Genital *Chlamydia Trachomatis* and *Neisseria Gonorrhoeae* among Reproductive Age Group Women Attending Jimma University Specialized Hospital: Prevalence and Risk Factors. Jimma, South West Ethiopia

By

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ABSTRACT

Back ground: *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections are the most common bacterial and treatable sexually transmitted diseases in both men and women. The reproductive outcome is more severe in women. If left untreated they may leads to various long term sequela including ectopic pregnancy, infertility, and pelvic inflammatory disease. Due to the asymptomatic nature of these infections, there is no clear picture about their prevalence. Therefore, the present study was aimed at determining the prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoea* infections and associated risk factors among reproductive age group women in Jimma.

Methods: *- A cross-sectional study was conducted from May to July, 2013 at Jimma University Specialized Hospital. A total 159 Women visiting the gynaecology outpatient department were included. Socio-demographic and selected risk factor were collected using semi structured and pre-tested questionnaire. Cervical swab specimens were collected for laboratory investigation. Chlamydia trachomatis was detected using Chlamydia antigen detection method (chromatographic test kit, Standard Diagnostics Inc, Korea). Modified Thayer Martin medium was used for isolation of Neisseria gonorrhoeae at 37^0c in 10% carbon dioxide rich atmosphere. Standard microbiologic technique was followed strictly. Percentage, frequencies and statistical association was computed. P-value of <0.05 was considered statistically significant.*

Results: *- One hundred and fifty nine women (15-49 years of age) with the mean age of* 25.60 years (SD ± 6.6), *attending the Gynaecology outpatient department were enrolled. Out of these 16.4% were positive for Chlamydia trachomatis and none were positive for Neisseria gonorrhoeae. More than half (66.0%) of the respondents were married and 28.9% and 16.4% were house wives and daily labourers, respectively. Among the study participant 79.2% did not have the habit of condom use during sexual activity, 76.7% had a history of pregnancy and 56.8% had no clinical symptoms. Level of education, occupational status and presence of clinical symptoms were significantly associated with Chlamydia trachomatis infection.*

Conclusion: - *Although no Neisseria gonorrhoeae isolate was found among symptomatic and asymptomatic women, there was high prevalence of Chlamydia trachomatis. Hence, further in-depth inquiry is required to implement screening of Chlamydia trachomatis among all reproductive age women who visit the gynaecological outpatient department regardless of the presence of clinical symptoms.*

 Key words*: - Chlamydia trachomatis, Neisseria gonorrhoeae, antigen detection, prevalence.*

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LIST OF ABBREVIATIONS

EB -   Elementary body

HIV -   Human Immune Deficiency Virus

JUSH- Jimma University Specialized Hospital

CD- Cluster of Differentiation

CDC-   Centre for Disease Control

DFA- Direct Fluorescent Staining

EIA-   Enzyme Immune Assay

IL-   Interleukin

LGV-   Lymphogranuloma venereum

MBL- Mannose Binding Lectin

NAAT- Nucleic Acid Amplification Test

OPD-   Out Patient Department

PCR- Polymerase Chain Reaction

PID-   Pelvic Inflammatory Disease

PMN- Polymorphonuclear Neutrophil

RB-   Reticular body

SOP-   Standard Operating Procedure

STI-   Sexually Transmitted Infection

WHO- World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 Back ground

Sexually transmitted infections (STIs) are major global causes of acute illness, infertility, long-term disability and death with serious medical and psychological consequences to millions of men, women and infants in the world. Genital Chlamydia, gonorrhoea, trichomoniasis, hepatitis virus, HIV and syphilis are among the common STIs (1, 2).

Now days there are over 30 bacterial, viral and parasitic pathogens that can cause sexually transmitted infection. Among these, *Chlamydia trachomatis (C. trachomatis)* and *Neisseria gonorrhoeae (N. gonorrhoeae)* are most prevalent throughout the world. Globally, there is an estimated annual incidence of 105.7 million cases of *C. trachomatis* and 106.1 million cases of *N. gonorrhoeae* (2).

Chlamydia trachomatis is an obligate intracellular Gram negative bacterium which belongs to the kingdom: Bacteria, Phylum: Chlamydiae, Class: Chlamydiae, Order: Chlamydiales, Family: Chlamydiaceae, Genus: Chlamydia. *C. trachomatis* inhabits mucosal surfaces, particularly infecting non-ciliated columnar, cuboidal, or transitional epithelial cells (3).

N. gonorrhoeae is a Gram negative fastidious, diplococcic that can grow and rapidly multiply in the mucous membranes of the mouth, throat, and anus of male and females, and the cervix, fallopian tubes, and uterus of the female reproductive tract. It is classified under the kingdom: bacteria, phylum: proteobacteria, class: beta proteobacteria, order: Neisseriales, family: Neisseriaceae, genus: Neisseria (3).

Infections with *C. trachomatis* and *N. gonorrhoeae* cause more damage and subsequent consequences to reproductive health of women than men. Mostly due to their asymptomatic nature, genital Chlamydia and gonorrhoea remain undetected and untreated. This results in complication leading to pelvic inflammatory disease, ectopic pregnancy, tubal factor infertility and adverse pregnancy outcomes (4, 5). In addition it result in hidden the actual prevalence of these two bacterial infections (6). Their prevalence and distribution are determined by various individual and social factors, cultural values, geography, demography, economics, health service, and political and legal structures (4, 7).

In order to prevent and control *C. trachomatis* and *N. gonorrhoeae* infection, it is important to identify and treat women both with and without symptomatic or women with mild infections; which are at increased risk for acquisition of this infection. Early diagnosis and treatment is the most cost effective measure of preventing long term sequela. In addition it is helpful to have clear picture of their prevalence (4). Diagnosis of *C. trachomatis* can be achieved by various techniques including cell culture, direct immunofluorescence, enzyme immune assay, direct DNA hybridization, most recently nucleic acid amplification test (NAATs) and immunochromatographic rapid tests . Whereas, *N. gonorrhoeae* can be diagnosed by culture NAATs (8).

In most developing countries the diagnosis relies only on syndromic approaches without laboratory support. This has increased miss diagnosis of asymptomatic infections as negative. Due to their affordability and the need for no big laboratory setup, rapid Chlamydia detection testes are method of choice to diagnose women of risk group like patients at gynaecological outpatient attendees (9, 10).

Being the most common organism the asymptomatic nature of infection with *C. trachomatis* and *N. gonorrhoeae* remain undetected and untreated resulting serious complications in women including fatal ectopic pregnancy, PID and adverse pregnancy outcomes. This indicates there is a need to screen women of reproductive age who are at risk of acquiring the infections. To decide whether or not to apply screening program in developing countries where advanced laboratory is not affordable; knowing the magnitude of the disease distribution in specific locality is immensely important for evidence based decision making purpose in different group of women including gynaecological outpatient attendees using inexpensive methods.

1.2 Statement of the problem

The classical bacterial STIs have not been given emphasis as public health priority and control efforts have contributed little to their prevention (11).

According to CDC report in February 2013 based on the estimate in 2008 there are about 110 million STI cases in United State. Out of which 59,569,500 are women. In the country there are about 20 million new STI infections each year. Of these estimate *C. trachomatis* and *N. gonorrhoeae* accounts 2, 860, 000 and 820,000 respectively (12).

Among population of adults between ages 15-49, years World health organization estimated number of cases of curable STDs in WHO regions of the world, in 2008. In WHO European Region, comprising 53 countries with and estimated population of 450.8 million, it was estimated that 17.3 million adults were infected with *C. trachomatis*, 1.0 million with *N. gonorrhoeae*. In South-East Asia Region, covering 11 countries with an estimated population of 945.2 million adults, it was estimated that there was 8.0 million cases of *C. trachomatis*, and 9.3 million *N. gonorrhoeae*. There were estimated cases of 3.0 million *C. trachomatis*, and 1.0 million *N. gonorrhoeae* in Eastern Mediterranean Region containing 23 countries with an estimated of 309.6 million. The WHO Western Pacific Region covers 37 countries with an estimated population of 986.7 million adults. Prevalence was estimated that 37.8 million adults were infected with *C. trachomatis*, 13.3 million with *N. gonorrhoeae*. In WHO African region with estimated population of 385.4 million adults between ages 15-49 there are about 92.6 million cases of curable sexually transmitted disease reported. Among these there were estimated cases of 9.8 million C. *trachomatis* and 8.2 million N. *gonorrhoeae* (2).

Infection *C. trachomatis* is the most common bacterial STI worldwide and has long been cause of mild to chronic infection. About 50-70% of infected individuals remain asymptomatic. Undiagnosed and untreated Chlamydia can ascend to the upper genital tract, where they colonize the endometrial mucosa and the fallopian tubes in women resulting in disease sequela. Up to two thirds of cases of tubal-factor infertility and one third of cases of ectopic pregnancy may be attributable to undiagnosed and untreated infection. Approximately 20% of women with PID later have decreased fertility (13-15).

Chlamydia during pregnancy is associated with: preterm labor, prolonged rupture of the membranes, low birth weight, neonatal death, and postpartum endometritis (16, 17). An infant born to a mother with active infection has a 50 to 75% risk of acquiring infection at any anatomical site. About 30 to 50% will have conjunctivitis, and at least 50 percent of infants with chlamydial conjunctivitis will also have nasopharyngeal infection. Chlamydial pneumonia develops in about 30 percent of infants with nasopharyngeal infection (15, 18, 19).

The asymptomatic nature of this bacterium has forced many countries, specially developed ones, to implement screening of reproductive age women. Importance of screening has been demonstrated in areas where screening programs reduced both the prevalence of *C. trachomatis* and rates of pelvic inflammatory disease. However, it is important to have prevalence estimate of the infection in women to decide whether to implement screening. Because of test acceptability and their high risk to infection made women who attend health care facilities are an important choice to get prevalence estimate among women (20-22).

On the other hand, though gonococcal infections have existed as STD for so long; it has never been regarded as intractable disease. Gonococcus can also be vertically transmitted during labor and is still a leading cause of infectious neonatal blindness in the developing world. It remains a major public health problem due to rapid acquisition of resistance to multiple antibiotics (23, 24).

In Ethiopia there are various reasons for increased prevalence of STIs; increased population migration, high prevalence of unprotected sex with multiple partnerships, gender inequality and poverty are among many. In addition the diagnosis of STIs is based on syndromic approach since 2001. Also very little epidemiological studies have been documented (25-27).

To date there is scarcity of information on the prevalence of the C. *trachomatis* in Ethiopia and there are only few studies conducted on the prevalence of *N. gonorrhoeae* (27). To the best of our knowledge, there is no study conducted on the prevalence of C. *trachomatis* and *N. gonorrhoeae* infection in Jimma among reproductive age women except one study conducted in 2006 on sero-prevalence of *C. trachomatis* antibody (28). So it is imperative to undertake this study to determine the prevalence of infection with C. *trachomatis* and *N. gonorrhoeae*.

1.3 Significance of the study

Infection with *C. trachomatis* and *N. gonorrhoeae* can lead to severely complicated infection of the reproductive tract and adverse pregnancy outcomes. Early detection and treatment of symptomatic and asymptomatic women is necessary to avoid unnecessary complication. Research gap exists whether to support or avoid routine *C. trachomatis* screening program among women in Ethiopia. There is no evidence on the burden of both infections among reproductive age group women in Jimma in particular. Therefore this study helps as a base line prevalence of *C. trachomatis* and *N. gonorrhoeae* infection and associated risk factors among the reproductive age group women. It will also give evidence to policy makers whether to consider screening program for these pathogens is needed.

CHAPTER TWO: LITERATURE REVIEW

2.1 *Chlamydia trachomatis*

2.1.1 *History of Chlamydia trachomatis*

Chlamydias were found in 1907 by German radiologist Ludwig Halberstadter and the Austrian zoologist Stanislaus Von Prowazek in Java, Indonesia. They considered these organisms were responsible for trachoma, which was a global disease at that time. Originally, they were considered neither protozoa nor bacteria, and then regarded as viruses. In the 1960s, they were recognized as bacteria (29).

In 1999, the order *Chlamydiales* was reclassified and the family *Chlamydiaceae* is now divided in two genera, *Chlamydia* and *Chlamydophila*. The genus *Chlamydia* comprises the species *Chlamydia trachomatis* (pathogen of man), *Chlamydia suis* (swine) and *Chlamydia muridarum* (hamsters and mice). The *Chlamydophila* include the species, *Chlamydophila pneumoniae* (man) *Chlamydophila* abortus (ruminants and swine), *Chlamydophila pecorum* (ruminants, swine and marsupials), *Chlamydophila felis* (cats), *Chlamydophila psittaci* (birds and poultry) and *Chlamydophila caviae* (guinea pigs) (29).

Based on monoclonal antibody typing of the major outer membrane proteins (MOMP), there are about 18 serological variants (serovars) of C. trachomatis. These include, A, B, Ba and C which are the causative agents of trachoma, the leading cause of infectious blindness worldwide. Serovars Ba and C are also rarely associated with urogenital infections. Serovars D to K, Da, Ia, Ja and rarely Ba and C are responsible for sexual transmitted diseases worldwide. The lymphogranuloma venereum (LGV) serovars L1-L3 and L2a, along with serovars D and G, are prevalent in anorectal infections unlike other genital serovars (30).

2.1.2 *Microbiology of Chlamydia trachomatis*

Chlamydia trachomatis is an obligate intracellular gram-negative bacterium, whose cellular biosynthetic capacity is much more limited even than the reckettsia. It was originally thought that it was "energy parasite", obtaining not only biosynthetic intermediate but also adenosine triphosphate (ATP). But the finding of gene sequence encoding protein for ATP synthesis put this hypothesis under question mark. The bacteria show a characteristic biphasic developmental cycle: metabolically inert elementary bodies (EBs) and actively dividing

reticulate bodies (RBs) which thrive within a host-derived vacuole termed as inclusion. The infection cycle starts with the entry of the infectious particle (EB) into an epithelial cell. EB-laden cytoplasmic vacuole migrates to the peri-Golgi region. Then it differentiates into a non-infectious but metabolically active RB. After replication, progeny RBs differentiate back to EBs for exiting (exocytosis) the infected cells and disseminate to adjacent cells (3, 30).
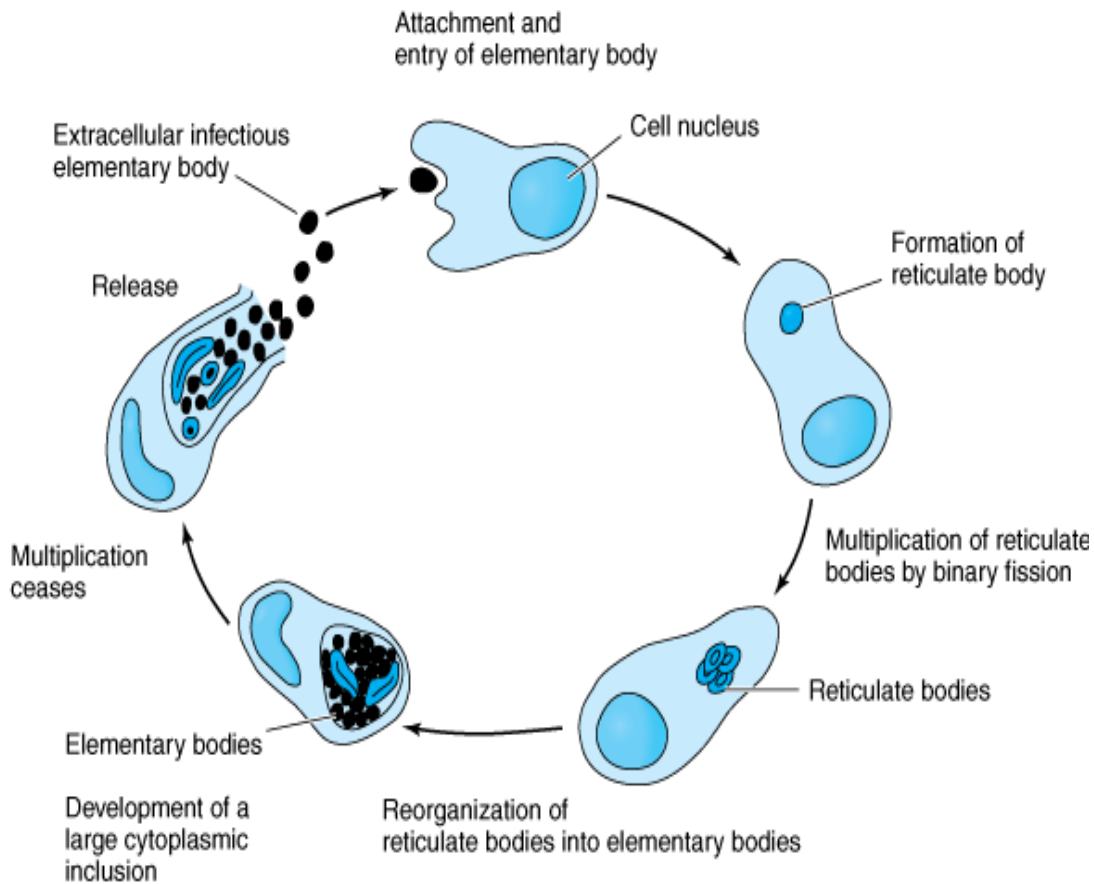
Figure 1. Life cycle of Chlamydia trachomatis

(Adopted from: Levinson W: Review of Medical microbiology and immunology 10th edition: Http://www.accessmedicine.com)(31).

2.1.3 *Pathogenesis and immunity*

Although the pathologic outcome of *C. trachomatis* infection is well established; the mechanism of pathogenesis is still unrevealed in most part. The pathogenesis thought to be attributed to the bacteria biological makeups (virulence factors) including: Polymorphic outer membrane protein (B, D, and H which are strongly immunogenic having the capacity to elicit pro-inflammatory cytokine response), Chlamydial type three secretion system (promote the delivery of organism effector protein in to the target cell), and Chlamydial stress response protein which contribute for inflammation and cytotoxicity but, absent in LGV strain. On the

other hand the host immune response to the intracellular organism is important factor thought to contribute to the pathogenesis (22, 32, 33).

There are two paradigms on pathogenesis of *C. trachomatis*. Richard Stephens first theorized "cellular paradigm of *C. trachomatis* pathogenesis" that states, "the inflammatory process of chlamydial pathogenesis are sufficient to account for chronic inflammation and the promotion of cellular proliferation, tissue remodelling and scaring which is the ultimate cause of disease sequela" in other word the production of cytokines , including IL-1 and IL-8, in response to infection causes tissue destruction leading to the development of tubal infertility and ectopic pregnancy. This idea is strongly supported by the finding that, activation of inflammatory mediators from non-immune host cells infected in vitro *(32, 34)*.

"Immunological paradigm", according to the Macaque and his colleague model, reinfection results in rapid infiltration of the lymphocytes which will result in the formation of lymphoid follicle and notable tissue destruction. Subsequent infection of the pocket will result reactivation of lymphocyte with focal area of epithelial destruction and the formation of fibrosis resulting in extension of follicle formation in the deep stroma. Nevertheless the exact mechanism still needs proof. Also in this case direct inoculation of non human primate has shown genital tract destruction increased with repeated infection which is mediated by immune cell. But we should not forget they are naturally different and the result might be cumulative effect (32, 34, 35).

Concerning immunity against *C. trachomatis* infection, epithelial cells of the reproductive tract are the first line of defence. Initial infection of epithelial cells causes a cascade of events leading to the increased production of pro-inflammatory cytokines and chemokines including IL-1, IL-8, IL-12, IL-6, and GM-CSF and cell adhesion molecules which induce recruitment of innate immune cells such as natural killer cells, dendritic cells (DCs), and neutrophils. In addition to epithelial cell innate immune response involved the mannose binding lectin (MBL). This plays early microbial detection and blocks the attachment of organisms to host cells. Its activity has been shown in the increase susceptibility of host with defective MBL (35, 36).

Though it is not protective against re-infection Chlamydia responsive CD_4^+ Th-1, IFN-γ producing cell plays indispensable roll in limiting disease progression. Also CD_8 T cell has been shown to protect against genital infection in mice. There is significant data demonstrating

that both CD_4^+ and CD_8^+ T cells are involved in controlling *C. trachomatis* infection via the secretion of interferon gamma. Recently it has been shown that examination of cytobrush samples from the endocervix demonstrated that women infected with C. trachomatis had an increase in CD_3^+, CD_4^+ and CD_8^+ cells, and neutrophils and an increase in recruitment of myeloid and plasmacytoid DCs (32, 37).

2.1.4 *Clinical feature*

The predominant clinical features include urethritis, cervicitis and proctitis. In pregnant women can predispose to low birth weight, preterm delivery. After delivery it can lead to endometritis in the mother and conjunctivitis and pneumonia in infants (19). Some of the clinical manifestations are:-

Cervicitis:-It is the most common infection in women and it is generally asymptomatic. Patients report nonspecific symptoms such as vaginal discharge or postcoital bleeding speculum examination may reveal mucoprulant cervical discharge, eroded feable appearance or may appear normal. **Urethritis: -** Women with acute infection may complain of dysuria slight discharge in urine or urinary frequency. It is asymptomatic and women may notice her symptom in suprapubic area and occurs after voiding is finished (19).

Lymphogranuloma venereum (LGV):- is caused by serovar L1, L2, or L3, typically presents with 1 or more genital ulcers or papules, followed by the development of unilateral or bilateral, fluctuant, inguinal lymphadenopathy (buboes) LGV proctitis, a less common form of LGV that occurs in both men and women, may present with rectal ulcerations, purulent or sanguineous anal discharge, tenesmus, and lower abdominal cramping or pain. Prolonged infection may result in perirectal abscesses, anal fissures, fistula formation, and such constitutional symptoms as fever, malaise, generalized fatigue, and weight loss (19, 36).

Fitz-Hugh-Curtis syndrome: - inflammation of the liver capsule associated with genital tract infection due to ascension of the causative organism to the upper genital tract and dissemination to the liver. It occurs in up to one fourth of patients with PID. Classically present as sharp, pleuritic right upper quadrant pain, and usually accompanied by signs of salpingitis. This syndrome is also caused by gonoccocal infection which was primarily considered as the only cause (38, 39).

2.1.5 *Epidemiology of Chlamydia trachomatis*

Chlamydia trachomatis is one of the most prevalent STI in the world. The significant concern regarding Chlamydia is that 50-70% of infected women are asymptomatic. There is a high prevalence of co-infection with other STIs in untreated infection about 50%. After lower genital tract Chlamydia, risk of developing PID infection varies considerably and is up to 30% (40). Repeat or multiple infections increase the likelihood of long term sequela, with a 2 to 4.5 fold increase in the risk of ectopic pregnancy, a 4.5–6.4 fold increase in the chance of PID development and may result in miscarriage (34, 41-43).

2.1.5.1 *Prevalence of Chlamydia trachomatis*

There are various studies done to determine the distribution of *C. trachomatis* among reproductive age women. Most studies conducted on women are institution based and have been source of prevalence. A study conducted from 2000 – 2009 in Italy on sexually active women age 15-55 years of age who underwent testing for endocervical *C. trachomatis* showed the mean prevalence of 5.2% (44).

A study conducted in India on gynaecologic out patients for the evaluation of in-house PCR assay for detection of *C. trachomatis* has revealed of prevalence 23.0% (45). A 14.3% (40/280) prevalence was found in a research on the prevalence of *C. trachomatis*, *Ureaplasma parvum* and *Ureaplasm urealyticum* in Japan (46). A cross sectional study done on married women in the middle east community, attending primary and secondary care centres including in a large nationwide cervical abnormalities screening survey found prevalence of 2.6% (47). Another study entitled "Detection of *C. Trachomatis* among infected women", which was conducted in Saudi Arabia using IgA detection, and ELISA revealed a prevalence *C. trachomatis* to be 25% and 20% respectively (48).

In 2002, Fallah *et.al* investigated a group of women with cervicitis in Tehran, Iran and found the prevalence of 9 % (49). In study done in Mosul, Iraq, the prevalence among symptomatic and asymptomatic women was 15.9% and 18.7% respectively. The overall prevalence was 17% (50). A study conducted in Sabzevar, Iran in on pregnant women has shown the prevalence of 15.81 % (51).

A study evaluated the prevalence of *C. trachomatis* in Nigeria among 164 women between 14 and 45 years of age found out 92 (56.1%) tested positive (52). Study conducted on pregnant

women in Benin City Nigeria showed a prevalence of 13.3% (53). Another study in Nairobi Kenya found infection rate of *C. trachomatis* infection to be 6.0% (54). Study conducted in Addis Ababa on has shown the prevalence of 1.2% (55).

2.1.5.2 *Chlamydia trachomatis prevalence and associated risk factors*

The infections with *C. trachomatis* vary in women with different age group; different studies have shown that women of younger age have higher prevalence and the prevalence decreases with age (56-59). According to a study by Mawak *et.al*, the infection rate was slightly higher in women within the age group 25-29 (17.68%) than in the age group 20-24 (15.24%) (52). A study conducted on gynaecologic out patients in evaluation of in-house PCR assay for detection of *C. trachomatis* has revealed highest prevalence in the age range of 18-33(45). Statistically significant association between ages under 20 and more than one life time partner was found in Italy (44).

Marital status is another factor indicated to be a risk factor for acquiring *C. trachomatis.* Women who are not currently in a relationship are less likely to be reported being diagnosed with infection (60). In a study conducted on women showed there was higher percentage of infection in married women than single ones with prevalence of 38.41% versus 17.07%, respectively (52). In contrast, a study done in Ireland in 2004 showed women with single marital status had higher risk of *C. trachomatis* with the prevalence of 11.2% (61).

A study done on 200 obstetrics and gynaecology outpatient department attendee in Portugal, has revealed a prevalence of *C. trachomatis* has statistically significant association with having educational level of 8 year and less (62). Also in Brazil, it was found that there was a statistically significant association between Chlamydia prevalence and lower education (63). In addition, condom use during sexual activity has been shown to be determining factor in prevention of C. *trachomatis* (64, 65).

2.1.6 *Diagnosis of Chlamydia trachomatis:*

2.1.6.1 *Direct detection*
Due to its obligate intracellularity, cell culture remains a reference method (about 100% specificity). However, it is not recommended for routine use due to its lack of sensitivity, its technical complexity and the long turn-around time. *C. trachomatis* can be detected by using antigen-based detection methods, particularly direct fluorescent staining with monoclonal

antibodies (DFA) and enzyme immunoassay (EIA) (62). EIA tests are more reproducible than DFA and the sensitivity of the best EIA is comparable to that of culture but lower than that of nucleic acid amplification tests (NAATs) due to cross-reactions with the lipopolysaccharide of other microorganisms (30).

Recently, a rapid antigen detection tests are produced to diagnose *C. trachomatis* infection. When compared with the gold standard test (NAATs), they have performance with sensitivity 83.5%, specificity 98.9%, positive predictive value 86.7%, and negative predictive value 98.6%. However, NAATs are the tests of choice for the diagnosis of *C. trachomatis* genital infections. In everyday clinical practice, several commercial NAATs are available and make use of different technologies: PCR and real-time PCR. Nevertheless, they have limitation because of their higher cost (64).

2.1.6.2 *Indirect detection*

A recent review by Pearson suggested that serology is useful only in some cases of *C. trachomatis* infection and in sero-epidemiological studies. On the other hand, recent evidences showed that anti- *C. trachomatis* immunoglobulin A in association with interleukin 8 (IL-8) evaluation appear to be the best immunologic markers of chronic chlamydial prostatitis status so the bacteria can be diagnosed using this methods (30).

2.1.7 *Treatment prevention and control*

Effective antibiotic treatment is available. The antibiotic of choice includes tetracyclines, macrolides, sulfonamides, some fluoroquinolones, and clindamycin. Nevertheless, if infertility develops, there is no simple treatment. Safe sexual practice, abstinence from sex until treatment is complete, follow-up test to make sure that treatment has cleared the infection and treatment of sexual partner can prevent the infection. The infection can be control by screening non symptomatic people especially sexually active women and treating them if they are found infected (4, 41).

2.2 *Neisseria gonorrhoeae* (the gonococcus)

2.2.1 *Microbiology, pathogenesis and epidemiology*

It is Gram negative non capsulated diplococcus measuring 0.6 to 1.0 μm in diameter. Its outer membrane consists of phospholipids, lipolysaccharide and proteins, which are typical of Gram-negative bacteria. Its sensitivity to temperature, aridity, and ultraviolet rays make *N.*

gonorrhoeae a relatively fragile organism. It causes sexually transmitted disease giving rise to intense local inflammation and a range of clinical manifestations. The bacteria tend to inhabit distinct mucosal niche in the human urogenital tract. *N. gonorrhoeae* is acquired through sexual contact and establishes infection in the urogenital tracts by interacting with non-ciliated epithelial cells (39).

The key structures at the interface between the host and *N. gonorrhoeae* are the protruding surface proteins that are known as pili (fimbriae) also called type IV pilus is an important colonization factor. In addition, Opa and Opc are expressed in the greatest abundance in the bacteria which help the bacteria to interact with the host cell (66).

The invasion of the urogenital tract mucosal cell results in the influx of polymorphonuclear leukocytes (PMN) and leads to inflammation. However, infection of the lower female genital tract is typically asymptomatic. *N. gonorrhoeae* engulfed by PMN are secreted in PMN-rich exudates. Regardless of the anatomic site that is infected, gonococcus promotes an inflammatory response that is characterized by the recruitment of PMNs. The cervical secretions of women with gonorrhoea also contain PMNs. The Bacteria in gonorrhoeal secretions are found attached to and within PMNs (39, 67).

Humans are the only known reservoir for N. gonorrhoeae. It is transmitted through contact with mucous membranes harbouring the bacteria, which often occurs during sexual contact. *N. gonorrhoeae* binds onto CD4 surface glycoproteins on cells. Its ability to bind to immune cells and prevent immune response allows for future reinfection, since the body does not develop immunological memory against this bacterial species. Its DNA transformation and conjugation capabilities allow for N. gonorrhoeae to become resistant to antibiotic treatment (4, 2, 41)

A study conducted in Awassa among symptomatic women has revealed the prevalence of *N. gonorrhoeae* to be 5% (68). On the other hand, a study conducted on endocervical specimen has shown the prevalence of zero percent (69). Study conducted on 617 women on the prevalence of *N. gonorrhoeae* and *C. trachomatis* has have yielded the prevalence of 0.8% (70).

2.2.2 Treatment, prevention and control

The antibiotic of choice includes ciprofloxacin, norfloxacin and spectinomycine. Safe sexual practice, abstinence from sex until treatment is complete, follow-up test to make sure that treatment has cleared the infection and treatment of sexual partner can prevent the infection. The infection can be control by screening non symptomatic people especially sexually active women and treating them if they are found infected (4).

2.2.3 Diagnostic methods for Neisseria gonorrhoeae

Microscopy

A direct smear for gram staining may be performed as soon as the swab specimen is collected from the urethra, cervix, vagina or rectum. The swab should be rolled gently on to the slide to preserve cellular morphology and over and area of less than $1cm^2$. The sensitivity and specificity for urethral smears is 90% to 95%, while 50% to 70% sensitivity and over 90% specificity for endocervical smears (71).

Culture

Preferred laboratory method for diagnosis of gonorrhoea is isolation and identification of the agent. Culture is important for antibiotic susceptibility testing, surveillance purpose, and detection of treatment failure. Primary isolation should be made on selective agar medium like modified Thayer-Martin, Martin Lewis, and New York City medium (70).

Non-culture diagnostic method (Nucleic acid detection)

These methods are important in a more rapid and specific diagnosis of the pathogens. These methods permit the use of specimens that are unsuitable for culture, such as urine and vaginal swabs that can be obtained from patients without discomfort. Nucleic acid methods are suitable for detecting *N. gonorrhoeae* in specimens that may not contain viable organisms due to long transportation time or exposure to extreme temperature conditions. Highly sensitive amplification methods may have cross contamination problems and are expensive (70).

CHAPTER THREE: OBJECTIVES OF THE STUDY

3.1 General objective

- ❖ To determine the prevalence of *C. trachomatis* and *N. gonorrhoeae* infection and asses possible risk factor among women in the reproductive age group, at JUSH, 2013.

3.2 Specific objectives

- ❖ To determine the prevalence of *C. trachomatis* infection among women in the reproductive age group.

- ❖ To determine the prevalence of *N. gonorrhoeae* infection among women in the reproductive age group

- ❖ To assess possible risk factors associated with *C. trachomatis* and *N. gonorrhoeae* among women in the reproductive age group.

CHAPTER FOUR: MATERIALS AND METHODS

4.1 Study area and study period

The study was conducted from May to July 2013 in Jimma University Specialized Hospital gynaecological outpatient department. The hospital provides services for about 9000 inpatient and 80, 000 outpatient attendances a year coming to the hospital from the catchment population of about 15 million people. The gynaecological outpatient department gives service for about 20 clients a day using two OPDs. In the first OPD medical and Health officer intern students work on all patients who visit the department. The second room serve as consultation room (72).

4.2 Study design

Cross-sectional study design was used.

4.3 population

4.3.1 *Source population*

The source population was all reproductive age women attending the gynaecological clinic in JUSH.

4.3.2 *Study population*

All women in the reproductive age who visited the gynaecological OPD during study period who meet the inclusion criteria.

4.4 Inclusion and exclusion criteria

4.4.1 *Inclusion criteria*

All women who attended the gynaecological OPD for diagnosis and treatment of reproductive tract infections, termination of pregnancy and counselling during the study period.

4.4.2 *Exclusion criteria*

Women having taken antibiotics two weeks prior to or within the data collection period.

4.5 Sample size and sampling techniques

4.5.1 *Sample size determination*

The sample size was determined by using single population proportion formula as stated below. The prevalence of *N. gonorrhoeae* 12.2% from a study conducted in Addis Ababa (55) was used for estimation of the sample. Taking 95% confidence interval and ±5% marginal error, sample size (n) is determined using the following statistical formula.

$$n= \frac{(Z_{1-\alpha/2})^2 \, p \, (1-p)}{d^2}$$

Where, P= Prevalence rate of 12.2%,

n = Sample size,

Z = 95% confident interval

d= Bond on sampling error tolerated between the sample and population: ±5%

α = Critical value at 95% confidence interval of certainty (1.96)

Thus, totally 162 women were included.

4.5.2 *Sampling procedure*

All consecutive consenting women coming to the gynaecology outpatient department during the study period were enrolled provided that inclusion criteria were full filled to achieve the intended sample size.

4.6 Measurements
4.6.1 Study variables

4.6.1.1 Dependent variables
  ➢ prevalence of *C. trachomatis*
  ➢ prevalence *of N. gonorrhoeae*

4.6.1.2 Independent variables
  ➢ Socio-demographic characteristics(age, educational status, income , occupational status, and residence)

- ➢ Behavioural factor(age at first sexual intercourse, number of sexual partner, habit of condom use and contraceptive use)
- ➢ Clinical factors(history of abortion, number of abortion, history and number of pregnancy)
- ➢ STI symptoms and clinical diagnosis

4.6.2 Data collection instrument

4.6.2.1 Questionnaire

A semi-structured questionnaire having both closed and open ended questions was prepared. The questionnaire incorporated socio-demographic and socio-economic information, reproductive histories, and behavioral factors of the women. The Laboratory format was also used to record the laboratory test results. Prior to data collection, the questionnaire was pre-tested on 5% of the total sample size in similar setup outside the study area and appropriate changes were made according to the feedback received. The questions were checked for clarity, completeness, consistency, sensitiveness and setting of time required to conduct interviews and the questions which posed difficulty or unclear were rephrased and corrected. Unnecessary questions were excluded and missed questions were incorporated where necessary.

4.7 Data collection procedure

Data on socio demographic and selected risk factors for STIs was collected using semi structured questionnaire by interview. The data was collected by trained data collector who works in the clinic. Two endo-cervical swabs for Chlamydia antigen test and *N. gonorrhoeae* culture and microscopy were collected by experienced and trained nurse; and physician working at the clinic. Then the specimen was processed in accordance with standard operating procedures (SOP) by the principal investigator. The laboratory work up was done according to the flow chart below shown on **figure-2.**

4.7.1 *Specimen collection*

Endo-cervical swab specimen for *N. gonorrhoeae* culture and gram staining was collected using sterile plastic shaft nylon fibber swab and was inoculated immediately on gonococcal culture medium (Modified Thayer Martin medium which is selective for gonococcus). Then inoculated medium put in to candle extinction jar and transported with in 1 hour to the Jimma

University Medical Microbiology laboratory. For the detection of *C. trachomatis* antigen, the endo-cervical swab specimen was collected by sterile swab provided by the manufacturer*(Standard Diagnostics Inc, Korea)* and placed in to sample collection tube containing 300 micro litre of reagent A (sample digesting reagent) (73-76).

4.7.2 *Specimen processing*

All specimens were processed at the site of specimen collection following Standard Operational Procedures (SOP). Sample for *C. trachomatis* antigen detection, briefly after two minute of addition of reagent 300 micro litre A, 600 micro litre of reagent B was added and the swab was removed (after 2 minutes again) by squeezing then discarded. The collected specimen was tested using Rapid diagnostic test device (having a sensitivity and specific of 93.1% and 98.8% respectively), which detect Chlamydia outer membrane protein, manufactured by Standard Diagnostics Inc, Korea and result was interpreted within 15 minutes accordingly. Internal quality control samples were run in parallel with sample to assure the accuracy of the test result. Specimen for culture was inoculated immediately on sterile Modified Thayer Martin Medium plates on the same day and incubated at $37^{o}C$ in 10% CO_2 (candle extinction jar). Growth was checked every 24 hours for 72 hours. Colony morphology, gram staining of the colony was used as a tool of identification (44-45).
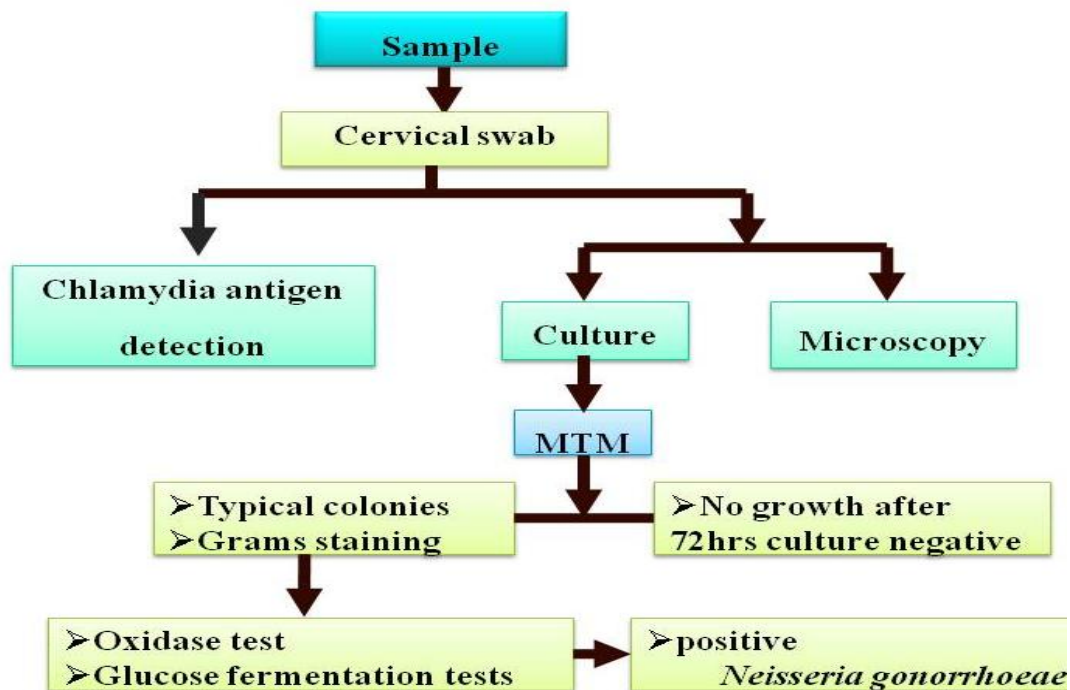
Figure- 2. Simplified laboratory flow chart for isolation of *Neisseria gonorrhoeae*.

4.8 Data Quality assurances

The questionnaire was prepared in English version and translated to Afan Oromo and Amharic then translated back to English to confirm the correctness of the translation by language expert. Pre-test was done in Shenen Gibe Hospital gynaecologic and obstetric outpatient department and the necessary amendment was made. Standard Operating Procedure (SOPs) was prepared according to different text books and manuals (annex-IV). It was strictly followed during the course of reagent and culture media preparation, specimen processing, culturing of specimen and microscopic examination. A generous gift of *N. gonorrhoeae* (ATCC 49226) Control strain was obtained from Ethiopian Health and Nutrition Research Institute for internal quality control purpose of *N. gonorrhoeae* culture process. At the end of each day the collected data was checked for completeness.

4.9 Data management and analysis,

After coding, the data was entered Epi Data version 3.1 and then data was checked for completeness, inconsistency and outliers by looking at their distribution. Incomplete and inconsistent data was excluded from the analysis. Data was analyzed using SPSS version 16.0 for Windows. Descriptive statistics was used to describe the study sample. The results were then expressed as frequency; mean and percentage.

Binary logistic regression model was employed to analyze the adjusted effect of each independent variable on the outcome variables. Based on purposeful selection of variables in logistic regression, Preliminary bivariate analysis for each independent variable was performed to start with; and those variables significant at P-value of 25% at the bivariate regression were then selected to Multivariate analysis model. Multivariate analysis was done to identify the independent predictors of the outcome variable. Odds ratios were calculated with a 95% confidence interval to determine the strength of association and statistical significance at two sides' P-value of $\leq 5\%$.

4.10 Ethical considerations

Ethical clearance was obtained from Jimma University, ethical review board. Official request letters were submitted to JUSH administration office and permission letter was obtained to conduct the research. The purposes and the importance of the study were explained & informed consent was secured from each participant. Confidentiality was maintained at all levels of the study. Participant's involvement in the study was on voluntary basis. Participants who were unwilling to participate in the study & those who wished to quit their participation at any stage were informed to do so without any restriction. Study subjects with positive result were treated.

4.11 strategy for dissemination and utilization of the study findings

The results of the study will be submitted to the Department of Laboratory Sciences and Pathology, College of Public Health and Medical Sciences, Jimma University. It will be also presented to Jimma University scientific community through thesis defence. After that the final report will be disseminated to concerned bodies. The paper will be considered for publication on peer reviewed journals.

CHAPTER FIVE: RESULTS

From the total intended sample size of 162, 159 women who visited Jimma University Specialized Hospital gynaecological outpatient department were involved in the study with response rate of 98.14%. From the total 159 women, 26 (16.4%) were positive for *C. trachomatis* and none were positive for *N. Gonorrhoeae* in this study.

5.1 Socio-demographic characteristics of the respondents

The mean age of the women was 25.60 (SD ± 6.6) years. Residence wise, 64.8% (103/159) of the respondents reside in urban areas. Majority of the respondents were Oromo (59.7%) followed by Amhara (17.6%). Seventy one (44.7%) and 64 (40.3%) respondents were Muslim and Orthodox, respectively. More than half (66.0%) of the respondents were married and 46 (28.9%) and 26 (16.4%) were house wives and daily labourers, respectively (**Table 1**)

Table 1: Socio-Demographic Characteristics and *Chlamydia Trachomatis* Infection among Women of Reproductive Age Group Who were Attending Gynaecology OPD at JUSH, South West Ethiopia; May- July, 2013 (n=159)

Variables	Response rate n (%)	Positive n(%)
Age		
15-19yrs	27 (17.0)	6 (3.8)
20-24yrs	46 (28.9)	8 (5.0)
25-29yrs	47 (29.6)	6 (3.8)
30-34yrs	19 (11.9)	4 (2.5)
35-49	20 (12.6)	2 (1.3)
Residence		
Rural	56(35.2)	6 (3.8)
Urban	103(64.8)	20 (12.6)
Ethnicity		
Gurage	2(1.3)	0 (0.0)
Tigre	7(4.4)	3 (1.9)
Others*	27(17.0)	7 (4.4)
Amhara	28(17.6)	3 (1.9)
Oromo	95(59.7)	13 (8.2)
Religion		
Protestant	24 (15.1)	6 (3.8)
Orthodox	64 (40.3)	12 (7.6)
Muslim	71 (44.7)	8 (5.0)
Occupation		
Private business owners	4 (2.5)	1 (0.6)
NGO employee	5 (3.1)	1 (0.6)
Unemployed	5 (3.1)	1 (0.6)
Merchant	11 (6.9)	1 (0.6)
Farmer	14 (8.8)	0 (0.0)
Government employee	24 (15.1)	5 (3.2)
Students	24 (15.1)	6 (3.8)
Daily labourers	26 (16.4)	6 (3.8)
Housewife	46 (28.9)	5 (3.2)
Marital status		
Widowed	2(1.3)	0 (0.0)
Divorced	6(3.8)	1 (0.6)
Unmarried	46(28.9)	10 (6.3)
Married	105(66.0)	15 (9.4)
Level of education		
Unable to read and write	37 (23.3)	6 (3.8)
Able to read and write	15 (9.4)	1 (0.6)
Grade1-8	38 (23.9)	8 (5.0)
Grade9-12	44 (27.7)	6 (3.9)
Grade12+	25 (15.7)	5 (3.1)

Others* = Kafa, Yem, Wolayta, Dawuro, Sidama, Konita, and Sheka.   **n** =number

5.2: Behavioural Factors

Of all study participants 51.6% (82/159) of them started their first sexual intercourse at age of 15-19 years whereas, 20.8% (33/159) of respondents engaged in sexual activity at age before 15 years. One hundred fifteen (72.3%) respondents had one sexual partner and the rest had two and more than two sexual partners in their life time. Concerning to their habit of condoms use more than half (79.2%) of the study participants responded that they had no habit of using condoms during their sexual activities. There was higher prevalence of infection seen among women who do not have habit of condom use, 16.7% (21/126).

Totally 50.3% (80/159) of the women participated in this study have used contraceptive methods in their past experience of which 42.5% (34/80) and 38.8% (31/80) of them used pills and Depo respectively. Regarding current contraceptive method use, 37 (23.3%) were using at least one type of modern contraceptive methods, among which 32.4% (12/37) and 29.8 %(11/37) of the study participants were using pills and Depo respectively. There was no *Chlamydia trachomatis* infection among in women using IUCD **(Table 2)**.

Table 2: Behavioural Factors and Chlamydia infection in Reproductive Age Group Women Attending Gynaecology OPD at JUSH, South West Ethiopia; May- July, 2013 (n=159)

Variables	Response category	n (%)	Positive n(%)
Age at first intercourse			
	< 15	33 (20.8)	6 (3.8)
	15-19	82 (51.6)	10 (6.3)
	20 and above	44 (27.6)	10 (6.3)
Number of sexual partner			
	One	115 (72.3)	18 (11.4)
	Two and above	44 (27.7)	8 (5.0)
Condom use			
	Yes	33 (20.8)	5 (3.2)
	No	126 (79.2)	21 (13.2)
Ever used contraceptives			
	Yes	80 (50.3)	15 (9.5)
	No	79 (49.7)	11 (6.9)
Type of contraceptives			
	Pills	34 (21.3)	6 (3.8)
	IUCDs	8 (5.0)	0 (0.0)
	Implant	7 (4.4)	2 (1.3)
	Depo	31 (19.5)	7 (8.0)
Current use			
	Yes	37 (23.3)	8 (5.1)
	No	122 (76.7)	18 (11.3)
Type of contraceptives			
	Pills	12 (7.5)	2 (1.3)
	IUCDs	10 (6.3)	0 (0.0)
	Implant	4 (2.5)	1 (0.6)
	Depo	11 (6.9)	6 (3.8)

n =number

5.3: Reproductive History

A total of 76.7% (122/159) have had at least one pregnancy. Thirty eight (23.9%) of them had history of abortion in their life time, among these 74.5% (26/38) had experienced abortion at least one time. There is a high prevalence of Chlamydia trachomatis seen in women having a history of pregnancy more than five which is 25% (2/8) **(Table 3)**.

Table 3: Reproductive History and Chlamydia infection among Reproductive Age Group Women Attending Gynaecology OPD at JUSH, South West Ethiopia; May- July, 2013 (n=159)

Variables	Response category	n (%)	Positive n(%)
History of pregnancy	Yes	118 (76.7)	20 (12.6)
	No	41 (23.3)	6 (3.8)
Number of pregnancy	1-3	97 (86.8)	16 (13.2)
	3-5	13 (8.2)	2 (1.3)
	>5	8 (6.9)	2 (1.3)
History of Abortion	Yes	38 (23.9)	6 (3.6)
	No	121 (76.1)	20 (12.6
Number of abortion	1	26 (16.4)	5 (3.2)
	2	11 (6.9)	2 (1.3)
	3	1 (0.6)	0 (0.0)

n =number

5.4: STI Symptoms and Clinical Diagnosis

Clinical symptoms of the study participants were assessed and 56.0% (89/159) of them were asymptomatic. Out of 159 women participated in this study, 20.8% (33/159) and 25.8% (41/159) were diagnosed for STIs and unwanted pregnancy, respectively. Those who presented clinical symptom had high prevalence of infection. Patients with vaginal discharge had highest rate of infection which is 29.2% (7/24); patient with lower abdominal pain 25.0% (5/20); and 19.2% (5/26) of patients with vaginal bleeding were positive for *C. trachomatis*, while there was no infection was seen in women having PID **(Table 4)**.

Table 4: STI Symptoms, Clinical Diagnosis and Chlamydia infection among Reproductive Age Group Women Attending in Gynaecology OPD at JUSH. South West Ethiopia May- July, 2013 (n=159).

Variables	Response category	n (%)	Positive n(%)
STI symptoms	Lower abdominal pain	20 (12.6)	5 (3.2)
	Vaginal discharge	24 (15.0)	7 (4.4)
	Vaginal bleeding	26 (16.4)	5 (3.2)
	Non symptomatic	89 (56.0)	9 (5.9)
Clinical diagnosis	Cervical cancer	10 (6.3)	1 (0.6)
	PID	11 (6.9)	0 (0.0)
	STIs	33 (20.8)	6 (3.6)
	Mayoma	18 (11.3)	2 (1.3)
	Unwanted pregnancy	41 (25.8)	8 (5.0)
	Others*	46 (28.9)	9 (5.9)

Others*- urinary tract infection, those who came to change IUCD, cervical polyp, incomplete abortion, check up patients. **n =**number

5.4: Association between Genital *Chlamydia Trachomatis* Infection and Risk Factors.

To identify the factors associated with the prevalence of genital *C. trachomatis*, binary logistic regression was performed on our dichotomous dependant variable. Based on purposeful selection rule of variables for regression analysis, variables with P-value of less than 25% at preliminary bivariate analysis were selected as candidate for multivariate analysis. Accordingly; residence, level of education, occupational status and clinical symptoms, which were found to have a P-value of less than 25% in preliminary bivariate analysis, were selected as candidate variables and entered into multivariate logistic regression analysis (**Table 5**).

In multivariate logistic regression, level of education, occupational status and STI symptoms showed statistically significant association with the prevalence of *C. trachomatis*. The likelihood of detecting *C. trachomatis* is 0.082 times (AOR=0.082, 95% CI: 0.007-0.909; P-value=0.042) lower in women with vaginal discharge, 0.004 times (AOR=0.004, 95% CI: 0.000-0.090; P-value=0.001) lower in those with lower abdominal pain, 0.039 times (AOR=0.039, 95% CI: 0.003-0.463; P-value=0.010) lower in those with vaginal bleeding when referred to women without disease symptom. The detection rate of *C. trachomatis* is

likely 0.053 times (AOR = 0.053, 95% CI: 0.004-0.679; P-value = 0.024) lower in students compared to house wives and 0.074 times (AOR = 0.074, 95% CI: 0.006-0.975; P-value = 0.048) lower in an illiterate women, 0.082 times (AOR = 0.082, 95% CI: 0.010-0.677; P-value = 0.020) lower in primary school complete women related to high school complete women(**Table 5**).

Table 5: The Regression Estimate of Predictor Variables on Prevalence of *Chlamydia Trachomatis* Among Reproductive Age Group Women Visiting Gynaecological Outpatient Department of JUSH, South West Ethiopia, May- July, 2013 (n=159).

Variables	Response category	Freq (%)	AOR	P-value
Highest Educational status	Un able to read & write	37(23.3)	0.074 (0.006-.975)	0.048
	Able to read and write	15(9.40	0.214 (0.007-6.141)	0.368
	Grade 1-8	38(23.9	0.082 (0.010-0.677)	0.020
	Grade 9-12	44(27.7)	1	1
	Grade 12+	25(15.7)	0.421 (0.054-3.267)	0.408
Occupational status	G. Employee	24(15.1)	0.164 (0.012-2.280)	0.178
	Farmer	14(8.8)	2.0598 (0.000-0.00)	0.998
	Merchant	11(6.9)	0.289 (0.009-9.192)	0.482
	House wives	46(28.9)	1	1
	Daily labourers	26(16.4)	0.402 (0.030-5.371)	0.491
	Students	24(15.1)	0.053 (0.004-0.679)	0.024
	NGO employee	5(3.1)	0.021 (0.013 -1.687)	0.084
	Un employed	5(3.1)	*0.079* (0.003-1.966)	0.122
	Private business owners	4(2.5)	0.104 (0.003-3.158)	0.194
STI symptoms	Vaginal discharge	24(15.0)	0.082(0.007-0.909)	0.042
	Lower abdominal pain	20(12.6)	0.004(0.000-0.090)	0.001
	Vaginal bleeding	26(16.4)	0.039(0.003-0.463)	0.010
	Non symptomatic	79(56.0)	1	1
	Constant		0.000	0.999

Freq- frequency

CHAPTER SIX: DISCUSSION

Chlamydia trachomatis has long been known to infect human causing mild to chronic infections. The infection and destruction of the cervical, endometrial and fallopian tube columnar epithelial cell may result in infertility and create risk of ectopic pregnancy or cause damage to pregnancy. Due to asymptomatic nature of the infection and lack of screening tests there is no clear picture about the prevalence of infection in most developing countries (6, 9).

If left untreated they may leads to various long term sequela including ectopic pregnancy, infertility, and pelvic inflammatory disease. Due to the asymptomatic nature of these infections, there is no clear picture about their prevalence. Therefore, the present study was aimed at determining the prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoea* infections and associated risk factors among reproductive age group women in Jimma(4-6).

Prevalence studies are very important to health care planner giving a clear picture about the prevalence of infection. This will aid in planning intervention like screening sexually active adolescents and young adults for Chlamydia. Due to test acceptability and high risk of infection the most practical and feasible way to obtain the prevalence estimate is the population at health facility. There are different studies conducted in various parts of the world including Sub Saharan Africa with reported prevalence ranging from 2.6% to 56% (45-53).

In the present study the overall prevalence of *C. trachomatis* is 16.4 %. This high prevalence of *C. trachomatis* may indicate hidden dissemination and presence of the infection among women of reproductive age (41). This finding is comparable with a study in Japan 14.3% (46), and the findings of studies in Iran which were documented as 17.0% and 15.4 % in 2007 and 2011 respectively (50, 51).

This result is higher than the study conducted in Nairobi Kenya on prevalence of genital Chlamydia infection in urban women of reproductive age; with the prevalence of *C. trachomatis* infection to be 6.0% and Addis Ababa which was 1.2% (54, 55). The possible reason for such discrepancy may be due to the characteristics of the study participant, socio-cultural, geographical difference, methodological variation and difference laboratory specimen and sensitivity of diagnostic tests.

However, the finding of this is lower than study conducted in India on gynaecologic out patients with prevalence of 23.0% (45), and Saudi Arabia with 25% prevalence (48). Also

another study conducted in Jose, Nigeria which is 56.1% has shown higher prevalence than the present study (52). These differences may be because of variation sensitivity of laboratory method, the sociodemographic and behavioural characteristics of the population studied and geographical difference.

In general identifying the overall prevalence of *C. trachomatis* has its own benefit in order to control the distribution and avoid disease complication among women of reproductive age. This will aid planning early diagnosis and treatment of these groups which are prone to complications during pregnancy and in entire life. Therefore the high prevalence of *C. trachomatis* may be an indicative for the health care provider to plan preventive measures.

Chlamydia can be linked to various socio-demographic characteristics of the population including age, marital status, level of education, occupation of women in reproductive age group (9, 17, 18).

Regarding the age group there was high prevalence of infection in the age groups between 15-19 (22.2%) and 20-24(17.4%). This population comprises the adolescent and young adults. This high prevalence may be explained by various reasons: younger women are unlikely to control over their sexual choice and are less able to negotiate protective behaviour like condom use with their partner (59). They are anatomically susceptible to infection because of their immature reproductive epithelial cells. These columnar epithelial cells extend to the vaginal surface of the cervix where they are not protected by cervical mucus that makes them especially susceptible to invasion by sexually transmitted organism including Chlamydia trachomatis. There are also social and contextual factors that increase susceptibility of these age groups. Our finding is in agreement with other studies (56).

Risky behaviour can increase the acquisition of *C. trachomatis* infection. This includes having multiple sexual partners, use of condom. Even though there is no statistically significant association there is high prevalence of *C. trachomatis* in women with multiple sexual partners which are 27.7% of the total sample size. The high prevalence (18.2%) is an indicative of the contribution of this risky behaviour in the epidemiology of the infection.

Studies showed consistent condom use can associated with up to 90% reduction on in the prevalence of *C. trachomatis* among women. In our study 79.2% of women do not have the habit of condom use and 80.0% of positive women have not the habit of condom use during

sexual inter course. This is similar with the finding of a study conducted in 2006, in Jimma where there was high rate of non use of condom and increased antibody prevalence against the bacteria (28). This may be explained by their reluctance to use condom (65). This indicates work should be done on advocacy of condom use.

There was high prevalence of *C. trachomatis* in women who undergo termination of pregnancy. Over all 19.5% of women who visited the OPD for termination were positive and majority of them were asymptomatic, it suggests the presence *C. trachomatis* infection in pregnant women. This finding is supported by study done in Nigeria on antenatal care attendees with prevalence of 13.3% (53). This should be considered carefully, because infection of pregnant women can result adverse out come in both the mother and neonate (18, 19). In general identifying the contributing factor is important for health care sector in designing of preventive methods and controlling of the infection.

Response to our study questionnaire reveal that 23.1% (6/ 26) women infected with *C. trachomatis* had history of abortion. Although we do not have evidence that previous abortion is because of *C. trachomatis* there is evidence that the infection can cause abortion and premature delivery (18).

Individuals with asymptomatic infection remain undiagnosed during syndromic approach which in practice in most developing countries including Ethiopia (9, 27). Finding from this study showed that 34.6% of Chlamydia positive women were asymptomatic. This indicates more than one third of *C. trachomatis* infected women miss diagnosed as negative using syndromic management.

Besides, statistical analysis showed that -there was higher chance of detecting *C. trachomatis* in those without symptom than the symptomatic ones. Since both asymptomatic and symptomatic women have chance of developing complications, screening those women visiting gynaecological OPD at all time have its own contribution in the prevention and control of the infection and its sequale.

Findings from this study depicted that *C. trachomatis* infection was statistically associated with occupational status. Accordingly, there was higher prevalence of infection in house wives than students (AOR = 0.053, 95% CI: 0.004-0.679; P-value = 0.024). In addition, there was higher prevalence of *C. trachomatis* in high school complete women than those who have

completed primary school education, and those who were unable to read and write. This result is in contrast with findings from the study conducted in Portugal and Brazil (62, 63).This might have been attributed to residual confounding due to unmeasured sexual characteristics such as frequency of sexual contact, the characteristics of sexual partners (husband or boy friend), cultural influence, socioeconomic status difference and the other related characteristics of the studied women. Therefore further in-depth inquiry is required to substantiate contributing factors for the acquisition of infection.

This study also tried to determine the prevalence of *N. gonorrhoeae* among the study participants using MTM from cervical swab. Unfortunately there was no *N. gonorrhoeae* isolated. This is study is in line with prevalence of 0.0% and 0.8% in study conducted in Iran (2013) and Croatia in 2009 respectively (69, 70). This finding is contrary to findings from a study conducted in Hawassa Referral Hospital with the prevalence of 5.1% (68). This discrepancy might be due to difference in the characteristic of the women studied, sampling technique, methodological variation, difference in sample size and all study participants in the case of contrary study were symptomatic, while in our case were both symptomatic and asymptomatic.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1. Conclusion:

This study depicted that there is 16.4% prevalence rate of *C. trachomatis* infection among reproductive age women and no *N. gonorrhoeae* isolated. There was statistical association between, educational status; with high infection rate in women having high school education than those with elementary and no education; occupation, where house wives have higher rate of infection than students and the presence of clinical symptom in which there is high probability of detection in asymptomatic women than those who present with clinical symptoms.

7.2. Recommendation and future direction:

1. Designing and implementing policy focusing on screening of reproductive age group women for *C. trachomatis* in health care setup should be considered.

2. Health promotion and health education on STI prevention should be implemented.

3. Women education and employment has to be strengthened in Ethiopia since housewives had high prevalence of Chlamydia.

4. It would be very important to conduct research on the hospital as well as the community to get the clear picture of the distribution of the bacterial and viral causes of STIs which includes both women and men.

5. Conducting research on the area using other more sensitive laboratory techniques like nucleic acid detections and using comprehensive tools that measures behavioral factors will be of very important to evaluate the prevalence of STIs, including *C.* trachomatis.

6. Longitudinal studies should be conducted to evaluate the incidence of *C. trachomatis* among women of reproductive age will be also important to evaluate screening program application feasibility.

STRENGTH AND LIMITATION OF THE STUDY

❖ As this study is cross sectional study which measures both the exposure and outcome; it is difficult to link between the cause and the outcome, or understand the natural history of *C. trachomatis* infection.

❖ Only takes the outpatients of gynecological department but others like antenatal care clinic, family planning clinic are not included.

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ANNEXES

ANNEX I. INFORMATION SHEET AND CONSENT FORM
Title of the Research Project

Genital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among women of reproductive age group: prevalence and risk factors at JUSH. South West Ethiopia

Name of Principal Investigator: Dagnamyelew Tilahun

Name of the Organization: Jimma University, College of public Health and Medical Sciences

Name of the Sponsor: Jimma University

Information sheet and consent form prepared for women attending the JUSH gynaecological OPD who is going to participate in Research Project.

Introduction

This information sheet and consent form is prepared with the aim of determining the magnitude of *Chlamydia trachomatis* infection and *N. gonorrhoeae* among reproductive age group women at Jimma University Specialized Hospital 2013 and to forward the possible solutions in controlling the infection to concerned bodies. The research group includes the principal investigator, and advisors from Jimma University.

Research description

This is a study focusing on detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in women attending the gynaecological OPD at Jimma University Specialized Hospital. urogenital *C. trachomatis* and *N. gonorrhoeae* infection that is implicated to be one major causes of a disease among women called Pelvic Inflammatory Disease (PID) and other medical condition like tubal infertility, preterm labor and prolonged rupture of membrane. These diseases are mostly asymptomatic in nature.

Its main aim is to detect and assess the prevalence of Chlamydia trachomatis and *N. gonorrhoeae* in both symptomatic and asymptomatic patients. This will help to know the magnitude of infection by these bacteria in both symptomatic and non symptomatic women. That will help in knowing the prevalence of infection and to consider planning screening program for these infections.

The laboratory examination requires collection of cervical swab by employing standard procedure and using sterile speculum (used for routine gynacological procedure) and using swab.

Risks

There will be no foreseeable risks to you except that you may fill discomfort while collecting cervical sample.

Benefits

There will be no special benefits to you except the laboratory test results. The laboratory findings would be judiciously used in conjunction with the clinical Findings to initiate appropriate treatment for your medical problem.

Confidentiality

Privacy during interviewing and confidentiality of information are guaranteed. In case you know one of the researchers, you can be interviewed by someone else or withdraw from the study. Concerning your laboratory sample will be collected and tested confidentially and result will be known by the examining physician only. The information collected will only be accessible to the research team.

Compensation

No compensation will be available for your time and any inconvenience but we are very grateful to you for taking part in this study.

Contacts

If you have any questions now please feel free to ask me. In case you have any later on, you can contact the principal investigator, <u>Dagnamyelew Tilahun</u>, on the telephone number - 0910166320. Mr ketema Abdissa telephone number 0912035503 and Wro. Haimanot Tassew telephone number 0917804249. If you have any issues pertaining to your rights and participation in the study, please contact the Chairperson of the Institutional Review Board, Jimma University School of Public Health and Medical sciences on the telephone number 0471120945.

Voluntary Participation

Participating in this study is voluntary. You have the right to refuse to take part and can withdraw at any point without any penalty.

Participant: I understand all the conditions above and have agreed to take part in this study of my own free will.

Participant name…………………………………………………………………

(Signature / mark)………………………………………………………………..

Researcher / research assistant's signature……………………………………………

Any other witness………………………………………………………………..

INFORMATION SHEET CONSENT FORM AMHARIC VERSION

የጥናት መረጃ መስጫ ቅፅ

ጤና ይስጥልኝ

የተመራማሪ ስም: ዳኛምየለው ጥላሁን

የአማካሪዎች ስም: ከተማ አብዲሳ

ሐይማኖት ታስው

ተቋም: የጅማ ዩኒርሲቲ የህክምናና ጤና ሳይንስ ኮሌጅ የህክም ላብራቶሪ ሳይንስ እና ፓቶሎጂ ትምህርት ክፍል::

ስፖንሰር: ጅማ ዩኒቨርሲቲ

የጥናት ርዕስ : በጅማ ዩኒቨርሲቲ ስፔሻላይዝድ ሆስፒታል የክላሚዲያ ተራኮማቲስ እና ናይሴሪያ ጎኖሪያ (*CHLAMYDIA TRACHOMATIS* **AND** *NEISSERIA GONORRHOEAE*) በሴቶች ላይ ያላቸው ስርጭት:: ጅማ ደቡብ ምዕራብ ኢትዮጵያ

ማብራሪያ: ይህ የጥናት መረጃ የተዘጋጀው በጅማ ዩኒቨርስቲ ስፔሻላይዝድ ሆስፒታል የማህፀን ህክምና ክፍሉ ለተለያዩ ምርመራዎች ለመጡ በጥናት ላይ ለሚሳተፉ ሴቶች የተዘጋጀ ነው::

መግቢያ: ይህ የጥናት መረጃ የተዘጋጀው የክላሚዲያ ተራኮማቲስ እና ናይሴሪያ ጎኖሪያ (*CHLAMYDIA TRACHOMATIS* **AND** *NEISSERIA GONORRHOEAE*) በሴቶች ላይ ያላቸውን ስርጭት በጅማ ዩኒቨርሲቲ ስፔሻላዝድ ሆስፒታል ለማጥናት አልሞ ነው:: ከጥናቱም የሚገኘው መረጃ የባከቴሪያዎቹን ስርጭት ለመቆጣጠር የሚፍትሔ እርምጃ ለመውሰድ ያገለግላል:: በጥናቱ ላይ አንድ የጥናቱ ዋና ተመራማሪ:: ሁለት የመረጃ ሠብሳቢዎ (በሙያ ነርስ/አዋላጅ ነርስ) እንዲሁም አንድ ሱፐርቫይዘር እና ሁለት አማካሪዎች ይሠተፋሉ::

የጥናቱ ማብራሪያ ጥናቱ ትኩረት ሚያደርገው በግብረ ሥጋ ግንኙነት አማካኝነት የሚተላለፈውን የክላሚዲያ ተራኮማቲስ እና ናይሴሪያ ጎኖሪያ (*CHLAMYDIA TRACHOMATIS* **AND** *NEISSERIA GONORRHOEAE*) የተሰኙ ባከቴሪያዎች በጅማ ዩኒቨርሲቲ ስፔሻላይዝድ ሆስፒታል የማህፀን ህክምና ክፍል በሚታከሙ ሴቶች ላይ ያላቸውን ስርጭት ማወቅ ላይ ነው:: እነዚህ ባቴሪያዎች በሴቶች ላይ የተለያዩ የመራቢያ አካላት ችግሮችን ያስከትላሉ:: ከነዚህም ውስጥ የማህፀን ህመም፤ ውርጃ እዲሁም ነፍስ ጡር ሴቶች ያለ ጊዜ ቀድመው እንዲወልዱ ሲያደርጉ:: በባከቴሪያዎች ከተያዙ እናቶች የሚወለዱ ህፃናትን ደግሞ ለአይን፤ አፍንጫና ጉሮሮ ህመም ይዳረጋሉ:: እነዚህ ባክቴሪያ በባህሪያቸው ምልከት ሳያሳዩ የመቆየት አቅም አላቸው:: በመሆኑም የነዚህን ባቴሪያዎች በሴቶች ላይ ያላቸውን ስርጭት ማወቅ አስፈላጊውን የመከላከያ መንገድ ለመቀየስ ዋነኛ ግብአት ነው::

የጥናቱ ቅደም ተከተል በመጀመሪያ ጥናቱ ላይ ለመሣተፍ ያንቺን ሙሉ ፈቃድ ይጠይቃል ለጥናቱ ተበለዉ ለተዘጋጁ ጥያቄዎች አጠር ያለ ምላሽ ትሠጪያለሽ:: በመቀጠልም በማንኛውም ጊዜ እንደሚደረግ የማህፀን ምርመራ ሐደትን ተከትሎ ከማፀንሽ በር ላይ በክፍሉ ውስጥ በሚሠራ ነርስ/ሐኪም አማካኝነት ናሙና ይወሰዳል:: ይህም ናሙና ለተለያዩ

ምርመራዎች የሚውል ሲሆን የምርመራ ውጤቱም ከ20 ደቂቃ እስከ ሁለት ቀን ይፈጃል በመሆኑም የመረመረሸ ሐኪም የምርመራውን ውጤት ተከትሎ አስፈላጊን ህክም ሊያደርግልሸ ይችላል፡፡

ሥጋትና ጉዳት ምርምሩ ጥያቄና ለምርመራ ከማህፀን በር ላይ ናሙና መውሠድን የሚጠይቅ ስለሆነ ናሙናው በሚወሰድበት ያለመመቸት ስሜት ሊሰማሸ ከመቻሉ ውጭ ምንም አይነት የጤና ችግር አያጋጥምሸም ለዚህም አስፈላጊን ጥንቃቄ ይደረጋል፡፡

ጥቅም ጥናቱ ላይ በመሳተፍሸ የተለየ ጥቅም አይኖረውም፡፡ ነገር ግን የላቦራቶሪ የምርመራ ውጤት ካሳየሸው የህመም ምልክት ጋር በማቀናጀት በመርማሪ ሀኪም አስፈላጊን ህክና እንዲያደርግልሸ ይረዳል፡፡

ሚስጥራዊነት ለጥናቱ የምትሠጫው ማንኛውም መረጃ በሚስጥር እንደሚዘ እናረጋግጣለን፡፡ የላቦራቶሪ ውጤትሸም ከመረመረሸ ሐኪም ውጭ ለማንም ይፋ አይሆንም፡፡ አድራሻሽን ወይም ስምሸን ያለመስጠት ባለሙሉ መብት ነሸ፡፡

የጥናቱ ተሣታፊ መብቶች በጥናቱ ላይ የምትሣተፈው ባንቺ ሙሉ ፈቃደኝነት ብቻ እንደሆነ እያሣወቅን በጥናቱ ላይ ያለመሣተፍ ወደ ጥናቱም ከገባሸ በሁዋላም በፈለገሸ ሠዓት አቋርጦ የመውጣትም መብት እንዳለሸ ልንገልፅልሸ እንወዳለን፡፡ በጥናቱም ላይ በመሣተፍ ማንም ተፅዕኖ አያሳድርብሸም በጥናቱም ላይ ያለሸን ጥያቄ ሁሉ የምትጠይቅሸን ነርስ/አዋላጅ ነርስ የመጠየቅና የመረዳት ባለሙሉ መብት ነሸ፡፡

በተጨማሪ መረጃ ማነጋገር ብትፈልጊ ማንኛውም ጥያቄ ቢኖርሽ አሁን ወይም ሌላ ጊዜ የሚከተሉትን ሠዎች በሚከተለው አድራሻ ማግኘት ትችያለሸ፡፡

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ወ/ሮ ሐይማኖት ጣሠው ስልክ. ቁ. 0917804249

INFORMATION SHEET AND CONSENT FORM (AFFAN OROMO)

Mata duree Qorannichaa

Qorannoo dhibeen Kiilamidiiyaa fi Niiseeriyaa Gonooree dubartoota JUSH keessatti tajaajilaman irratti qabu, Kibba Lixa Itoophiyaa, 2013.

Maqaa qorataa: Daanyamyellewuu Xilaahuun

Maqaa dhaabbataa: Yuuniveersiitii Jimmaatti Koollejjii Saayinsii Meedikaalaa fi Fayyaa Hawaasaa.

Maqaa Ispoonseeraa: Yuuniveersiitii Jimmaa.

Unka kun fedhii maamiltootn (dubartootni) kutaa yaala deddeebbii gadameessaa qorannoo kana irratti fedhii isaaniin, dhimma kana keessa beekuun irratti hirmaachuuf waadaa seenanii dha.

seensa

Kaayyoon Unki kun qophaa'eef inni guddaan hirmaattootni qorannoo mata duree **"**Qorannoo baay'ina dhibeen Kiilamidiiyaa Trachoomattis fi Niiseeriyaa Gonooree dubartoota JUSH keessatti tajaajilaman irratti qabu" jedhu fi bara 2013 geggeffamu irrati namootni hirmaatan fedhii isaanii kan ittiin mirkanneffatanii dha. Gareen qorannoo kana gaggeessu kan of keessattuu qabatu, qorataa 1^{ffaa} fi gorsitoota lama Yuuniveersiitii Jimmaa irraa.

Haala qorannichaa

Dhimmi guddaan qorannoon kun gaggeffamuuf, rakkoo dhibeen Kiilamidiiyaa Trachoomattis fi Niiseeriyaa Gonooree dubartoota JUSH keessatti tajaajilaman irratti qabu adda baasuu ta'a. Kiilamidiiyaa Trachoomattis fi Niiseeriyaa Goonnoreen haala fayyummaa dubartoota irratti dhibee madaa gadameessaa (pelvic Inflammatory Disease (PID)) fi rakkoolee fayyaa kanneen maseenummaa ujummoo gadameessaa, miixuu yeroo malee fi madaa meemberenii yeroo dheeraaf turuuf ka'uumsa olaanaa dha jedhama. Kanaafuu kaayyoo guddaan qorannoo kanaas sakata'iinsa baay'ina Kiilamidiiyaa Trachoomattis fi Niiseeriyaa Goonnoreen dubartoota mallattoo dhibee kanaa agarsiisaniis ta'ee kanneen hin argisiisnee irratti qabu haala barbaachisaan adda baasuu dha.Haaluma kanaan saamudni kan fudhatamu ujummoo gadaamessaa keessaa yemmuu ta'uu firiin qorannoo kanaa daqiiqaa 20 hanga guyyaa lamatti waan ba'uuf ogeessi fayyaa gadaamessaa namoota dhibee kana qabaniif qorichaa barbaachisaa ni kenna.

Qorannoon kun kan gaggeeffamu ji'a sadiif yemmuu ta'uu, akkaataan funaansa isaa maamila irraa saamuda fudhachuun Laaboraatooriin qorachuun raawwatama.

Miidhama

Qorannoo kanatti hirmaachuun miidhamni gama fayyaan mul'atuu fi isin irraa ga'uu danda'u tokko illee hin jiru**.**

Bu'aa

Bu'aan adda hirmaachuun argamu hin jiru. Haa ta'uu malee garuu, qorannoon kun tajaajilli isin argachaa jirtanii akka kana caalaa fooyya'uuf shoora olaanaa taphata.

Iccitii

Mirgi sagalee keessan bilisan kennuu fi Iccitiin isaa sirriitti eegama. Tarii dhoksaatti sagalee keessan lachuu yoo barbaadan mirga guutuu qabachuu keessan isinii mirkaneessaa odeeffannoon isin irraa argamu lakk. Dhoksaa(koodii) waan funaanamuuf odeeffannoo isin laatan eenyuu illee adda baasee beekuu hin danda'u.

Beenyaa

Beenyaan adda yeroo keessaniifis ta'ee haala biraaf kaffalamu hin jiru. Garuu hirmaachuun keessan tajaajilichaa fooyyessuu keessatti qooda bakka hin buune qaba.

Fedhii hirmaachuu

Qorannoo kana irratti hirmaachuu dhiisuuf mirga guutuu qabdu. Kana malees erga jalqabdan giddutti kutuuf mirgi keessan eegama.

Teessoo

Yoo gaaffii qabatan amma bilisa taatanii na gaafachuu ni dandeessu. Kana malees booda qorataa 1^{ffaa}, **Daanyamyellewuu Xilaahuun** lakk. Bilbila kanaan booda gaafachuu ni dandeessu- **0910166320,** ykn **Obbo Katamaa Abdiisaa (0912035503)** fi **Addee Haayimaanoot Xaasoo (0917804249).** It dabalees waa'ee rakkoo mirga keessaniin waliin walqabatee yoo jiraate walitti qabaa Boordii Naamusa Qorannoo Yuuniveersiitii Jimmaa lakk. bilbila **0471120945** gaafachuu ni dandeessu.

Maamila hirmaate: Waantoota armaan oliitti caqasamee sirriitti hubadhee fedha koon hirmaadheera.

Mallattoo ……………………………………………………………………..

Qorataa/ gargaara qorataa………………………………………………….

Ragaan kan biraan yoo jiraate…………………………………………………

ANNEX II QUESTIONNAIRES AND DATA SHEETS

QUESTIONNAIRE ON DEMOGRAPHIC AND RISK FACTORS, OF THE STUDY PARTICIPANTS (English version).

I. **SOCIODEMOGRAPHIC FACTORS**

1. Code _____

2. Address/Residence /   1. urban_____   **2.**rural_____

3. Age in year _____

4. What is your religion?

    1. Orthodox   2. Protestant   3. Muslim

    4. Catholic   5. Others specify_____

5. What is your ethnicity?

    1. Oromo   2. Amhara   3. Tigre

    4. Gurage   5. Others specify_____

6. What is your marital status?

    1. Single   2. Married

    3.Widowed   4. Divorced

7.   Your educational status?

    1. Unable to read and/or write   2. Able only to read and/or write

    3. Primary school (1-8)   4. Secondary school (9-12)

    5. College and /or university (12+)

8.   Your Occupation

    1. Government employee   2. Farmer   3.Merchant

    4. House wife   5. Daily labour   6. Student

    7. NGO employee   8. Unemployed   9. Other (specify) _____

9.   Average monthly income of the family (ETB) _____

Part II. BEHAVIOURAL FACTORS AND REPRODUCTIVE HISTORY

1. Age at first intercourse _____

2. Number of life time partners_____

3. Have you ever used condom? _____

4. Did you use contraceptives methods previously? Yes___ No_____

5. If yes which method   Pills _____ IUCDs _____ Condom _____ Implant_____
   Injectables _____ _____

6. Did you use contraceptive currently? Yes___ No_____

7. If yes which method Pills _____ IUCDs _____ Condom _____ Implant_____ Injectables
   _____ Others specify_____

8. Did you become pregnant? Yes_____ No_____

9. If yes how many times you become pregnant? _____

10. History of termination of pregnancy: Yes___ No ___

11. If yes, frequency (number) _____

QUESTIONNAIRE ON DEMOGRAPHIC AND RISK FACTORS, OF THE STUDY PARTICIPANTS (Amharic version).

ማህበራዊ መረጃዎች

1. ስም (ኮድ) _____

2. አድራሻ/መኖሪያ   1. ገጠር   2. ከተማ

3. ዕድሜ_____

4. ሃይማኖት

     1. ኦርቶዶክስ   2. ፕሮቴስታንት   3. ሙስሊም
     4. ካቶሊክ   5. ሌላ (ይገለፅ)

5. ብሄር

     1. ኦሮሞ   2. አማራ   3. ትግሬ
     2. ጉራጌ   5. ሌላ (ይገለፅ)

6. የትዳር ሁኔታ

     1. ያላገባች   2. ያባች
     3. ባል የሞተባት   4. የተፋታች

7. የትምርት ሁኔታ

     1. ማንበብ መፃፍ የማትችል   2. ማንበብና መፃ የምትችል
     3. የመጀመሪያ ደረጃ (1-8)   4. ሁለተኛ ደረጃ (9-12)
     5. ኮሌጅ/ ዩኒቨርሲቲ (12+)

8. የሥራ ሁኔታ

     1. የመንግስት ሥራተኛ   2. ገበሬ   3. ነጋዴ
     4. የቤት እመቤት   5. የቀን ሥራተኛ   6. ተማሪ
     7. መንግስታዊ ያልሆነ ድርጅት ሥራተኛ   8. ሥራ አጥ   9. ሌላ (ይገለፅ)

9. አማካኝ ወርሃዊ ገቢ_____

ተጓዳኝ ሁኔታዎች

1. ለመጀመሪያ ጊዜ ወሲብ የጀመርሽበት ዕድሜ _____
2. ከምንያህል ሰው ጋረ ወሲብ ፈጽመሻል_____
3. በወሲብ ጊዜ ኮንደም ታውቂያለሽ አዎ_____ አይ_____
4. ካሁን ቀደም የወሊድ ትጠቀሚ መቆጣጠሪያ ነበር
5. አዎ ከሆነ ምን አይነት የወለደ መቆጣጠሪያ
   የወሊድ መቆጣጠሪያ እንክብል_____የማህፀን ቆብ ___ኮንዶም _____ በክንድ የሚቀበር_
   ___ምረፊ _____ሌላ ይጠቀስ_____
6. አሁንስ የወሊድ መቆጣጠሪያ እየተጠቀምሽ ነው አዎ_____ አይ_____
7. አዎ ከሆነ ምን አይነት
   የወሊድ መቆጣጠሪያ እንክብል_____የማህፀን ቆብ ___ኮንዶም _____ በክንድ የሚቀበር_
   ___ምረፊ _____ሌላ ይጠቀስ_____
8. አርግዘሽ ታወቂያልሽ   አዎ_____ አይ_____
9. አዎ ከሆነ ስንት ጊዜ
10. ከዚህ በፊት ፅንስ ተቋርጦብሽ ያውቃል? አዎ_____አይደለም
11. አዎ ከሆነ ም ያህል ጊዜ _____

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QUESTIONNAIRE ON DEMOGRAPHIC AND RISK FACTORS, OF THE STUDY PARTICIPANTS (Oromifa version).

QAJEELFAMA WALII GALAA (NAMA ODEEFFANNOO GUURUUF)

Gaaffilee jiran keessa deebii Kan ta'eef lakkoofsa isa fuula dura jirutti mari! Gaaffilen tokko deebii tokko ol qabachuu danda'u.

KUTAA I. ODEEFFANNOO HAWWAASUMMAA

1. Koodii gaaffiii_____

2. Bakki jireenya kee eessa?   1) Magaala   2) Badiyyaa

3. Umriiin kee meeqa?_____waggadhan

4. Amantiin kee maali?

    1) Ortoodoksii   2) Protestaantii   3)Musliima

    4) Kaatolikii   5) Kan biraa yoo ta'ee ibsii_____

5. Sablammii

    1) Oromoo   2) Amaara   3) Tigree

    4) Guraagee   5) Kan biraa yoo ta'e ibsi_____

6. Haala fuudhaa fi heerumaa

    1) Kan Hin heerumne   2) Kan Heerumte

    3) Kan abba warran irraa du'e   4) Kan Hiikte

7. Sadarkaa barumsaa

    1) Barrresuu fi dubbisuu kan hin dandenye

    2) Barrresuu fi dubbisuu kan dandessu

    3) Sadarka tokkoffaa Kutaa 1-8

    4) Sadarka lamaffaa Kutaa 9-12

    5) Kolleejjii Ykn Univarsiitii(12+)

8. Hojii/hojja

    1) Hojjettuu Mootumma   2) Qotee Bulaa   3) Daldaltuu

    4) Hojii mana keessaa kan hojjettu   5) Hojii Humna guyyaa kan hojjettu

    6) Barattuu   7) Dhaabbata miti Mootumma kan Hojjettu

    8) Hojii kan hin qabne   9) Kan biro yoo jiraate ibsi_____

9. Galii ji'aa ykn baatii giddugaleessatti qarshii Itiyoophiyaatin_____

KUTAA II. GAAFFIILEE RAGAA WAANTOOTA DHUKKUBA NAF- SAALATIIF SAAXILAN SASSABUF QOPHAA'AN.

1. Umrii meeqatti yeroo jalqabadhaaf wal qunnamtti nafsaalaa raawwatte?_____waggaadhan

2. Hanga yoonatti hiriyaa dhiiraa yookin kaadhimma meeqa qabatte jirta?_____

3. Wal quunnamttii saalaa Koondoomiin ala Raawwattee beektaa?   1) Eeyyee   2) Lakki

4. Kanaan duraa Karoora maatiitti fayyadamttee beektaa?   1) Eeyyee)   2)Lakki

5. Yoo eeyye jette maal fa'i?   1) Piilsii   2) Loopii(IUCD)   3) Kondomii   4) Kan irree jaal awwaalamu (Implant)   5)Lilmoodhaan kan kennamu(Depo) 6) Kan biro yoo jiraatee ibsi_____

6. Amma karoora maatiitti fayyadama jirtaa?   1) Eeyyee 2) Lakki

7. Yoo eeyyea jette maal fa'i?   1) Piilsii   2) Loopii(IUCD)   3) Kondomii   4) Kan irree   jala awwaalamu (Implant)   5)Lilmoodhan kan kennamu(Depo) 6) Kan biro yoo jiratee ibsi_____

8. Kanaan dura Ulfooftee ykn dadhabbii godhatte beektaa?   1) Eeeyyee   2) Lakki

9. Eeyye yoo jette si'a meeqa ulfooftee?_____

10. Ulfa ofirraa baaftee beektaa?   1)Eeyyee   2) Lakki

11. Eeyyee yoo jette si'a meeqa?_____

ANNEX -III STANDARD OPERATING PROCEDURES

Sample collection procedure

1. Moisten the speculum with sterile water, and insert it into the vagina.

   Note: Do not lubricate the speculum with a gel that may be bactericidal.

2. Cleanse the exo- cervix using a swab moistened with sterile physiological saline to remove excess mucus.

3. Pass a sterile nylon tip swab into the endocervical canal and gently rotate the swab to obtain a specimen for *N. gonorrhoeae culture* and remove the swab.

4. Insert the second swab and collect another swab specimen that will be used for the detection of Chlamydia trachomatis antigen.

5. Insert the swab 20–30 mm and rotate for 10-30 minutes against the endocervical wall to obtain columnar epithelial cell that harbour the organism.

6. Remove the swab gently and insert in to sample collecting tube containing reagent A according the instructional manual

7. For gonorrhoea, inoculated on culture medium in big zigzag shape.

8. Label medium with patient identification number.

9. The inoculated plate should be transported within one hour to the laboratory for incubation.

STANDARD OPERATING PROCEDURE-I

INSTRUCTIONS TO PREPARE MODIFIED THAYER MARTIN (MTM)

Principle

Peptones provide nitrogen, vitamins and amino acids. Corn starch absorbs any toxic metabolites that are produced; dibasic and monobasic potassium phosphates buffer the medium. Sodium chloride maintains osmotic balance. Agar is the solidifying agent. Chocolate Agar is prepared from GC agar medium base with the addition of 2% Hemoglobin. Hemoglobin provides hemin (X factor) required to enhance the growth of *Neisseria*. Addition of VCNT antibiotics will help to prevent other pathogenic bacteria, this make the medium selective.

> "Saprophytic" *Neisseria* are generally suppressed by selective media, the occasional recovery of *N. lactamica* on Thayer- Martin Selective Agar has been reported. Some strains of *Capnocytophaga* species may grow on this selective medium when inoculated with oropharngeal specimens.

Supplies

- ❖ GC agar Base (Oxoid)
- ❖ Distilled water
- ❖ Flask
- ❖ Sterile graduated cylinder
- ❖ Sterile Petri dishes with 100 mm diameter
- ❖ VCNT and Vitox
- ❖ Defibrnated Sheep blood

Equipment

- ❖ Balance
- ❖ Agar dispenser
- ❖ Bunsen burner
- ❖ Autoclave
- ❖ Hot plate
- ❖ Spatula
- ❖ Refrigerator
- ❖ Water bath

Procedure

1. Weigh 36 grams of the medium on a clean paper and transfer to one litre of distilled water
2. Mix thoroughly, heat with frequent agitation until the medium dissolves completely
3. Sterilize by autoclaving 15 lbs , $121^{o}C$ for 15 minutes
4. After sterilization , cool the medium to $50^{o}C$
5. Add 50 ml defibrinated sheep blood mix with gentle rotation
6. Heat the medium in water bath at $80 - 85^{o}C$ for $10 - 15$ minutes, mix the GC agar base and the blood by gentle agitation periodically until the medium becomes brown in color.
7. When the medium becomes cool add Vitox and VCNT according to the manufacturer instruction.
8. Dispense to sterile Petri dishes 20 ml amount
9. Allow the medium to solidify, write the date on medium and store in refrigerator

Shelf life

- 4-6 weeks providing there is no change in the appearance of the medium suggests contamination of deterioration

Limitations

1. GC agar medium bases are intended for use with supplementation.
2. Although certain diagnostic tests may be performed directly on the medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification.
3. GC agar medium bases have sufficient buffering capacity to offset the very low pH of the small amount of nutritive enrichments added. However, the pH of some media may have to be adjusted with 1% NaOH after the addition of these enrichments.

Procedural Notes

- Do not heat for prolonged time

STANDARD OPERATION PROCEDURE III
INSTRUCTIONS FOR THE PREPARATION OF CHOCOLATE AGAR.

Purpose: - This procedure provides instructions how to prepare chocolate agar.

Principle: - Chocolate agar supplemented with 10% sterile defibrinated blood is suited for isolating Neisseria species.

Supplies

1. Nutrient agar
2. Defibrnated sheep blood
3. Distilled water

Materials and Equipment

1. Balance, Autoclave
2. Hot plate and Bunsen burner
3. water bath
4. measuring cylinder and a flask
5. sterile Petri dish

Procedures

1. Use 1liter of nutrient agar
2. Add 50ml defibrnated sheep blood aseptically
3. Heat the mixture in water bath at $80\text{-}85^0$cfor 10 minutes
4. Mix the nutrient agar and the blood gently and periodically
5. When the mixture become chocolate -brown in color distribute about 20ml on sterile Petri dish aseptically
6. Store at $2\text{-}8\ ^0$c up to usage
7. Procedural Notes
8. Don't over heat after blood is added

Clinical Utility

It is a non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms including *Neisseria.*

SOP-IV INSTRUCTION FOR CHLAMYDIA ANTIGEN TESTING

Chlamydia antigen detection

Chlamydia antigen was detected using SD BIOLINE one step Chlamydia antigen rapid test kit (standard diagnostic INC). Sample processing and test procedure done according the manufacture instructions.

Extraction procedure/ preparing of extracted sample

1. Open the empty sample collection tube.
2. Take a reagent A up to the fill line/300µl and then, transfer into the tube.
3. Insert the patient swab into the tube containing reagent A.
   - ➢ Compress the bottom of the tube between the thumb and forefinger and twirl the swab 10 times.
4. Wait 2 mutes.
   - ➢ Compress the bottom of the tube between the thumb and forefinger and twirl the swab 10 times.
5. Hold the dropper vertically, draw reagent B solution up to the fill line/ about 600µl and then, transfer 600µl of reagent B into the tube.
   - ➢ Compress the bottom of the tube between the thumb and forefinger and twirl the swab 10 times.
6. Express the liquid from the swab by compressing the middle of the tube and pulling the swab up through it. Discard the swap and assemble dropping cap on the sample collection tube.

Test procedure: -

1. Remove the test device from the foil pouch and place on clean and dry surface.
2. Then add three drop of extracted sample from the tube to the round sample well on the test device.
3. Finally the result of the test was interpreted after 15 minutes. Some positive results may be seen earlier.

STANDARD OPERATING PRECDURE -V
MICROSCOPIC EXAMINATION

On First day

Swab samples

1. First inoculate the MTM then prepare the smear.
2. Label the slide with patient identifier.
3. Roll the swab gently across the slide surface, covering an area the size of a quarter. Do not rub vigorously since cellular distortion may occur.
4. Allow it to air dry.
5. Heat fix
   ➢ Pass air-dried smears through a flame two or three times. Do not overheat.
   ➢ Allow slide to cool before staining.
6. Flood the prepared slide with crystal violet for one minute.
7. Rinse the slide gently with tap water.
8. Flood the slide with Gram's iodine for one minute.
9. Rinse the slide gently with tap water.
10. Working with one slide at a time, flood the slide with decolorizer for 5 seconds and rinse with tap water. Repeat decolorization step for thick smears.
11. Flood the slide with safranin for one minute.
12. Rinse the slide gently with tap water.
13. Drain the slide in an upright position. Blot the back of the slide and place on a slide warmer or heating block to completely dry.
14. Scan 20-40 fields using oil immersion.

For suspected colonies a Gram stain was performed from culture. One colony will be picked up and mixed with one drop of saline on labelled slide and the procedure will continue from step-4 on ward.

STANDARD OPERATING PRECDURE - VI

CULTURE AND IDENTIFICATION

I. First day

1. Bring MTM at room temperature.
2. Label the medium with patient identification number.
3. Inoculate the original swab specimen to the plate by rolling the swab on the agar surface in "Z" pattern. This will sufficiently transfer the specimen.
4. Put the inoculated plate in candle jar.
5. Plates should then be delivered to the laboratory within 1 hour of inoculation.
6. Cross-streak the plate using a sterile wire loop.
7. Incubate in 10% CO_2 (candle jar) at 35-37°C and examine at 24 hours.
8. If no growth is observed, re-incubate plate for up to 72 hours.

II. Second day

Isolates should be gram stained. If gram-negative diplococci are present colonies should be tested for oxidase production. Suspicious colonies should be identified

1. Inoculate isolated colonies from culture to chocolate agar plate and observed for growth at 24 hours after incubation in 3-7% CO2 at 35-37°C.
2. If there is growth gram stain the suspected colony.
3. Perform oxidase test.

Annex IV: DECLARATION

I, the undersigned, hereby declare that this thesis finding is my original work and has never been presented for any degree in Jimma University or any other institutions of higher learning in Ethiopia. I also declare the duly acknowledgement of all material sources used for this thesis work.

Name of the student: _____

Signature: _____

Place: _____

Date of submission: _____/_____/_____

This thesis has been submitted for approval with my supervision as a University advisor.

1. **Name of advisor:** _____

       Signature: _____

       Place: _____

       Date of submission: _____/_____/_____

2. **Name of advisor**: _____

       Signature: _____

       Place: _____

       Date of submission: _____/_____/_____

Name of examiner: _____

       Signature: _____

       Place: _____

       Date of submission: _____/_____/_____

Name of Department head: _____

       Signature: _____

       Place: _____

       Date of submission: _____/_____/_____