
40
30
20
10
0
Percentage
Married
Single
Divorced/
Separated
Marital status
Gonococcal Infection
Chlamydial Infection

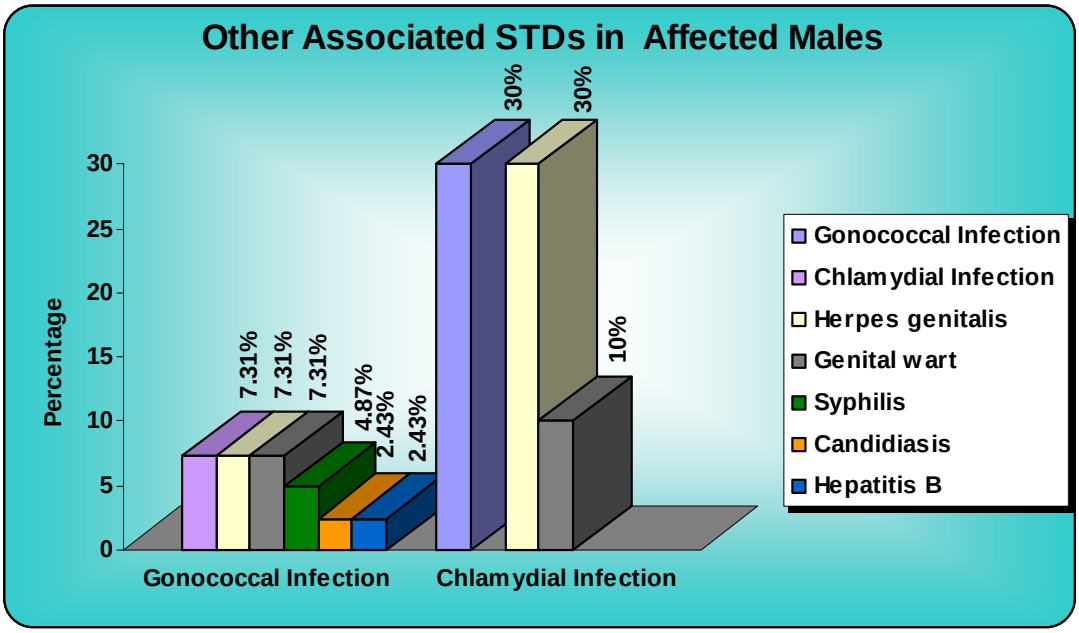

Marital status among Affected Females
83.34%
80%
2.78%
20%
5.56%
8.34%
90
80
70
60
50
40
30
20
10
0
Percentage
Married
Single
Divorced/
Separated
Widow
Marital status
Gonococcal Infection
Chlamydial Infection

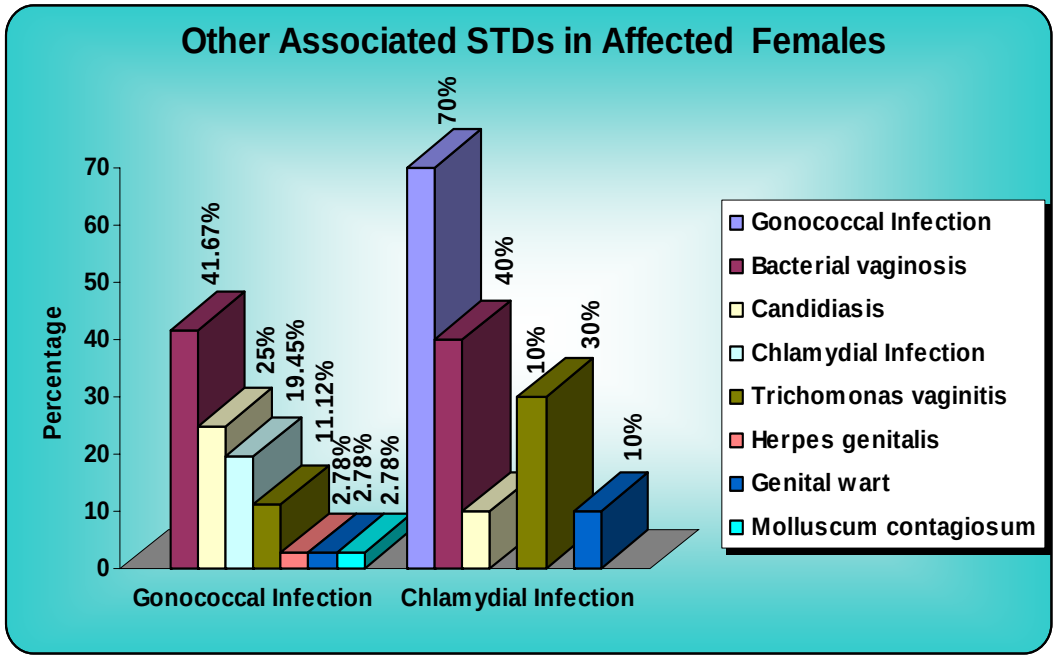

Presenting Complaints in Affected Males
Percentage
90
80
70
60
50
40
30
20
10
0
53.65%
29.26%
21.95%
7.31%
7.31 %
7.31%
4.87%
2.43%
2.43%
80%
20%
20%
30%
10%
10%
Gonococcal Infection
Chlamydial Infection
Burning micturition
Screening
Genital discharge
Genital ulcer
Skin rash
Loss of appetite
Growth genitalia
Genital itching
Sexual dysfunction


Presenting complaints in Affected Females
Percentage
60
50
40
30
20
10
0
50%
27.78%
22.23%
19.45%
8.34 %
8.34%
5.56%
5.56%
2.77%
50%
40%
20%
40%
10%
10%
Gonococcal Infection
Chlamydial Infection
Genital Discharge
Screening
Genital itching
Burning micturition
Genital Ulcer
Lower abdominal pain
Growth genitalia
Loss of appetite
Skin rash

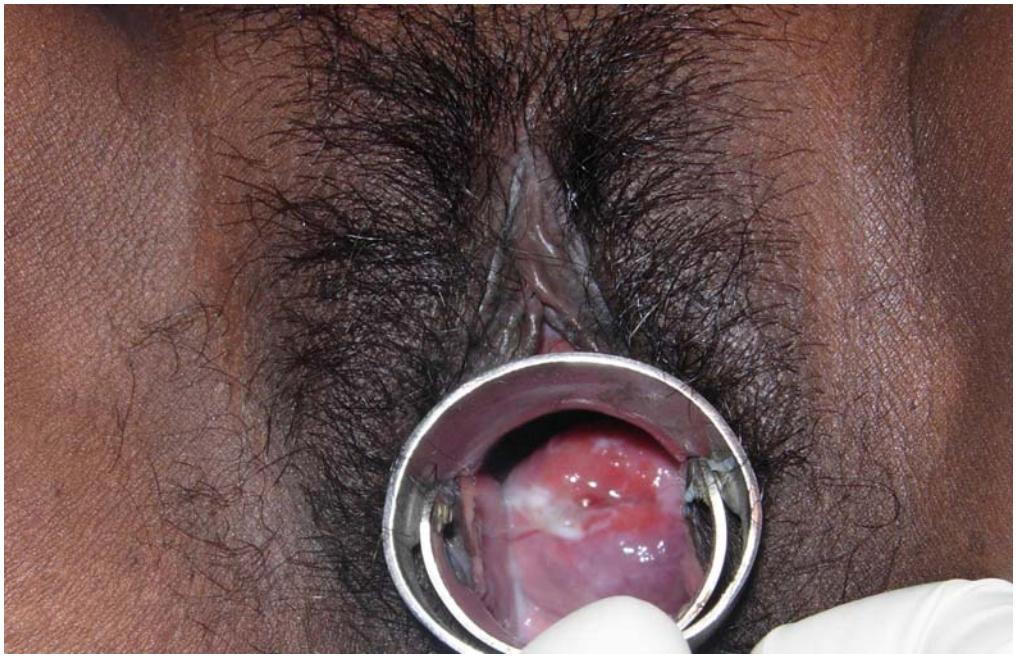

Clinical signs in Affected Males
Percentage
70
60
50
40
30
20
10
0
58.53%
14.63%
9.75%
7.31%
4.87%
4.87%
2.43%
2.43%
2.43%
2.43%
40%
10%
30%
10%
10%
Gonococcal Infection
Chlamydial Infection
No abnormal signs
Urethral discharge
Genital ulcer
Genital wart
Phimosis
Vitiligo
Balonoposthitis
Pearly penile papules
Dermatophytosis
Skin rash
Folliculitis


Clinical signs in Affected females
Percentage
80
70
60
50
40
30
20
10
0
58.34%
25%
16.67%
11.12%
2.78%
2.78%
2.78%
2.78%
2.78%
70%
20%
10%
10%
10%
10%
Gonococcal Infection
Chlamydial Infection
Cervical erosions
Cervix healthy
Cervical hypertrophy
Soddening of vulva
Genital ulcer
Genital wart
Molluscum contagiosum
Bartholin cyst
Folliculitis

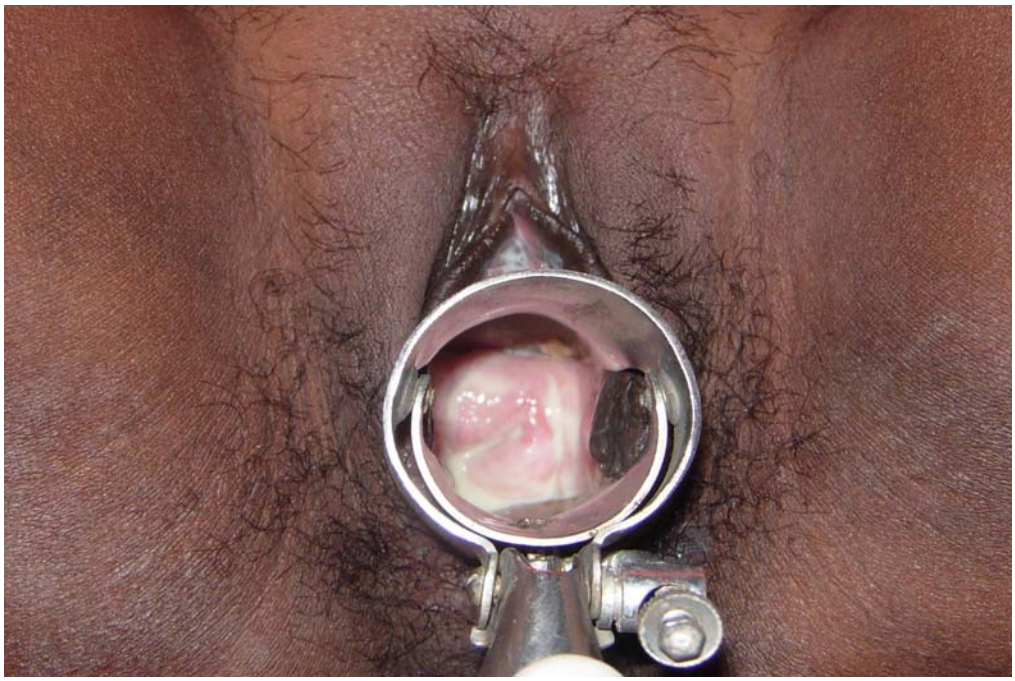

Other Associated STDs in Affected Males
Percentage
30
25
20
15
10
5
0
7.31%
7.31%
7.31%
4.87%
2.43%
2.43%
30%
30%
10%
Gonococcal Infection
Chlamydial Infection
Gonococcal Infection
Chlamydial Infection
Herpes genitalis
Genital wart
Syphilis
Candidiasis
Hepatitis B


Other Associated STDs in Affected Females
Percentage
70
60
50
40
30
20
10
0
41.67%
25%
19.45%
11.12%
2.78%
2.78%
2.78%
70%
40%
10%
30%
10%
Gonococcal Infection
Chlamydial Infection
Gonococcal Infection
Bacterial vaginosis
Candidiasis
Chlamydial Infection
Trichomonas vaginitis
Herpes genitalis
Genital wart
Molluscum contagiosum

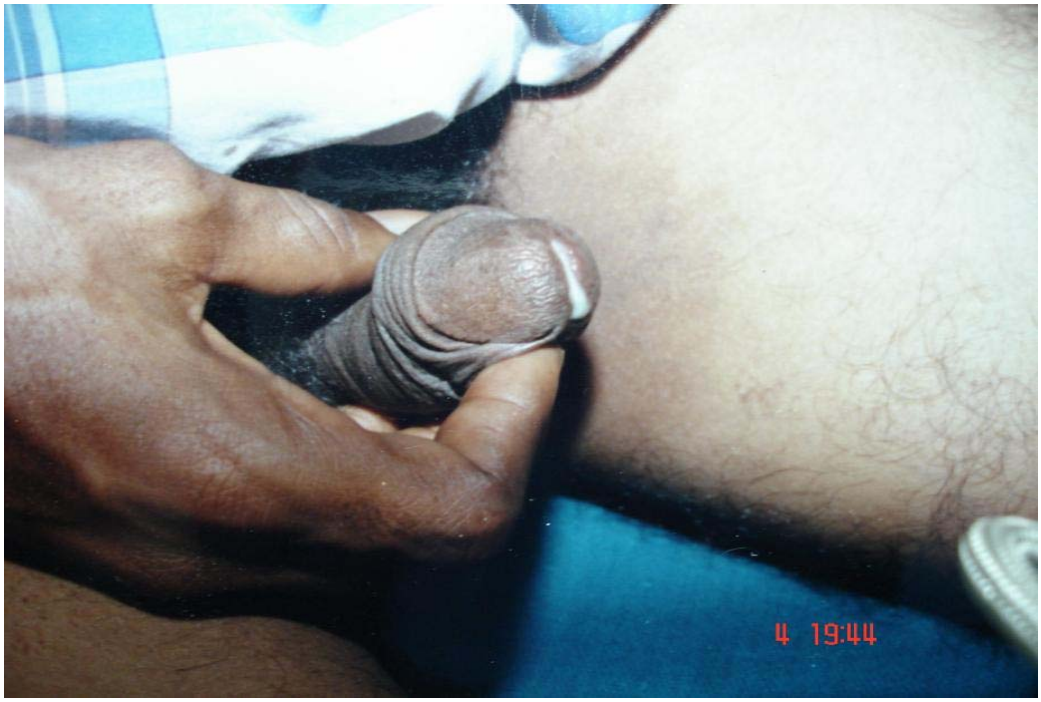
CERVICAL EROSION



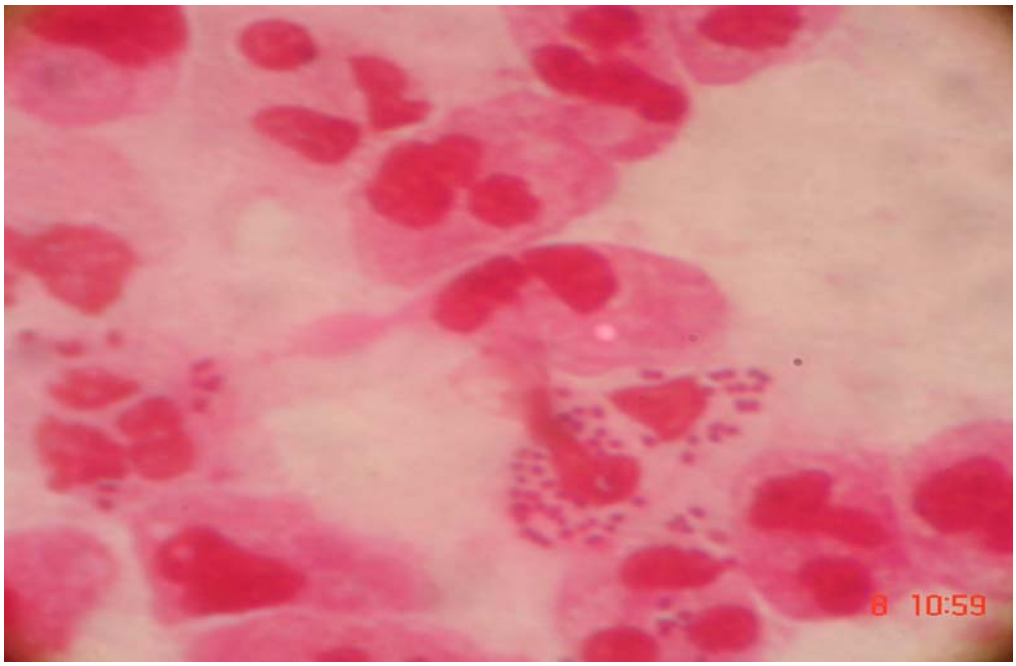
VAGINAL DISCHARGE



URETHRAL DISCHARGE



GONOCOCCI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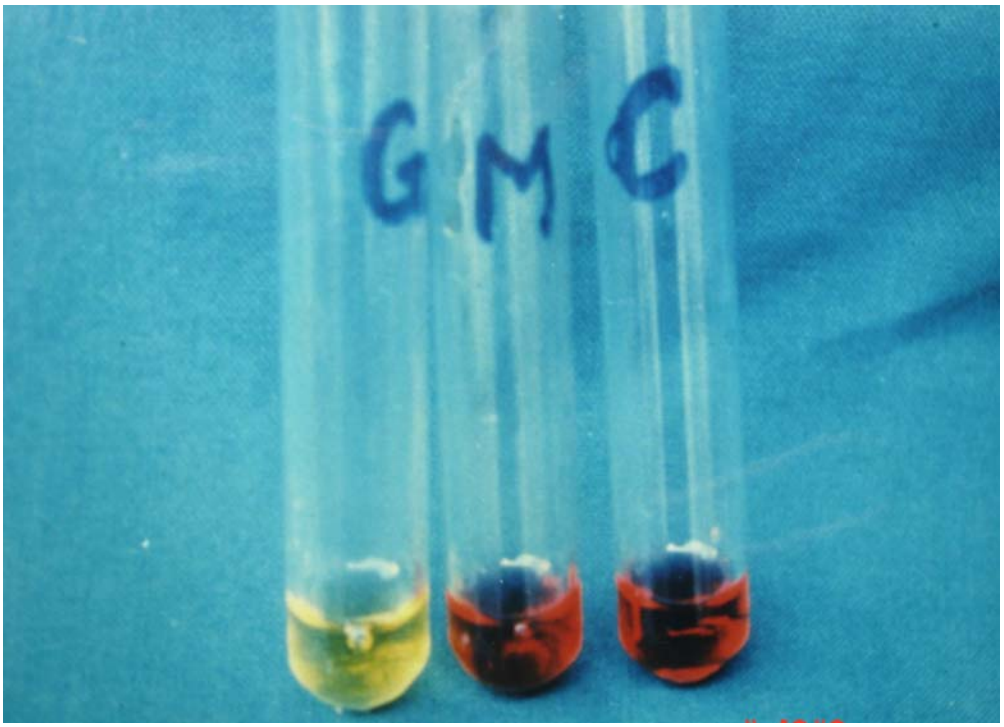
CULTURE FOR GONOCOCCI

SUGAR FERMENTATION-GONOCOCCI



IgM ELISA KIT

SPECIMEN COLLECTION KIT

SPECIMEN PREPARATION KIT


AMPLICOR CT/NG
Specimen Preparation Kit
CT/NG PREP
AMPLICOR®
CT/NG DIL
CT/NG DIL
CT/NG URINE WASH
CT/NG LYS

PIPETTES


20-200µl
1000µl
Nichipet EX
Nichipet EX
04.06.2007

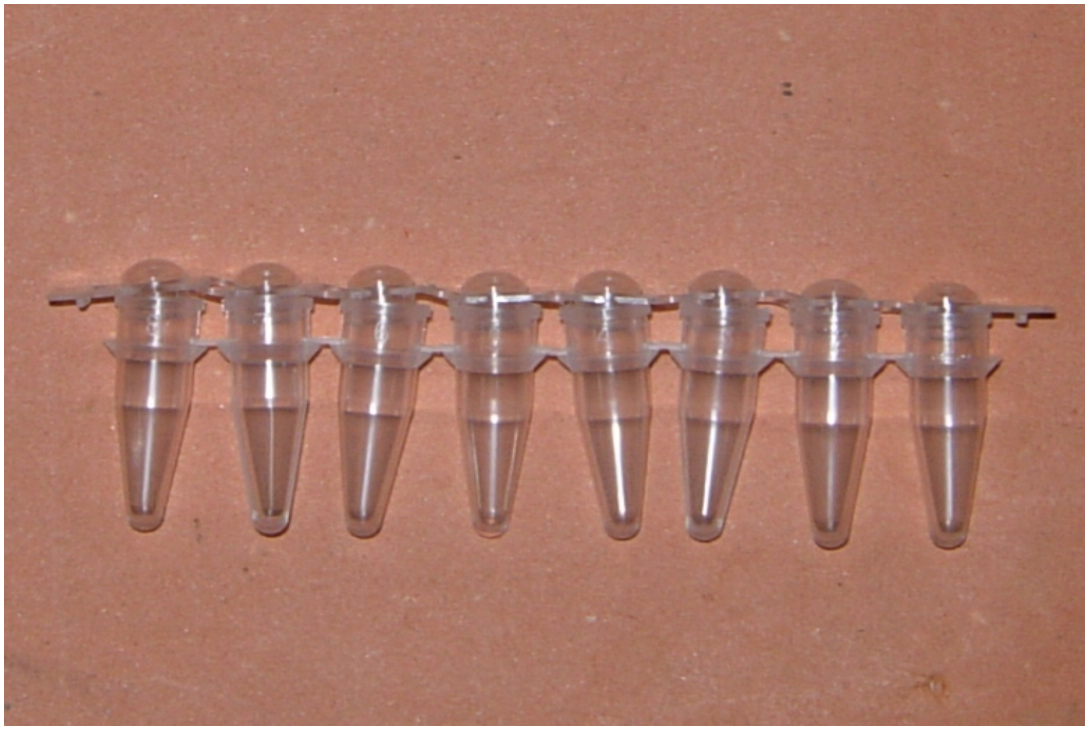
MICROCENTRIFUGE MACHINE

INCUBATOR


Thermostill-vx
TEMPERATURE
29.1
°C
POWER
OFF
ON
TIMER ON / OFF
TIME SET
03.12.2007 12:08

AMPLIFICATION KIT

PCR TUBES



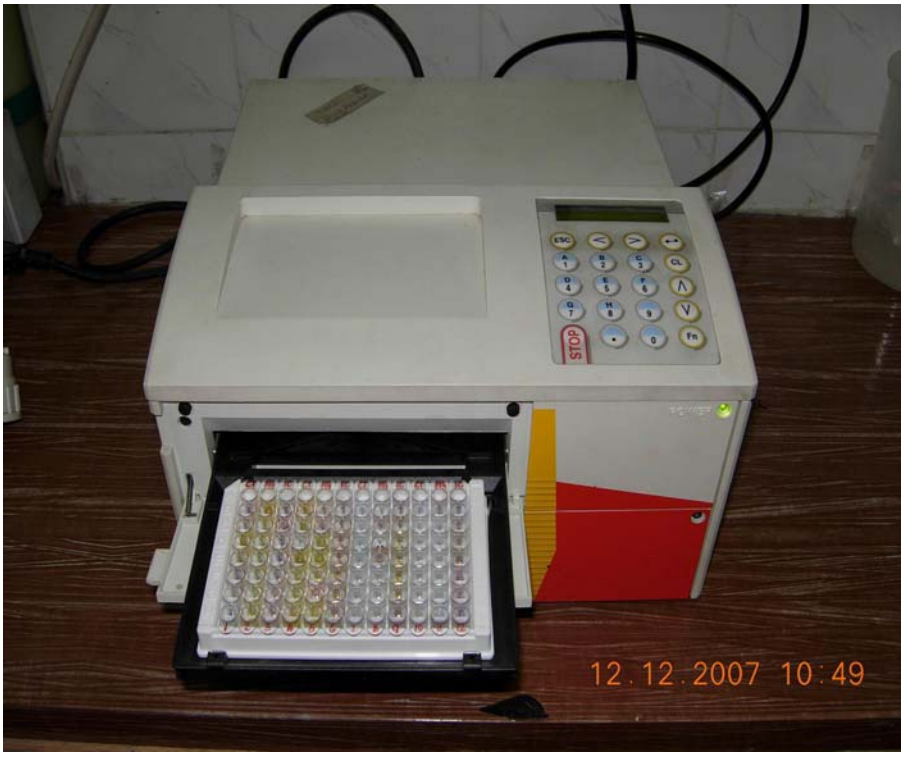
PCR THERMAL CYCLER

DETECTION KIT

ELISA INCUBATOR

MICROWELL PLATE WASHER

MICROWELL PLATE READER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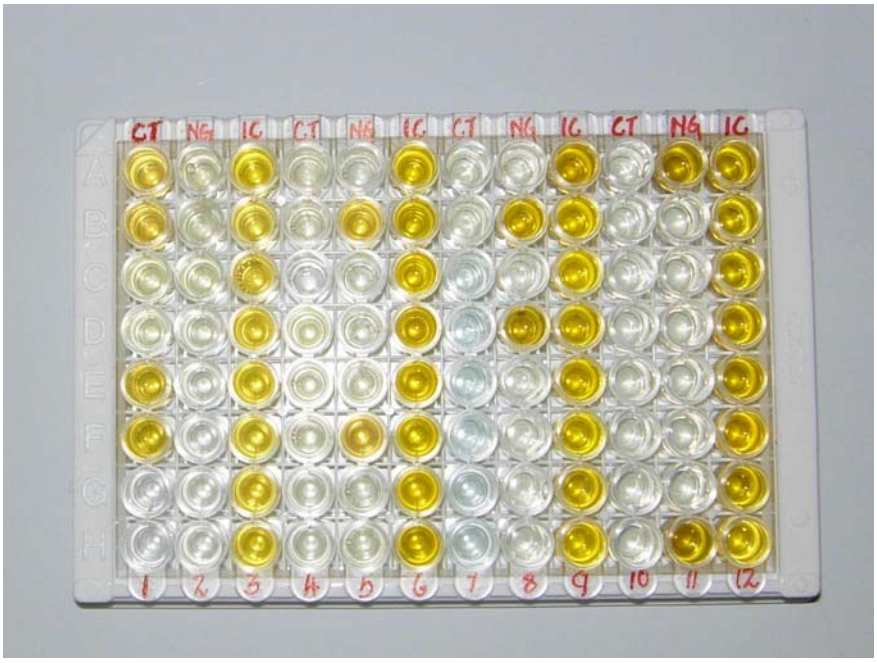
ELISA PLATE


CT NG IC CT NG IC CT NG IC CT NG IC
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BIBLIOGRAPHY

1. World health organization. Global Prevalence and Incidence of selected curable sexually transmitted infections:- Overview and Estimates, WHO, Geneva 2001.
2. Sexually Transmitted disease Surveillance 2006 supplement –GISP project Annual report 2006;Division of STD prevention April 2008,Department of Health and Human Services;Centers for Disease Control and Prevention.
3. Sexually Transmitted disease Surveillance 2006 supplement – Chlamydial Prevalence Monitoring project Annual report 2006;Division of STD prevention Revised May 2008,Department of Health and Human Services;Centers for Disease Control and Prevention.
4. World Health Organisation. Global Strategy for the prevention and care of STIs/RTIs– 2003 –2010. WHO DRAFT, 01, October 2003.
5. World Health Organisation Fifth –ninth world health assembly provisional agenta item 11.6 A59/11, 18 May 2006. Prevention and control of STIs; draft global strategy.
6. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines 2002. MMWR 2002;51 (No. RR-6)

7. StammWE, et al. Chlamydia trachomatis infections of the adult In: Holmes KK et al, (EDS). Sexually transmitted Diseases. Newyork, MC Graw- Hill, 1999: 407-422.
8. CDC, Recommendations, for the Prevention and management of Chlamydia trachomatis infections, 1993: MMWR 1993 –42 (No.RR-12): 1-39.
9. AkdumanD, et al Evaluation of a strand displacement amplification assay (BD Probe TCC- SDA), for detection of Neisseria gonorrhoeae in urine specimens. J. Clin microbiol 2002; 40; 281-283.
10. Page – Shafer K. et al. Increased Sensitivity of DNA Amplification testing for the detection of pharyngeal gonorrhoeae in men who have sex with men. Clin. Infec. Dis. 2002; 34; 173-176.
11. Livengood CH et al. Evaluation of COBAS AMPLICOR (ROCHE) accuracy in detection of Chlamydia trachomatis and Neiserria gonorrhoeae by Coamplification of Endocervical specimens: J clin microbiol; 2001; 39; 2929-2932.
12. World Bank.World Development Report; 1993; Investing in Health New York, Oxford University Press, 1993; 223.
13. Rajnarayan, et, al . Pattern of Sexually transmitted diseases in major hospital of Delhi. Indian J. Sex. Transm Dis 1996: 17: 76-78.

14. Chopra A, et al. Pattern and Changing trend of STDs at Patiala. Indian J. Sex. Transm Dis. 1999; 20: 22-25.
15. Jaiswal AK, et, al Pattern of sexually transmitted diseases in Jammu & Kashmir region of India. Indian J. Sex transm Dis 1998: 19: 113: 115.
16. Ranganayakulu B, et al Pattern of sexually transmitted diseases at Kurnool. Indian J. Sex Transm Dis 1998: 19: 117-121.
17. Khandpur S, Agarwal S, Kumar S, et al. Clinico epidemiological profile and HIV Seropositivity of STD patients. Indian J. Sex Transm Dis 2001: 22: 62-65.
18. Parmar J, Raval RC, Bilimoria FE. Clinical Profile of STDs at Civil Hospital Ahmedabad. Indian J Sex Transm. Dis 2001: 22:14-16.
19. Aggarwal K. Jain VK, Brahma D. Trend of STDs at Rohtak. Indian J. Sex Transm Dis 2002: 23: 19-21.
20. Divekar AA, Gogate AS, Shivkar LK, et al. Disease prevalence in women attending the STD clinic in Mumbai, India. Int J. STD AIDS 2000: 11:45-48.
21. Hook EW et al. Gonococcal infections. Ann Int Med 1985: 102: 229-243.
22. Judson FN. Gonorrhoeae. Med Clin North AM 1990: 74: 1353-67.

23. Hay RJ, Adriaanss BM. Bacterial Infection. In: Champion RH, Burton JL, Burns DA, Breathnach SM, eds. Rook Textbook of Dermatology oxford; Blackwell Sciences: 1998: p- 1140-1141.
24. Eschenbach DA, et al. Polymicrobial etiology of acute pelvic inflammatory disease. N. Engl J. Med 1975: 293: 166-171.
25. MC Cormack WM. Pelvic inflammatory disease. Nengl.J. Med 1994: 330: 115 –119.
26. Sherrard J. Modern diagnosis and management of gonorrhoeae Br. J. Hosp Med 1996: 55: 394-398.
27. Sackel SG, et al Orogenital contact and the isolation of Neisseria gonorrhoeae. Mycoplasma hominis and Ureaplasma Urealyticum from the pharnyx. Sex Transm Dis. 1979: 6: 64-68.
28. Rice PA, et al. Natural Serum bactericidal activity against. Neisseria gonorrhoea isolates from disseminated, locally invasive and uncomplicated disease. J. Immunol 1980: 124: 2105-2109.
29. Tice AW, et al. Pharyngeal gonorrhoea. JAMA 1981: 246: 2717-2719.
30. Donders GG, et al. The association of gonorrhoea and syphilis with premature birth and low birth weight. Genitourin Med 1993: 69: 98-101.
31. Philips DJ, et al. The sac –4 gene of Neisseria gonorrheoea and coexisting chlamydial infection. Sex Transm Infect 2000: 76: 400: 402.

32. Gastrin B, et al. Improved methods for gonococcal sampling and examination. Act a Pathol. Microbiol Scand 1968: 74: 362: 370.
33. Morse SA, et al: In Topley and Wilson's systemic Bacteriology, Microbiology and Microbial Infections Vol. 2 (Balows A, et al.Eds) 9th ed. London, Edward Arnold 1998: 877-900.
34. Chacko CW, et al. A new convenient egg enriched medium for the culture diagnosis of gonorrhoeae. Indian J. Dermatol Venereol Leprol 1966: 32;41.
35. Reichart CA, et al. Comparison of GC. Lect and Modified Thayer Martin medium for isolation of Neisseria gonorrhoeae. J. Clin microbiol 1989: 27; 808-811.
36. Schachter J et al,Enzyme immunoassay for diagnosis of gonorrhoeae.J Clin Microbiol,1984;19;57-59.
37. Granato PA, et al, use of the Gen-Probe PACE system for the detection of Neisseria gonorrhoea in urogenital sample. Diagn Microbiol Infect Dis 1990:13: 217-221.
38. Roymans R, et al. Evaluation of an in house polymerase chain reaction for detection of Neisseria gonorrhoeae in urogenital samples J. Clin Pathol 1999: 52: 411-414.

39. Martin DH, et al. Multicenter Evaluation of AMPLICOR and automated COBAS AMPLICOR CT /NG tests for Neisseria gonorrhoeae. J. Clin Mirobiol 2000: 38; 3544-3549.
40. Birkenmeyer J, et al. Preliminary evaluation of the ligase chain reaction for specific detection of Neisseria gonorrhoeae J. Clin Microbiol 1992: 30: 3089-3091.
41. Totten PA, et al. DNA hybridisation technique for the detection of Neisseria gonorrhoeae in men with urethrits. J Infect Dis 1983: 148: 462-471.
42. Gerbase Ac, et al. Global epidemiology of sexually transmitted diseases Lancet 1998: 351 (suppl.3);2-4.
43. Wang SP, et al. Immunologic relationship, between genital lymphogranuloma venereum, and related organisms in a new microtiter indirect immunofluorescence test. AmJ Opthalmol 1970; 70; 367-374.
44. Caldwell HD, et al. Antigenic analysis of the major outer membrane protein of Chlamydia Spp. Infect Immun 1982; 35; 1024-1031.
45. Oriel JD, et al .The lack of effect of ampicillin plus probenecid givenfor genital infections with Neisseria gonorrhoea on associated infections with Chlamydia trachomatis J Infect Dis. 1976; 133; 568; 571.

46. Centers for Disease Control and Prevention.Sexually transmitted diseases treatment guidelines 2002;MMWR-51(No.RR-6).

47. Martin DH, et al, Pre-maturity and perinatal mortality in pregnancies complicated by maternal Chlamydia trachomatis infections. JAMA 1982: 247: 1585 –1588.

48. Ripa KT, et al. Cultivation of C. trachomatis in cycloheximide treated Mccoycells. J. Clin Microbiol 1977: 5: 328-331.

49. Thompson C, et al. Evaluation of a microdot immnoflouroescent antigen detection test of Chlamydia trachomatis – Genitourin Med 1994; 70: 262-264.

50. Patel, JD, et al, Direct detection of Chlamydia trachomatis in clinical specimens by a dot immunobinding technique using monoclonal antibody. J. Immunol methods 1988; 108; 279-287.

51. Schachter JS, et al. Chlamydia. In: Manual of Clinical Microbiology (Murray PR, et al Eds.) Washington. DC, ASM Press 1999: 795-806.

52. Vogels WHM, et al. Chlamydia trachomatis infection in a high risk population; comparison of polymerase chain reaction and cell culture for diagnosis and follow up J. Clin Microbiol: 1993: 31: 1103: 1107.

53. Palladino S, et al. Diagnosis of Chlamydia trachomatis and Neisseria gonorrhoeae in genitourinary infections in males by the Amplicor PCR assay of urine. Diagn microbiol Infect Dis 1999; 33: 41-46.

54. Mahoney JB, et al. Multiplex PCR detection of Chlamydia trachomatis and Neisseria gonorrhoeae in genitourinary specimens. J. Clin microbiol 1995; 33; 3049-3053.

55. Peeling RW, et al. Pooling of urine specimens for PCR testing; a cost saving strategy for C. trachomatis control programmes. Sex Transm Infect 1998; 74; 66-70.

56. Schachter JS, et al. Non Invasive tests for the diagnosis of C. trachomatis infection of the cervix. Application of LCR to first catch urine specimens of women. J Infect Dis 1995: 172: 1411-1414.

57. Van Doornam GJ,et al.Detection of C.trachomatis infection in urine samples from men and women by Ligase Chain Reaction.J clin Microbiol,1995;33:2042-2047.

58. Ferrero DV, et al. Performance of Gen-Probe AmPLIFIED C-trachomatis assay in detecting Chlamydia trachomatis in endocervical and urine specimens from men attending sexually transmitted disease and family planning clinics. J. Clin Microbiol 1998; 38; 3230-3233:

59. Shah JS, et al. Novel ultrasensitive Qß replicase amplified hybridization assays for detection of C. trachomatis, J. Clin Microbiol 1995: 33; 2718-2724.

60. Chlamydia trachomatis IgM ELISA, CAIOBIOTECH INC; 10461 Austin Dr, Spring Valley, CA, 91978.

61. Cohen MS. Sexually transmitted diseases enhance HIV transmission; a hypothesis no longer. Lancet 1998; 351 (Suppl-III); 57.

62. Wasserheit JN. Epidemiological Synergy, interrelationships between HIV infection and other sexually transmitted diseases. Sex Transm Dis 1992;19;61-77

63. HoJL, et al. Neutrophils from human immunodeficency virus Seronegative donors induce HIV replication from HIV-infected patients mononuclear cells and cell lines; an in vitro model of HIV transmission facilitated by chlamydia trachomatis J. Exp. Med 1995; 181; 1493-1505.

64. Moss GB, et al Human Immunodeficiency virus DNA in urethral secretion in men; Association with gonococcal urethritis and CD4 T cell depletion J. Infect Dis 1995; 172; 1469-1474.

65. Polymerase Chain Reaction-From Wikipedia,the free encyclopedia.org/wiki/polmerase_chain _reaction.

66. Principle of Polymerase chain reaction-usersurgent.be/~avierstr /principle /pcr.html.
67. Information about PCR, types of PCR, -protocols and methods for PCR, Primer design, bioinformatics and databases for PCR. PCR station (www. Pcrstation. com).
68. Orle KA, et al. Simultaneous PCR detection of Haemophilus ducreyi, Treponema Pallidum and herpes simplex virus types 1 and 2 from genital ulcers. J.Clin Microbiol: 1996; 34;49:
69. AMPLICOR Chlamydia trachomatis / Neisseria gonorrhoeae (CT/NG) test – for invitro diagnostic use – Roche Molecular Systems, INC., Branchburg, NJ 08876 USA . A member of the Roche group.
70. Mullis, K.B.and Faloona, F.A.1987. Specific Synthesis of DNA in-vitro via a Polymerase –catalysed chain reaction methods in Enzymology 1553;335-350.
71. Miyada, C.G. and Bom, T.L. 1991. A DNA Sequence for the discrimination of Neisseria gonorrhoeae from other Neisseria species. Molecular and cellular probes 5; 327-335.
72. Longo, M.C., Berninger, M.S. and Hartley, J.L. 1990. Use of Uracil DNA glycosylase to control carry-over contamination in Polymerase chain reactions – Gene 93; 125-128.
73. Prevalence of Chlamydial and Gonococcal infections among young adults in the United States: William C. Miller, MD, PhD, MPH: JAMA. 2004; 291: 2229-2236.

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INSTITUTIONAL ETHICAL COMMITTEE
GOVERNMENT GENERAL HOSPITAL & MADRAS MEDICAL COLLEGE
CHENNAI-600 003

Telephone:044-25305000
Fax: 044-25305115

Lr.Dis.No. 3798/P&D3/Ethics/Dean/GGH/08 **Dated:** 15/02/08

Title of the Work : "A Study on the prevalence of chlamydia Trachomatis and Neisseria Gonorrhoeae using Polymerase chain Reaction"

Principal Investigator : Dr. M.S. Sridevi

Department : Institute of Venereology, MMC, Ch.3.

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 15/02/08 at the Conference Hall of the Dean, Tower Block I, Government General Hospital, Chennai-600 003.

The members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The principal investigator and their team are directed to adhere the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
4. You should not deviate form the area of the work for which I applied for ethical clearance.
5. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions.
6. You should abide the rules and regulations of the institution(s).
7. You should complete the work within the specific period and if any extension of time is required, you should apply for permission again and do the work.
8. You should submit the summary of the work to the ethical committee on completion of the work.
9. You should not claim funds from the Institution while doing the work or on completion.
10. You should understand that the members of IEC have the right to monitor the work with prior intimation.

SECRETARY
IEC, GGH, Chennai.

CHAIRMAN
IEC, GGH, Chennai.

DEAN
GGH &MMC, Chennai