PREVALENCE AND ASSOCIATED RISK FACTORS OF *NEISSERIA GONORRHOEAE*, *TREPONEMA PALLIDUM* AND *TRICHOMONAS VAGINALIS* AMONG JIMMA UNIVERSITY STUDENTS SUSPECTED FOR SEXUAL TRANSMITTED INFECTIONS, ETHIOPIA

BY

RAHEL TAMRAT

A THESIS SUBMITED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCES, JIMMA UNIVERSITY INSTITUTE OF HEALTH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (MSc) IN MEDICAL MICROBIOLOGY

FEBRUARY, 2017

JIMMA, ETHIOPIA

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

Prevalence and Associated Risk Factors of *Neisseria gonorrhoeae*, *Treponema pallidum* and *Trichomonas vaginalis* among Jimma University Students Suspected for Sexual Transmitted Infections, Ethiopia

By

Rahel Tamrat

ADVISORS:

1. Tesfaye Kassa (PhD): School of Medical Laboratory Sciences, Jimma University

2. Mr. Zewdineh S/Mariam (MSc): School of Medical Laboratory Sciences, Jimma University

3. Mr. Mulatu Gashaw (MSc): School of Medical Laboratory Sciences, Jimma University

February, 2017

Jimma, Ethiopia

ABSTRACT

Background: *Sexually transmitted infections (STIs) are major global cause of acute illness, infertility, long-term disability and death with serious medical and psychological consequences for millions of men, women and infants. Globally, one-third of 340 million new STI cases occur in people under 25 years of age every year. There is little or no evidence on the burden of STIs mainly due to Neisseria gonorrhoeae, Trichomonas vaginalis and Treponema pallidum among university students in Ethiopia. Therefore the present study was aimed to provide data regarding the problem in Jimma university main campus students. It also attempted to help in selection of an appropriate antimicrobial agent for the treatment of N. gonorrhoeae which has been demonstrated as multidrug resistance.*

Method: *A health facility based cross-sectional study design was conducted at Jimma University main campus students clinic from April to October 2017among 189Jimma university main campus students suspected for sexual transmitted infections. Socio demographic and risky sexual behaviors were collected by using structured self-administered questionnaire. Urethral discharge and endocervical/vaginal swab were collected by the attending nurses. Antimicrobial sensitivity test was done by using Kirby-Bauer disk diffusion test. Microscopic examination of wet mount preparation was done for the diagnosis of T. vaginalis and serological tests for syphilis were performed using a rapid immunochromatographic kit (XIAMEN, China). Data was entered and analyzed using SPSS Version 20.Descriptive statistics, Fisher exact, Chi-square tests and logistic regression was carried out to assess the risk factors for STIs.*

Results: *The overall prevalence of STI among Jimma university main campus students suspected for sexual transmitted infections was 14.3%. Neisseria gonorrhoeae accounted for 7.4%, T. vaginalis for 4.8% and T. pallidum, 3.7%. The prevalence of gonococcal infection in males was higher than in females accounting 15.5% and 3.8 % respectively (p= 0.012). All patients with trichomoniasis were females. Having had sex after taking alcohol was significantly associated with STIs (p=<0.05). All N. gonorrhoeae isolates were resistant to penicillin and tetracycline.21.4% isolates were resistant to ciprofloxacin and whereas two (14.3%) isolates were resistant to ceftriaxone and cefixime*.

Conclusion: *In this study the prevalence of Neisseria gonorrhoeae, T. vaginalis and T. pallidum was relatively high. Students who have had sex after taking alcohol were more likely affected by STI. The concerned bodies need to focus on giving health education on risky sexual behaviors for STIs. Further study is recommended.*

Keywords *N. gonorrhoeae, T. vaginalis, T. pallidum, Prevalence, Sexual transmitted infections*

ACKNOWLEDGMENT

Always I will give thanks and praise to our Almighty GOD, for not only his all blessings in my life but also for fulfilling his promises as assured in his Word.

I‘m very grateful for Jimma University Institute of Health Sciences, School of Medical Laboratory Sciences for giving me this chance to conduct this study.

My great gratitude extends to my advisors Tesfaye Kassa (PhD), Zewdineh S/Mariam (MSc) and Mulatu Gashaw (MSc) for their cooperation and unreserved willingness to share valuable scientific comments and guidance.

I would like to acknowledge all study participants and all JUMC staffs specially Mr. Dawit Abera and Mr. Amanuel Teferi for their intensive support.

Finally, I would like to thank all Jimma university main camps students’ clinic staffs specially Mr. Behailu Kereyu and Mr. Daniel Firehun for their unreserved support during data collection and my lovely family for their countless encouragement and a kind support.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLE

ACRONYMS & ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
AST	Antimicrobial Susceptibility testing
ATCC	American type culture collection
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
DGI	Disseminated Gonococcal Infection
DALYs	Disability-adjusted life years
EIA	Enzyme Immune Assay
FTA-ABS	Fluorescent treponemal antibody-absorbed
GC	Gonococcus
HIV	Human immunodeficiency virus
JUSC	Jimma University Student clinic
MSM	Men who have Sex with Men
MTM	Modified Thayer Martin medium
NG	*Neisseria gonorrhoeae*
NAATs	Nucleic acid amplification tests
PID	Pelvic inflammatory disease
PCR	Polymerase Chain Reaction
QA	Quality assurance
QRNG	Quinolone resistant *N. gonorrhea*
RTI(s)	Reproductive tract infection(s)
SOPs	Standard operating procedures
SPSS	Statistical Product and Service Solutions
STD(s)	Sexually transmitted disease(s)
STI(s)	Sexually transmitted infection(s)
TPELISA	*Treponema pallidum*Enzyme-Linked Immunosorbent Assay
TPPA TP	*Treponema pallidum*Particle Agglutination Assay
TRUST	Toluidine Red Unheated Serum Test
TV	*Trichomonas vaginalis*
WHO	World Health Organization

OPERATIONAL DEFINITIONS

Risky sexual behavior*:* Jimma University main campus students suspected to have sexually transmitted infections who experienced at least one of risky sexual behaviors such as having multiple sexual partners, sexual contact with commercial sex worker, or have experience of unprotected sex (inconsistent condom use), having sex after alcohol or substance use

CHAPTER ONE: INTRODUCTION

1.1 Back ground

The term sexually transmitted diseases (STDs) refers to a variety of clinical syndromes and infections caused by pathogens that can be acquired and transmitted through sexual activity(1). On the other hand, sexually transmitted infections (STIs) are infections that are mainly transmitted from person-to-person through sexual contact. There are more than 30 different sexually transmissible etiologies including bacteria, viruses and parasites .These are responsible for multiple sexually transmissible diseases such as gonorrhea, chlamydial infection, syphilis, trichomoniasis, chancroid, genital herpes, human immunodeficiency virus (HIV) infection and hepatitis B infection. Some of the above conditions, especially HIV and syphilis, can also be transmitted from mother to child during pregnancy and childbirths as well as through blood products(2).Out of the 30 recognized STIs, four of them, namely, syphilis, gonorrhea, chlamydia, and trichomoniasis have been found to be curable (3).

More than a million people acquire a sexually transmitted infection (STI) every day. WHO estimates that 499 million new cases of curable STIs occurred in 2008 among 15–49 year-olds globally: 106 million cases of gonorrhea, 11 million cases of syphilis, and 276 million cases of trichomoniasis. Men and women were similarly likely to acquire new STIs (2).

N. gonorrhoeae is the second most commonly reported sexually transmitted bacteria next to *C.trachomatis* in the United States. Currently, extended-spectrum cephalosporin is the only first-line antimicrobials recommended for the empirical treatment of uncomplicated gonorrhea in many countries. In the United States, the prevalence of isolates with reduced susceptibility to cefixime (CFM) has resulted in dual treatment with ceftriaxone (CRO) plus azithromycin (AZM) or doxycycline being the only Centers for Disease Control and Prevention (CDC) recommended treatment regimen. *N. gonorrhoeae* strains with reduced susceptibility to extended-spectrum cephalosporin have been reported worldwide and treatment failures with oral and injectable extended-spectrum cephalosporin have been reported in Europe, Africa, Asia, Australia and North America (4, 5).

Currently, extensively drug-resistant *N. gonorrhoeae* strains has been reported in Japan(6), France(7), and Spain (8) that displayed high-level resistance to cefixime and ceftriaxone. Resistance to commonly-prescribed antibiotics of *N. gonorrhoeae* is an expanding global problem resulting in diminishing treatment options for gonorrhea(9). Loss of utility of several drugs such as sulfonamides, penicillin and tetracycline, for treatment of gonorrhea, were reported in both developed and developing countries (10).

T. vaginalis is a flagellated, anaerobic protozoan and is the most common non-viral sexually transmitted pathogen. Available epidemiological data suggests that more women than men are often infected and in both sexes, most infections are asymptomatic, with symptomatic and severe infections more common in women than men(11) and it is capable of invading and colonizing the heavily defended host urogenital mucosa from both sexes, breaking through the primary innate defenses and withstanding induced innate and adaptive responses (12).

Syphilis, a sexually transmitted infection, is caused by the spirochete bacterium *Treponema pallidum*(TP) subspecies *palladium*, which is strongly contagious and capable of infecting multiple tissues and organs that produce complex clinical manifestations and TP cannot be cultured in vitro; therefore, serological tests for syphilis are key elements of the current diagnostic and therapeutic monitoring .Among the curable STIs, syphilis has the lowest global incidence but accounts for the greatest number of DALYs (13-15).

With the occurrence of resistance to commonly prescribed antibiotics in both developed and developing countries the current treatment guidelines for all sexually transmitted infection including *N. gonorrhoeae* in African countries follows the syndromic case management based on the WHO recommendation (16).

1.2 STATEMENT OF THE PROBLEM

STIs are a major global cause of acute illness, infertility, long-term disability and death with serious medical and psychological consequences of millions of men, women and infants. Although most STIs are asymptomatic, some cause genital symptoms that have an important impact on quality of life. Gonorrhea and trichomoniasis can cause vaginal discharge syndromes in women and urethritis in men. The magnitude is high in all regions, with highest rates in World Health Organization regions of America and Africa (2, 17).

Untreated bacterial STIs in women result in pelvic inflammatory disease in up to 40% of infections; and 1 in every 3 of these will result in infertility. Chronic pelvic pain from untreated bacterial STIs is an important cause of health care visits among women. In sub-Saharan Africa, untreated genital infection may be the cause of up to 85% of infertility among women seeking infertility care (18, 19).

Several STIs increase the risk of both acquiring and transmitting HIV. Both ulcerative (syphilis) and inflammatory (gonorrhea, trichomoniasis) curable STIs may also be associated with an increased risk of HIV acquisition, by up to two- to three-fold (20).

STIs also pose a substantial economic burden. In the United States, approximately $3 billion in direct medical costs were spent in 2008 to diagnose and treat 19.7 million cases of STIs and their complications, excluding HIV and pregnancy-related outcomes like still birth (21).

The psychological consequences of STIs include stigma, shame and loss of self-worth. STIs have also been associated with relationship disruption and gender-based violence in addition, the direct physical, psychological and social consequences of STIs have a major impact on quality of life and are prime indicator of the quality of global sexual and reproductive health care (19, 22).

Several risk factors exist that make certain populations more prone to STIs than others. While these risk factors are not shared by all STIs, there are many commonalities such as young age is a risk factor common to many STIs. Adolescents and young adults (15–24 years old) make up only 25% of the sexually active population, but represent almost 50% of all new acquired STIs (20, 23)

Globally, one-third of the 340 million new STIs cases occur per year in people under 25 years of age. Each year, more than one in every 20 adolescents contracts curable STIs .In developing countries such as sub Saharan Africa the burden of STI is very high with 108 million STIs occurring every day and it is estimated that 80‑90% of the global burden of STIs occur in the developing world where there is limited or no access to diagnostic facilities(24)

Ethiopia is the second most populous nation in Africa. About 84% of the population lives in rural areas, and young people (aged 15–24) represented one of the country's largest groups, comprising about 35% of the population (25, 26).

In the STI surveillance study which was conducted from January - June 2013 in 8 health facilities located in Amhara, Oromia and Addis Ababa by EHNRI in collaboration with CDC,E about 16% of the STI patients were co-infected with HIV (8.1% male and 21%female) and HIV prevalence is higher on STI patients with lower abdominal pain (41%) and genital ulcer (24.5%). Young people, in the age group 20-34 yrs, were the highly affected ones (68.2%), with a larger proportion being females (61%) (27).

University students are in the youth age category and are exposed to risky sexual behaviors such as un protected sexual intercourse leading to STIs(28).The risky behaviors may further be worsened by the fact that university students are too many in number, lack facilities for sexual and reproductive health services and live away from their parents and free from parental control. In addition ,some are subjected to wide spread substance use and peer-pressure that aggravate the risky behaviors (29).

According to a cross sectional study conducted in Wolaita Sodo University, Southern Ethiopia self-reported STIs prevalence in the past 12 months prior to the survey was 19.5% among students. Out of the 35.3% students who were sexually active, 46.0% used condom infrequently, 24.8% had sex with causal sexual partners and 13.9% had sexual intercourse with commercial sex workers. Students who had sexual contact with commercial sex workers in the last 12 months were at increased odds of developing sexually transmitted infections (Adjusted OR=4.7,95%CI: 1.2, 8.6) (30).

Currently, the primary approach to treating STIs is syndromic management. Providing appropriate and effective treatment requires up-to-date information about susceptibility patterns. Antimicrobial resistance in *N. gonorrhoeae* is already a major obstacle in disease management, whether it is syndromic or based on etiological diagnosis. The continued emergence of resistance in this organism threatens to further complicate management strategies. Sensitization on drug use and adopting preventive measures and continuous education on safer sexual behavior through health care authorities would lead to reduction in the prevalence of *N. gonorrhoeae* and resistance to antimicrobial (31).

The drug resistance varies greatly among countries. Therefore, having prevalence's data as well as the drug susceptibility pattern within consecutive year is important especially for gonorrhea, the highly drug resistant bacteria (32).

There is no adequate data about the prevalence of. *N. gonorrhoeae*, Syphilis and *T. vaginalis* infection and associated risk factors and anti-microbial susceptibility of *N. gonorrhoeae* among university students in Ethiopia, Even if available it is often incomplete because; clinical presentation are not specific enough for diagnosis based solely on symptoms and there is also lack of proper reporting mechanisms. Therefore, this study was aimed at determining the prevalence of. *N. gonorrhoeae, T. pallidum* and *T. vaginalis* infection and asses associated risk factors and anti-microbial susceptibility of *N. gonorrhoeae* among Jimma university main campus students.

1.3 Significance of the study

There is lack of enough evidence on the burden of *N. gonorrhoeae, T. pallidum, T. vaginalis* infection and associated risk factors and anti-microbial susceptibility of *N. gonorrhoeae* among university students in Ethiopia particularly in Jimma University. Therefore, the outcome of this study will serve as base line information on prevalence of *N. gonorrhoeae, T. pallidum, T. vaginalis* infection and associated risk factors and anti-microbial susceptibility pattern of *N. gonorrhoeae* among Jimma University main campus students. Conducting this study among youth/university students/ in particular can be an important input to design control and prevention strategies of STIs. It can provide additional input in the selection of an appropriate antimicrobial agent for treatment of *N. gonorrhoeae*.

CHAPTER TWO: LITERATURE REVIEW

2.1 Epidemiology and Associated Risk Factors of STIs

Adolescents and young adults often have the highest rates of incident STIs and account for a disproportionate number of new infections(33).It should be noted that global estimates, especially for the curable STIs, have relied on the few regions with systematic STI surveillance along with a relatively small number of prevalence studies among discrete populations (n = 180, WHO 2008 estimates) (2).

Fewer data exist from areas with limited laboratory infrastructure. However, despite data limitations, it is clear that the number of global STIs is large: available estimates suggest that well over a million people acquire an STI every day (2).

World health organization estimated number of cases of curable STDs in WHO regions of the world, in 2008 among population of adults between ages 15-49, years. In WHO European Region, comprising 53 countries with an estimated population of 450.8 million, it was estimated that 1.0 million adults were infected with *N. gonorrhoeae*, 0.3 million with syphilis and 14.3 million with *T. vaginalis.* In Region of Americas comprises 35 countries with an estimated population of 476.9 million adults 3.6,million with *N. gonorrhoeae,*6.7 million with syphilis and 57.8 million with *T. vaginalis* were infected. In South-East Asia Region, covering 11 countries with an estimated population of 945.2 million adults, it was estimated that there was 9.3 million *N. gonorrhoeae,* 12.3 million syphilis and 28.7 million *T. vaginalis*. There were estimated cases of 1.0 million *N. gonorrhoeae,* 1.6 million syphilis and 13.2 *T. vaginalis* in Eastern Mediterranean Region containing 23 countries with an estimated population of 309.6 million adults. In WHO Western Pacific Region covers 37 countries with an estimated population of 986.7 million adults. Prevalence was estimated that 13.3 million adults were infected with *N. gonorrhoeae,* 1.2 million with syphilis and 30.1 million with *T. vaginalis*. 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 8.2 million *N. gonorrhoeae,*14.3 million syphilis and 42.8 million *T. vaginalis* (2).

In the study conducted in Russia using nucleic acid amplification tests, the prevalence of *N. gonorrhoeae* and *T. vaginalis* were 2.5% and 1.2%. Men displayed riskier sexual behaviors and worse knowledge and attitudes regarding safe sex compared to women, with the most distinguishing features being younger age at first intercourse ($P < 0.0005$), higher numbers of sex partners during lifetime ($P = 0.001$) and latest 6 months ($P < 0.0005$), more frequently consuming alcohol ($P < 0.0005$), poorer knowledge of STI ⁄ HIV prevention measures ($P < 0.0005$) (34).

In a cross sectional study conducted in Abuja, Nigeria among students of tertiary educational institution the prevalence of *Neisseria gonorrhoeae* was 2.9%, syphilis 2.4%, and *Trichomonas vaginali*s 4.70% .All the students with trichomoniasis were females; Among students with gonorrhoea, 80% were males (35).

As study conducted in Uganda among patient attending St. Mary's Hospital Lacor-Gulu from Jan 2007-Dec 2011 showed prevalence of *Neisseria gonorrhoeae* was 59% among patients coming with STI symptoms (23.3%) isolates were resistant to Ciprofloxacin (36).

In the study conducted in University Of Port Harcourt among Under Graduate Female Students Nigeria using Strand Displacement and Amplification Technique the prevalence of *Neisseria gonorrhoeae* was 5%. Some of the associated risk factors elicited were; having multiple sexual partners, irregular use of condom and past history of sexually transmitted infections (37).

In study conducted in rural area of southern Mozambique out of the 170 (50.3%) subjects who were consuming alcohol, 41.8% were reported to have STIs which was a statistically significant . Of the 153 (45.3%) respondents who were chewing khat, 54 (35.3%) reported to have STIs; 29 (8.6%) used shisha (a less active narcotic smoked/sucked through a tube like apparatus) and 37 used cigarettes (32.4% had STIs) (38).

In a cross-sectional study conducted between May and September 2013 among women attending family planning clinic in Nairobi, Kenya the prevalence of *T. vaginalis* and *N. gonorrhoeae* were

0.4 % and 0 %.All the infected women were reported having had only one sexual partner in the previous 1 year (39).

In the STI surveillance study which was conducted from January - June 2013 in 8 health facilities located in Amhara, Oromia and Addis Ababa by EHNRI in collaboration with CDC-E, a total of 636 STI cases were reported from eight sentinel surveillance sites and the commonest syndrome was vaginal discharge (50%), followed by urethral discharge (31%), genital ulcerative disease (9%), lower abdominal pain (7.3%) and two syndrome were present in few patients (3%) (27).

According to a cross sectional study conducted between April and August 2014 among STI clinic clients in Gondar town hospitals and health centers,the prevalence of *T. palladium* ,*N. gonorrhoeae* and *T. vaginalis* were 30%, 20.8% and 14.2% respectively (40).

In A cross-sectional study conducted in Gambella, Ethiopia the prevalence of *N. gonorrhoeae* was 11.3 %.The prevalence of gonococcal infection in males was four times higher than in females accounting 16.0 and 5.0 % respectively ($p = 0.049$). It was also higher (18.9 %) in 20–24 years age group ($p = 0.439$). Alcohol intake ($p = 0.013$), less frequent condom use ($p = 0.031$), and multiple sex partners ($p = 0.024$) were associated with increased odds of infection (41).

2.2 *N. gonorrhoeae*

2.2.1 Microbiology and pathogenesis of *N. gonorrhoeae*

It is measuring 0.6 to 1.0 μm in diameter non capsulated Gram negative diplococcus bacterium. It possesses a distinctive Gram-negative outer membrane consists of proteins, phospholipids and lipopolysaccharide. It is a fairly fragile organism, vulnerable to temperature changes, drying, UV light, and other environmental conditions .It is an obligate human pathogen cause's 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 (42).

The main 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 (43).

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 bacteria in gonorrhoeal secretions are found attached to and within PMNs (42, 44).

2.2.2 Diagnostic methods for *Neisseria gonorrhoeae*

2.2.2.1 Microscopy

As soon as the swab specimen is collected from the urethra, cervix, vagina or rectum a direct smear for gram staining may be performed then 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 (45).

2.2.2.2 Culture

The current preferred laboratory method for diagnosis of gonorrhea 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 (46).

2.2.2.3 Nucleic acid detection

These methods are rapid, highly sensitive and specific for the detection of these organisms in clinical specimens and 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 (46).

2.2.3 Treatment of *Neisseria gonorrhoeae*

The CDC's treatment guide lines recommended for uncomplicated gonococcal infections are Cefixime (Suprax) 400mg orally, Ceftriaxone (Rocephin) 125 mg intramuscularly, Ciprofloxacin (Cipro) 500 mg orally, Levofloxacin (Levaquin) 250 mg orally and Ofloxacin (Floxin) 400 mg orally.and all medications are administered as a single dose (47).

2.2.4 Antimicrobial Susceptibility of *Neisseria gonorrhoeae*

In a resistance surveillance of *Neisseria gonorrhoeae* study conducted in Germany between October 2010 and December 2011, high resistance rates were found for ciprofloxacin (74%) and tetracycline (41%). Penicillin non-susceptibility was detected in 80% of isolates. The rate of azithromycin resistance was(6%), while all strains were susceptible to spectinomycin, cefixime, and ceftriaxone (48).

According Gonococcal Antimicrobial Susceptibility Program under the World Health Organization South East Asia Region continuing in India and neighboring countries and it was observed that in the Indian laboratories, penicillin resistance varied from 20% to 79%, tetracycline resistance from 0% to 45.6%, and ciprofloxacin from 10.6% to 100%. At Sri Lanka, gonococci showed resistance towards penicillin (96.8%) and ciprofloxacin (8.2%). Bangladesh reported *N. gonorrhoeae* with ciprofloxacin (76%), penicillin (33%), and tetracycline (50%) resistance and decreased susceptibility to ceftriaxone (1.5%) (49).

In study on Antimicrobial Susceptibility of *Neisseria gonorrhoeae* from 2004–2006 in Bangui, Central African Republic; Yaoundé´, Cameroon; Antananarivo, Madagascar; and Ho Chi Minh Ville and Nha Trang, Vietnam Ciprofloxacin was highly effective in Africa, but nearly all strains in Vietnam were resistant to this drug. Overall, ceftriaxone and spectinomycin were the best antibiotics, with one strain resistant to spectinomycin in Antananarivo and one strain resistant to ceftriaxone in Ho Chi Minh Ville (50).

In the study, conducted in Kashan, Iran among married women referred to the obstetrics and gynecology clinics in from December 2012 to May 2013 using modified Thayer Martin and all isolates were tested for their susceptibilities to antimicrobials using the Kirby Bauer-disk diffusion techniques. *N. gonorrhoeae* was detected in 2.38% of studied cases (95% confidence interval [CI] 1.5-3.26%). All isolates were resistance to ceftriaxone, penicillin G, ciprofloxacin, cefepime, and two isolate (28.5%) showed intermediate sensitivity to tetracycline(51)and in the same study conducted in south India from January 2013 to December 2015The overall prevalence of gonorrhoea was 9%.and resistance to Penicillin was 100% , Tetracycline 84% ,Ciprofloxacin 53.8%,Cefoxitin 61.5%,Ceftriaxone7%and Cefixime were 15% in HIV positive case (52).

In retrospective study conducted in Bahir Dar Ethiopia the prevalence of *N. gonorrhoeae* was 8.2% and the percentage of *N. gonorrhoeae* isolates non-susceptible to ceftriaxone, ciprofloxacin, tetracycline and penicillin G was 27.8%, 40.9%, 92.6% and 94.4% respectively (53) and in Hawassa the prevalence of *N. gonorrhoeae* 5.1% and most of the cases 45.5%were in age group of 20-24 years and the identified organism had low level susceptibility to quinolones ciprofloxacin 55%, ofloxacin 64% and lomefloxacin 64% respectively (54).

As a study conducted from January 2012 to December 2014 in Hawassa University Referral Hospital, Ethiopia the antimicrobial resistance pattern of *N. gonorrhoeae to* penicillin G was 100% and ceftriaxone were 33.3% (55).

2.3 Treponema pallidum

2.3.1 Microbiology and pathogenesis *Treponema pallidum*

Treponema pallidum is a motile helical bacterium with a central protoplasmic cylinder, cytoplasmic membrane, peptidoglycan and outer membrane, which resembles a Gram-negative bacterium .Its size ranges from a length of 6 μm to 20 μm and a width of 0.10 μm to 0.18 μm, which means light microscopy is inadequate for its visualization, however, it can be viewed using dark-field microscopy(56).

Furthermore, *T. pallidum* is typically deprived of a few common features, such as a lipopolysaccharide and an iron acquisition mechanism(57).However, differs with regard to a

paucity of surface exposed outer membrane proteins capable of eliciting a host response and has demonstrated the ability to evade the host immune system effectively by the process of antigenic variation.(58, 59) And is responsible for a chronic multistage disease, which can present a myriad of clinical complications when untreated, such as neuro syphilis, congenital syphilis, gummas and cardiovascular syphilis (60).

2.3.2 Epidemiology of *Treponema pallidum*

Infectious syphilis continues to be an important public health burden with a global prevalence estimate of 36 million cases and over 11 million new infections annually(61). In a cross-sectional study conducted among male university students who have sex with men in Beijing, China from September 1 to December 31, 2007 the prevalence of syphilis was 7.0% (62).

In other study conducted among students at the Copper belt University Riverside campus, Kitwe Zambia the prevalence of syphilis was 6.5% (males accounted for 3.6% while females accounted for 2.9% the number of students who had sex under influence of alcohol were independently associated with syphilis and number of partners was independently associated with syphilis. And in study conducted from February to October 2009 in Benin ,Nigeria among apparently healthy tertiary educational institution students using RPR and TPHA the prevalence of syphilis was 15.4 % (63, 64).

2.3.3 Laboratory diagnosis of *Treponema pallidum*

2.3.3.1 Microscopy

Dark field microscopic examinations and tests to detect *T. pallidum* directly from lesion exudate or tissue are the definitive methods for diagnosing early syphilis (65).

2.3.3.2 Serological tests

Non-treponemal tests detect antibodies (reagins) that react with lipoidal particles containing cardiolipin and it is used as qualitative assays for screening in the traditional algorithm or as quantitative assays to assess the response to treatment. Screening tests are performed using undiluted serum. The non-treponemal antibodies are detected by the rapid plasma reagin (RPR)

test, the Venereal Disease Research Laboratory (VDRL) test, and the toluidine red unheated serum test (TRUST).Results are reported as non-reactive or reactive. Some specimens give a granular or 'rough' appearance (66, 67).

Treponemal tests which are directed against *T. pallidum* polypeptides have been used for diagnostic purpose. Treponemal antibody are detected by immunofluorescence in the fluorescent treponemal antibody-absorbed (FTA-ABS) test or by agglutination in the *T. pallidum* hemagglutination (TPHA) or *T. pallidum* particle agglutination (TP-PA) test. Traditionally, *T. pallidum* infection has been diagnosed using a non-treponemal screening test, with reactive results confirmed using treponemal serologic tests. Treponemal tests detect both IgM and IgG antibodies. The antibodies detected by treponemal assays appear up to a few weeks earlier than those detected by non-treponemal tests (68, 69).

In contrast to the non-treponemal tests, the treponemal tests are considered to be more specific. However, rare false-positive treponemal results have been recorded, which may be transient and of unknown cause, or associated with connective tissue disorders(70, 71).

2.3.3.3 Molecular tests

Molecular methods are not commonly used in the detection of *T. pallidum* in a clinical setting but can be considered a complimentary technique to be used in combination with conventional dark-field microscopy or serology(69).

The application of molecular methods in the detection of *T. pallidum* DNA has the advantage of being a diagnostic method with the ability to characterize strains susceptible to macrolide antibiotics and the sensitivity of PCR detection assays has been found to vary depending on the specimen types and the stage of disease(72, 73).

2.3.4 Treatment of *Treponema pallidum*

Penicillin G, administered parenterally, is the preferred drug for treating persons in all stages of syphilis. The preparation used (i.e., benzathine, aqueous procaine, or aqueous crystalline), dosage, and length of treatment depend on the stage and clinical manifestations of the disease(1).

2.4 *Trichomonas vaginalis*

2.4.1 Microbiology and pathogenesis of *Trichomonas vaginalis*

Trichomonas vaginalis is a flagellated protozoan possessing five flagella, four of which are located at its anterior portion. The fifth flagellum is incorporated within the undulating membrane of the parasite, which is supported by a slender non contractile costa. This parasite varies in size and shape, with the average length and width being 13 and 10 μm. Its life cycle is simple and involves only the direct transmission of viable trophozoite. Unlike many protozoan parasites, it possesses trophozoite form and lacks cyst stage(74).It attaches to the vaginal epithelium using several *T. vaginalis* adhesins, substances that enable the attachment to epithelial surfaces, have been identified that mediate this binding after binding; it triggers detachment of cells through proteolytic activity, cytotoxicity and apoptosis (75, 76).

2.4.2 Epidemiology of *Trichomonas vaginalis*

An estimated 7.4 million new cases of *T. vaginalis* infection are reported in the United States each year. Prevalence ranges between 2.2% for young women (<20 years) compared with 6.1% in women >25 years. Male prevalence was lower for both age categories, with a reported 0.8% among men< 20 and 2.8% in males >25 year (77).

In cross-sectional study conducted in southwestern, Brazil from January to June 2013 using wet mount the prevalence of *T. vaginalis* was 9% among .women reporting two or more sexual partners in the last year were 3.3 times more likely to acquire the parasite, and those in use of oral contraceptives were 2.7 times more likely to have *T. vaginalis* (78).

According to study conducted in Onitsha community, Nigeria among women the overall prevalence of *T. vaginalis* infection was 17.5% (79).And in study conducted from April to May 2016 among undergraduate female students of Babcock University, Ilishan-Remo, Ogun State, Nigeria the prevalence of *T. vaginalis* was 12.5% (80).

2.4.3 Laboratory diagnosis of *T. vaginalis*

2.4.3.1 Microscopy

Wet mount microscopy has been used for many decades to diagnose TV. The test is inexpensive, low technology and is point of care; however, it is insensitive, particularly in men. Sensitivities range from 50–70 % depending on the expertise of the reader and should be read within 10 min of collection(81).

2.4.3.2 Culture

Culture was considered the gold standard method for diagnosing *T. vaginalis* infection before molecular detection methods became available. culture has better sensitivity than wet mount, in women it is also more expensive and time consuming, has a sensitivity of 75%–96% and a specificity of up to 100%.However, it demonstrates poor sensitivity in men (82, 83).

2.4.3.2 Molecular Testing

Nucleic acid amplification tests, such as PCR or transcription-mediated amplification (TMA), are generally more sensitive than non-amplified tests. TMA assay has been compared with an earlier widely used research PCR assay and was found to be extremely sensitive and specific (84).

2.4.4 Treatment of *Trichomonas vaginalis*

Metronidazole and Tinidazole can cure TV. Systemic therapy is preferred over topical applications to achieve adequate drug concentrations in non vaginal sites such as the urethra and periurethral glands. Cure rate for single 2-g dose oral metronidazole is 90% to 95%, whereas Tinidazole cure rate approaches 100% (85).

CHAPTER THREE: OBJECTIVES OF THE STUDY

3.1 GENERAL OBJECTIVE

- To determine the prevalence and assess associated risk factors of *N. gonorrhoeae, T. pallidum* and *T. vaginalis* infections and Antimicrobial Susceptibility Pattern of *N. gonorrhoeae* among Jimma university main campus students suspected to have sexually transmitted infections and presenting at the student clinic from April to October 2017.

3.2 Specific objectives

- To determine the prevalence of *Neisseria gonorrhea* among Jimma university main campus students suspected for sexual transmitted infections.
- To determine the prevalence of *T. vaginalis* among Jimma university main campus students suspected for sexual transmitted infections.
- To determine the prevalence of *T.pallidum* among Jimma university main campus students suspected for sexual transmitted infections.
- To determine the antimicrobial susceptibility pattern of *N. gonorrhoeae* isolates among Jimma university main campus students suspected for sexual transmitted infections.
- To assess associated risk factors of STI among Jimma university main campus students presenting to the student clinic with genitourinary complaints.

CHAPTER FOUR: METHODS AND MATERIALS

4.1 Study area

The study was conducted at Jimma University main campus student clinic. Jimma University is located in Oromia regional state, 352 km from Addis Ababa, in the southwest of the country and is one of the public higher education institutions in Ethiopia. The university currently has three campuses: (I) Main Campus; (II) College of Agriculture and Veterinary Medicine; and (III) Kitofurdisa campus (Institute of Technology/IOT). Our study was conducted at the Main Campus student clinic which gives service for 11,068 regular under graduate students in the four collages (College of Health Sciences, College of Natural Science, College of Social Science and Law and college of Business and Economics) (86).

4.2 Study design and Study period

Health facility based cross-sectional study design was conducted from April, 2017 to October 2017.

4.3 Population

4.3.1 Source population

The source population was all Jimma university main campus students who attended the student clinic during the study period.

4.3.2 Study population

All Jimma university main campus students suspected to have sexually transmitted infections and presenting to the student clinic during the study period.

4.4 Eligibility Criteria

4.4.1 Inclusion criteria

All Jimma university main campus students who attended the student clinic for diagnosis and treatment of sexually transmitted infections and gave their consent to be enrolled in the study during the study period.

4.4.2 Exclusion criteria

- Students who have a history of antibiotic and anti-protozoan treatment during the past two weeks prior to enrollment in or conduct of the study

4.5 Sample size and sampling technique/sampling procedure

I. Sample size

The sample size was determined by using single population proportion formula as stated below. Taking 95% confidence interval and ±5% marginal error, sample size (n) is determined using the following statistical formula.

$$n = \frac{\left(\mathrm{Z}\ 1 - \frac{\alpha}{2}\right) 2\ \mathrm{p}\ (1 - \mathrm{p})}{\mathrm{d}2}$$

$$n = \frac{(1.96)2 \text{ x } 0.113\text{x } (\ 1 - 0.113)}{(0.05)2}$$

n= 154

Where, P= Prevalence rate of *N. gonorrhoeae* 11.3% form pervious study of Gambella (41)

n = Sample size,

Z = Z = 95% confident interval

d=margin of sampling error tolerated (0.05)

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

Thus, considering 10% non-respondent rate the total sample size was **169**

II. Sampling technique

All Jimma university main campus students suspected for sexual transmitted infection coming to the university student clinic during the study period were consecutively enrolled provided that inclusion criteria were fulfilled to achieve the intended sample size.

4.6 Measurements

4.6.1 Study variables

4.6.1.1 Dependent variables

- Prevalence of *N. gonorrhoeae* ,*T. pallidum* and *T.vaginalis*
- Antimicrobial susceptibility pattern of *N. gonorrhoeae*

4.6.2 Independent variables

- Socio-demographic characteristics (age, sex, faculty/college, year of study)
- Sexual behaviors (number of sexual partner ,habit of condom use, sex after alcohol, habit of condom use after taking alcohol , sex with commercial sex workers)
- Substance use (alcohol use, *khat* chewing habit)

4.7 Data collection

4.7.1 Socio-demographic, sexual behavior and patient identification

A pre-tested structured self-administered questionnaire was used to collect socio-demographic data and risky sexual behaviors by attending nurses. (Annex II).

4.7.2 Laboratory data

I. Collection of clinical samples for *N. gonorrhoeae, T. vaginalis* and *T. pallidum*

Two cervical and one vaginal swab were taken from female students while three urethral swabs were taken from male students by attending nurses at Jimma university main campus student clinic. The first cervical and urethral swab were inserted aseptically in in Amies transport medium (Oxoid, UK) then transported to Jimma university medical center microbiology laboratory service and the second cervical and urethral swab were smeared on slide for gram stain at the student clinic laboratory immediately after collection. The first cervical and urethral swab were inoculated on seeded Modified Thayer Martin medium (MTM)(Oxoid,England) while rotating it to ensure that all surfaces of swabs tip come in contact with the medium and were incubated at 35-37^{0}C in the presence of 5% Carbon dioxide for 24-72 hours. Finally, if there is growth after the incubation gram staining and proper biochemical tests were done in Jimma university medical center microbiology laboratory for isolated microorganisms to identify *N. gonorrhoeae* (87).

The vaginal swab and the third urethral swab was placed in a tube containing sterile 0.5 ml physiologic saline and kept at room temperature for not longer than 30 minutes before examination to detect *T. vaginalis* trophozoites (88).

Venous blood collection: Five ml of venous blood was collected from the antecubital vein of each study participant into anticoagulant free sterile tubes. The blood was allowed to settle for 30 minutes and then centrifuged at 2000 rpm for 5 minutes and the serum was obtained. All serum samples were tested for *T. pallidumn* using one Rapid *immunochromatographic* test kit.

4.8 Specimen processing

4.8.1 Culture and Identification of *N. gonorrhoeae*

Urogenital specimens were inoculated on MTM medium suitable for isolating *N. gonorrhoeae.* The inoculated plate was incubated in 5% carbon dioxide incubator at 37^{0}C for up to72 hrs while being examined for growth every 24 hours of incubation. *N. gonorrhoeae* was suspected when small raised and grey shiny colonies grew on MTM. Single colony was sub cultured on a GC-

chocolate agar supplemented with 1%Vitox (Oxoid, England) and incubated at35-37^{0}C for overnight. For identification of the pathogen, Gram staining and standard biochemical tests (including oxidase, superoxol & carbohydrate utilization tests) and using Analytical Profile Index for Identification of *Neisseria* and *Haemophilus* (API NH) kit strips (BioMerieux, France) were performed. Isolates that were oxidase positive, superoxol positive and fermenting only glucose considered as *N. gonorrhoeae* (87).

4.8.2 Antimicrobial susceptibility testing of *N. gonorrhoeae*

Antimicrobial susceptibility was assessed by using Kirby-Bauer disk diffusion test, according to CLSI (89).From GC-chocolate agar culture medium 3-5 isolated colonies of bacteria was transferred and mixed in to a tube containing 2.5 ml sterile normal saline until the turbidity of the suspension become comparable to a 0.5 McFarland standard. The bacterial suspensions were swabbed evenly to distribute the bacteria over the entire surface of GC-chocolate agar with 1 % Vitox supplement using a sterile swab. The susceptibility of isolates to the following antimicrobial agents was assessed: penicillin (P10 IU), tetracycline (TE 30µg), ciprofloxacin (CIP 5µg), ceftriaxone (CRO 30µg), cefixime (CFM 5µg)and cefoxitin(FOX 30µg) (Oxoid ,England).Susceptible standard reference strain of *N. gonorrhoeae* ATCC 49226 were used as recommended by the Clinical and Laboratory Standards Institute (CLSI) 2016 for QC of susceptibility testing of gonococcal isolates (89).

4.8.3 Wet mount for *T. virginals*

Vaginal swab and urethral swab were inserted into a test tube and few drops of normal saline was placed. The swab (sample) was thoroughly mixed with the saline to get well homogenized sample and then the swab was discarded. A drop of homogenized sample was taken using a pipette and added on to a clean, grease-free slide, covered with cover slip and examined microscopically**.** The microscopic examination was began using the 10x objective noting cellular distribution and the 40x objective was used to identify the presence of motile *T. vaginalis* trophozoites.

4.8.4 Rapid immunochromatographic test for the diagnosis of syphilis

Rapid immunoassay was used for detection of Treponemal specific *IgM, IgG* antibodies. The test was performed using immunochromatographic kit manufactured by In Tec PRODUCTS, INC.(XIAMEN,China).Test protocol and result interpretation was done according to the manufacturer instruction (AnnexIII).

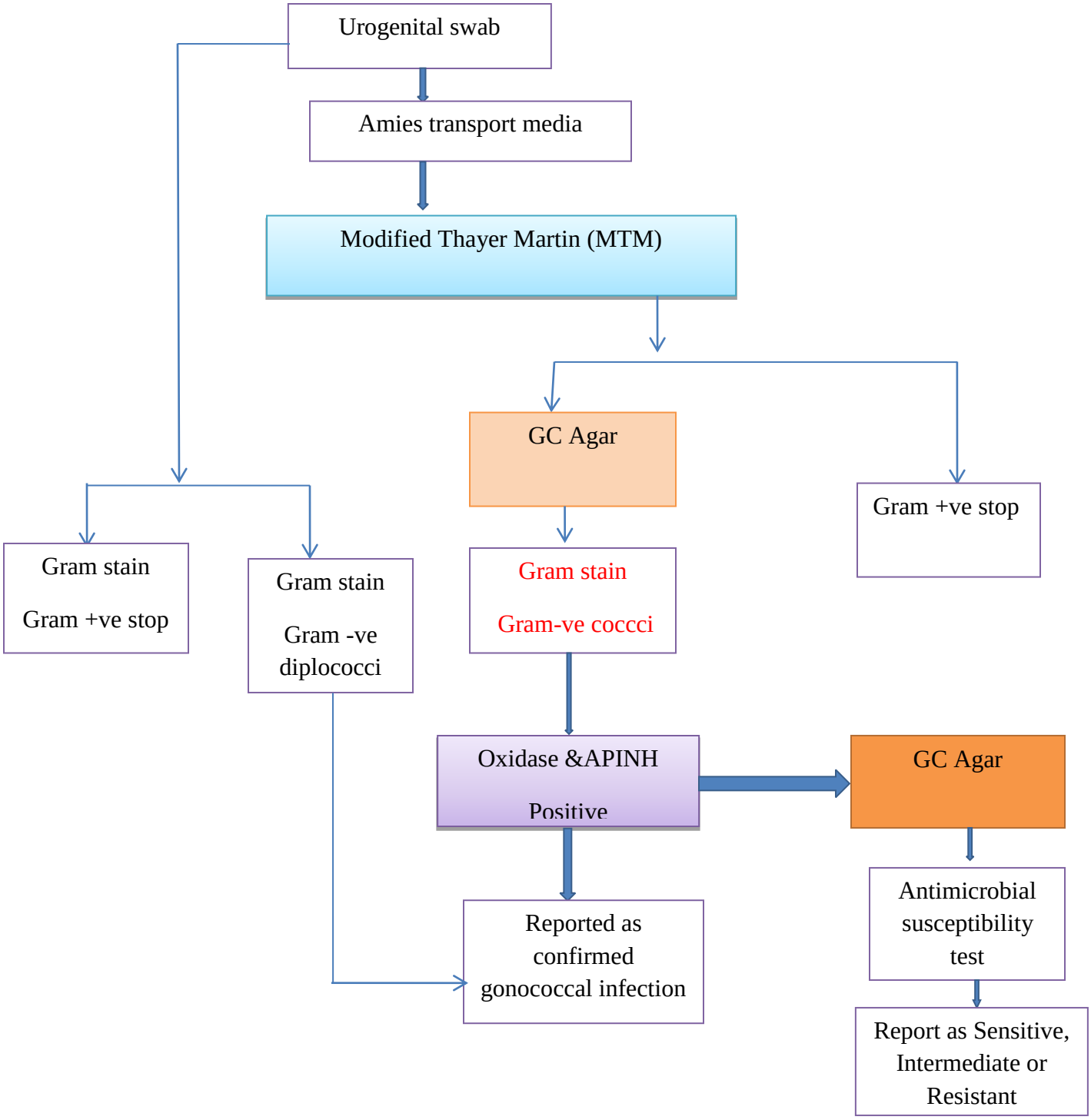


Figure 1. Flow chart for urogenital Specimen processing for *N. gonorrhoeae* detection collected from Jimma university main campus students suspected for sexual transmitted infections seen from April to October 2017at Jimma university main campus student clinic, Ethiopia.

4.9 Data quality assurance

The following measures were undertaken to control the quality of the data and laboratory investigation. Properly designed and pre-tested data collection questionnaire was used. Every day the collected data were cross checked for completeness, consistency and onsite corrective action was made. Standard operational procedures were strictly followed for sample collection; storage, transportation, analysis, and recording. All reagents were checked for being stored at appropriate temperature and used within specified shelf life. To avoid measurement bias, quality control was run along with the test sample according to the manufacturer's instruction and test procedures.

4.10 Data Analysis

Data were entered into Epi data version 4.0.2.101 software and exported into SPSS version 20.0 and the statistical analyses were performed by using SPSS version 20.0.Descriptive statistics, Fisher's exact test and Chi-square tests were carried out. 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 less than 0.25 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 $P < 0.05$ was reported with 95 % CI and AOR as determinant factor for STI.

4.11 Ethical Consideration

Ethical clearance was obtained from Jimma University Ethical Review Board. Support letter was obtained from school of medical laboratory sciences and dean of student service of Jimma University. As far as the confidentiality is concerned, all information's of the participant were kept confidential and never accessed by a third person. To keep the confidentiality no personal identifiers were used on data collection form. Written consent was obtained to participate in the study from each participant who was involved in the study.

CHAPTER FIVE: RESULT

Socio demographic characteristics:

A total of 189 students suspected for sexual transmitted infections were included in the study with 100% response rate from April to October, 2017. Fifty eight of the total 189(30.7%) students were males and the remaining 131 (69.3%) were females. Majority of the patients 75.7% (143/189) were twenty and greater than twenty years of age whereas the remaining forty six (24.3%) were in less than twenty years age group. The distribution of the students across the university colleges appeared as fifty two (27.5%) were from college of Natural Science and forty two (22.2%) from college of Public health and Medical sciences. Forty nine (29.5%) of the students were form study year one and sixty one (32.3%) were form study year II and the rest seventy nine (41.8%) were year III and above **(Table 1.)**.

Table 1.Socio-demographic characteristics of Jimma university main campus students suspected for sexual transmitted infections (n=189) seen at Jimma university main campus student clinic from April to October, 2017, Jimma; Ethiopia.

Socio-demographic characteristics		Frequency	percentage
Gender	Male	58	30.7%
	Female	131	69.3%
Faculty/college	Public health medical science	42	22.2%
	Social science and Law	51	27%
	Natural science	52	27.5%
	Business and Economics	44	23.3%
	Year I	49	25.9%
Academic year	Year II	61	32.3%
	Year III and above	79	41.8%
Age in years	<20 years	46	24.3%
	≥20 years	143	75.7%

Prevalence of *N. gonorrhoeae*, *T. pallidum* and *T. vaginalis*

From the total of 189 patients suspected for sexual transmitted infections 27(14.3%) patients were infected by one or more of the three STIs. The prevalence of *N. gonorrhoeae* was accounted for 7.4 % (14/189*), T. pallidium* for 3.7 %(7/189) and *T. vaginalis* accounted for 4.8 %(9/189) respectively. Two patients with *N. gonorrhoeae* were co-infected with syphilis and one patient with *N. gonorrhoeae* was infected with *T. vaginalis* **(Fig 2.)**.

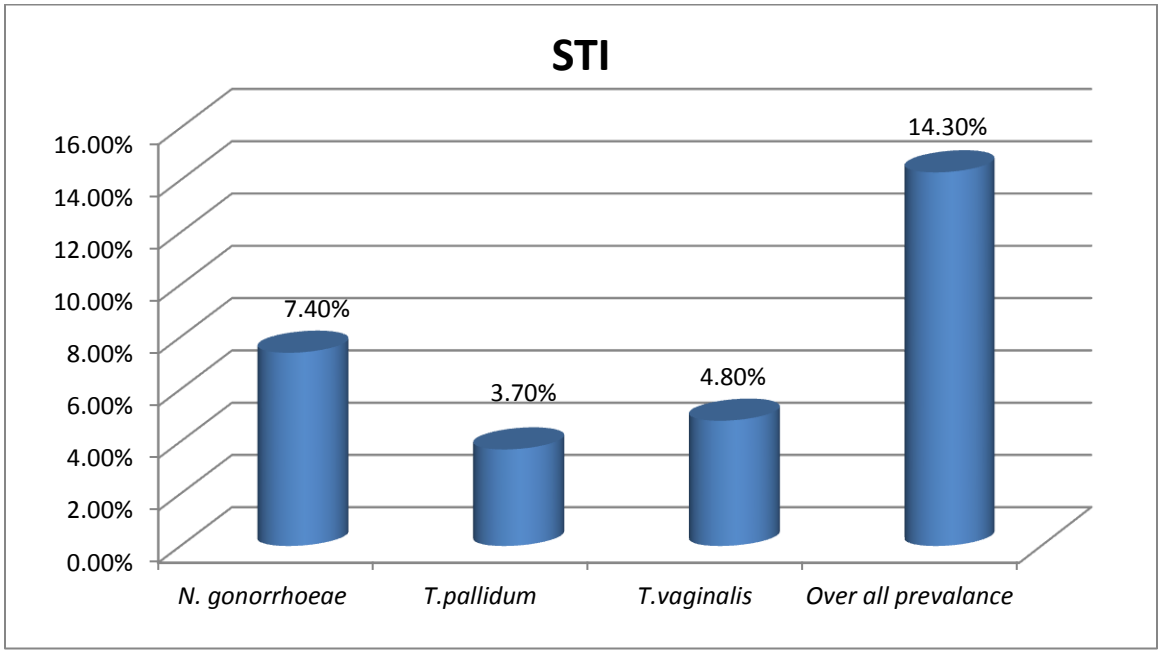


Figure 2. Prevalence of *N. gonorrhoeae, T. pallidum* and *T. vaginalis* infection among sexual transmitted infections suspected Jimma university main campus students who visited Jimma university main campus student clinic (n=189), Jimma ,Ethiopia, from (April-October 2017**)**

Prevalence of *N. gonorrhoeae*

Among a total of189 patient 14(7.4%) patients were confirmed to have gonococcal infection. Of the 14 patients who were confirmed to have gonococcal infection, nine (15.5%) were males and five(3.8%) were females. The prevalence of gonococcal infection was higher in male patients than females and the difference was statistically significant (P=0.012).Seven (8.9%) of 14 patients who had confirmed to have gonococcal infection were year III and above and there is no significant association between academic years and gonococcal infection (p=0.828). Twelve of the 14 patients who were confirmed to have gonococcal infection were in age group of ≥ 20

years of age .The differences in proportion of gonococcal infection with age groups was not statistically significant(p=0.524).**(Table2.)**.

Table 2.Distribution of *N. gonorrhoeae* infection in relation to Socio-demographic characteristics of sexual transmitted infections suspected Jimma university main campus students(n=189) seen at Jimma university main campus student clinic, Ethiopia from (April-October, 2017)

Socio-demographic characteristics		*N. gonorrohoea* infection			
		Positive (n=14) n (%)	Negative (n=175) n(%)	Total (n=189) n (%)	Fisher's Exact test p-value
Gender	Male	9(15.5)	49(84.5)	58(30.7)	
	Female	5(3.8)	126(98.2)	131(69.3)	0.012
Faculty/ college	Public Health &Medical Sciences	2(4.8)	40(95.2)	42(22.2)	
	Social Sciences & Law	3(5.9)	48(94.1)	51(27.0)	
	Natural Science	4(7.7)	48(92.3)	52(27.5)	
	Business& Economics	5(11.4)	39(88.6)	44(23.3)	0.730
Academic Year	Year I	3(6.1)	46(93.9)	49(25.9)	
	Year II	4(6.6)	57(93.4)	61(32.3)	
	Year III and above	7(8.9)	72(91.1)	79(41.8)	0.828
Age in years	<20 years	2(4.4)	44(95.6)	46(95.6)	
	≥ 20 years	12(8.4)	131(91.6)	143(91.6)	0.524

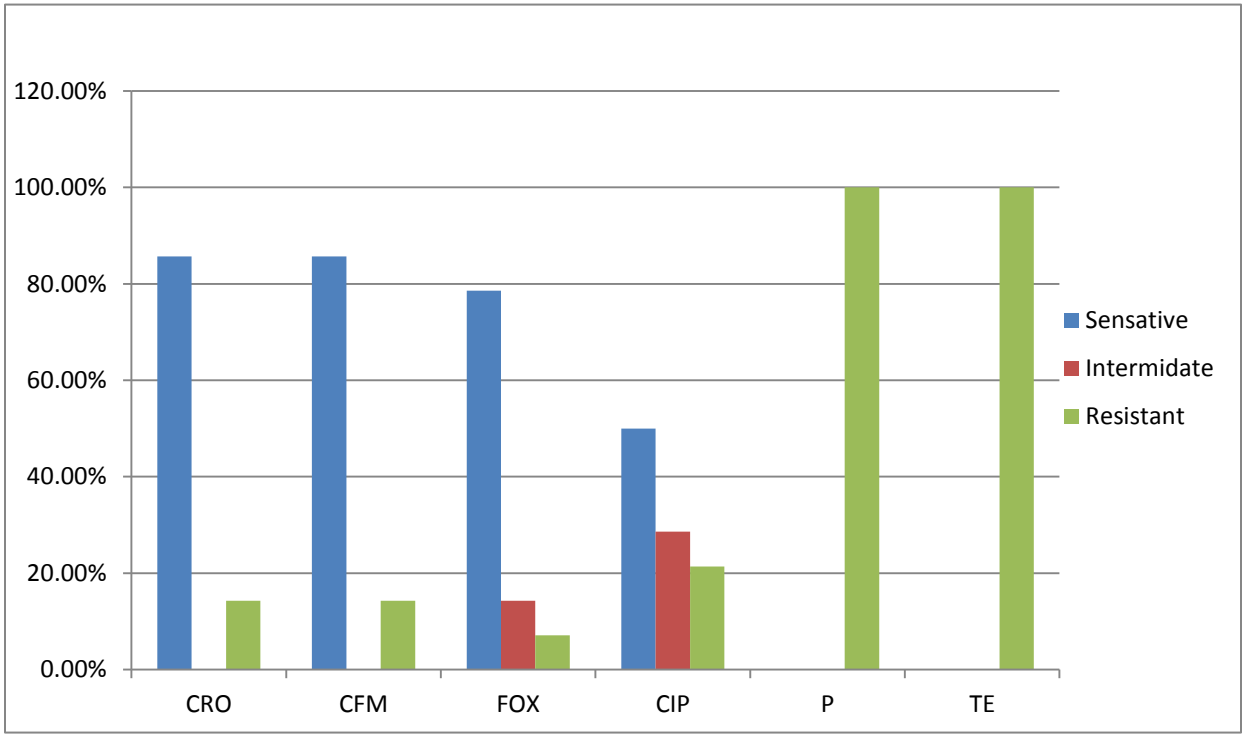
Among fifty six (29.62%) respondents who had more than one sexual partners, eleven (19.6%) were positive for gonococcal infection and it was significantly associated with gonoccocal infection (p=0.000).Among the total of 58 male patients, three male patients (5.5%) had sex with commercial sex workers from whom 2(66.7%) were infected with gonococcal infections and it was not significantly associated with gonococcal infection (p=0.060).Among forty(21.20%) patients have had sex after using alcohol eleven (28.2%) were positive for gonoccocal infection and it was significantly associated with gonoccocal infection (p= 0.000). The proportion of gonococcal infection among patients used condom after having alcohol were 16.7% and 32.1% among patients were not used condom after having alcohol and there is no association between gonoccocal infection and not using condom after having alcohol (p=0.451).In association with *khat* chewing among 189 patients 27(14.3%) were chew chat from this four (14.8%) were positive for gonococal infection and there is no association with gonoococcal infection *khat* chewing (p=0.120). **(Table3.)**.

Table 3.Distribution of *N. gonorrhoeae* infection in relation to risky sexual behaviors among sexual transmitted infections suspected Jimma university main campus students (n=189) seen at Jimma university main campus student clinic Jimma; Ethiopia from (April-October, 2017)

Sexual behavior		*N. gonorrhoeae* infection			
		Positive	Negative	Total	Fisher's Exact test p-value
Number of sexual partners(n=189)	one	3(2.3)	130(97.7)	133(70.4)	
	>1	11(19.6)	45(80.4)	56(29.6)	0.000
Ever used condom(n=189)	Yes	9(8.7)	97(91.3)	106(56.1)	
	NO	5(5.9)	78(94.1)	83(43.9)	0.586*
Had sex with CSW(n=58)	Yes	2(66.7)	1(33.3)	3(5.2)	0.060
	NO	7(12.7)	48(87.3)	55(94.8)	
Sex after alcohol use (n=189)	Yes	11(28.2)	29(71.8)	40(21.2)	0.000
	NO	3(2.0)	146(98.0)	149(78.8)	
Condom use after having alcohol (n=40)	Yes	2(16.7)	10(83.3)	12(30.0)	0.451
	NO	9(32.1)	19(67.9)	28(70.0)	
Chew *khat*(n=189)	Yes	4(14.8)	23(85.2)	27(14.3)	0.120
	NO	10(6.2)	152(93.8)	162(85.7)	

CSW=commercial sex worker ****=chi-square test***

Antimicrobial Susceptibility Testing for *Neisseria gonorrhoeae*: In our study the susceptibility patterns of isolated *Neisseria gonorrhoeae* (n=14) was done against six antimicrobial agents by the agar disc diffusion technique.85.7% *Neisseria gonorrhoeae* isolates were susceptible to ceftriaxone and cefixime, 78.6% and 50% were sensitive to cefoxitin and ciprofloxacin respectively. All isolates were resistant to penicillin and tetracycline. 21.4% of resistance was also seen against ciprofloxacin and 7.1% were resistant for cefoxitin. Moreover, intermediate resistance was seen in 28.6% for ciprofloxacin and 14.3% % of the cefoxitin **(Fig.3)**.



P: Penicillin, TE: Tetracycline, CIP: Ciprofloxacin CRO: Ceftriaxone; FOX: Cefoxitin; CFM: Cefixime

Figure 3. Antimicrobial Susceptibility Patterns of *N. gonorrhoeae* from sexually transmitted infection suspected Jimma university main campus students seen at Jimma university main campus student clinic, Ethiopia (April-October, 2017)

Prevalence of *T. vaginalis*

Of all study participants tested for *T. vaginalis* infection nine (6.9%) female patients were positive for *T. vaginalis*. There was no male patient positive for *T. vaginalis* and there was no significant association between *T. vaginalis* infection and female gender (p=0.059). With regard to college fifty one of patients were from college of social science and law from this five (9.8%) were positive for *T. vaginalis* infection there is no significant association between *T. vaginalis* infection and college. In relation to academic year forty nine patients were year I and from this five (10.2%) were infected with *T. vaginlais*. In association with age four (8.7%) patients were infected with *T. vaginalis* were in less than 20 years age group .There is no significant association between *T. vaginalis* infection, Academic years and age **(Table 4.)**.

Table 4. Distribution of *T. vaginalis* infection in relation to socio-demographic characteristics Jimma university main campus students (n=189) suspected for sexual transmitted infections seen at Jimma universty main campus student clinic, Ethiopia (April-October, 2017)

Socio-demographic characteristics		*T.vaginalis* infection			
		Positive (n=9) n (%)	Negative (n=180) n (%)	Total (189) n(%)	Fisher's exact test p-value
Gender	Male	0	58(100)	58(30.7)	0.059
	Female	9(6.9)	122(93.1)	131(69.3)	
Facility/ college	Public Health& Medical Sciences	2(4.8)	40(95.2)	42(22.2)	0.166
	Social Sciences & Law	5(9.8)	46(90.2)	51(27.0)	
	Natural Science	2(3.8)	50(96.2)	52(27.5)	
	Business &Economics	0	44(100)	44(23.3)	
Academic Year	Year I	5(10.2)	44(89.8)	49(25.9)	0.182
	Year II	2(3.3)	59(96.7)	61(32.3)	
	Year III and above	2(2.5)	77(97.5)	79(41.8)	
Age in years	<20 years	4(8.7)	42(91.3)	46(24.3)	0.225
	≥ 20 years	5(3.5)	138(96.5)	143(75.7)	

Regarding number of sexual partners of the nine *T. vaginalis* positive patients five (8.9%) were had more than one sexual partners. There was no significant association between *T. vaginalis* infection and number of sexual partners (p=0.128). From the nine *T. vaginalis* positive patients six patients (7.2%) were not ever used condom. There was no significant association between *T. vaginalis* infection and condom use. Form forty patients (21.2%) had sex after had used alcohol from this six (15.0%) were positive for *T. vaginalis* infection .There was significant association between *T. vaginalis* infection and having had sex after taking alcohol (p=0.003).Among the nine *T. vagialis* positive patients 3(10.7%) patients were not used condom after they had used alcohol .There was no significant association between using condom after taking alcohol and *T. vaginalis* infection. Twenty seven (14.3%) were chew chat from this 2(7.4%) were infected with *T. vaginalis* there was no significant association between chewing chat and *T. vaginalis* infection **(Table5.)**.

Table 5.Distribution of *T. vaginalis* infection in relation risky sexual behaviors among sexual transmitted infection suspected Jimma university main campus students (n=189) seen at Jimma university main campus student clinic, Ethiopia from (April-October, 2017)

Sexual behaviors		*T .vaginalis infection*			
		Positive n(%)	Negative n(%)	Total	Fisher's Exact test p-value
Number of sexual partners (n=189)	One	4(3.0)	129(97.0)	133(70.4)	0.128
	>1	5(8.9)	51(91.1)	56(29.6)	
Ever used condom	Yes	3(2.8)	103(97.2)	106	0.184
	NO	6(7.2)	77(92.8)	83	
Sex after taking alcohol	Yes	6(15.0)	34(75.0)	40(21.2)	0.003
	NO	3(2.0)	146(98.0)	149(78.8)	
Condom use after taking alcohol (n=40)	Yes	3(25.0)	9(75.0)	12(30.0)	0.341
	NO	3 (10.7)	25(89.3)	28(70.0)	
Chat chewing (n=189)	Yes	2(7.4)	25(92.6)	27(14.3)	0.619
	NO	7(4.3)	155(95.7)	162(85.7)	

Prevalence of *T. palidium*

Among 189 patients included in this study seven (3.7%) were positive for *T. palidium*. And the proportion of *T. palidium* infection were three (5.2%) for males and Four (3.1%) were for females. There was no significant association between gender and gonococcal infection (p=0.441).Three (5.9%) of the seven *T. palidium* positive patients were from college of social since and law (p=0.690) there was no significant association between *T. palidium* infection and college. Of the seven *T. palidium* infected patients three (6.1%) were year I students (p=0.296) and two (4.3%) of the seven syphilis positive patients were less than 20 years of age (p=0.678) there was no significant association between *T. palidium* infection, academic year and age group **(Table6.)**.

Table 6.Distribution of *T. palidium* infection in relation to socio-demographic characteristics of sexual transmitted infections suspected Jimma university main campus students (n=189) seen at Jimma university main campus student clinic, Ethiopia from (April-October, 2017)

Socio-demographic characteristics		*T. pallidum* infection		Total (n=189) n (%)	
		Positive (n=7) n (%)	Negative (n=182) n (%)		Fisher's Exact test p-value
Gender	Male	3 (5.2)	55(94.8)	58(30.7)	
	Female	4(3.1)	127(96.9)	131(69.3)	0.441
Faculty/ college	Public Health& Medical Sciences	2(4.8)	40(95.2)	42(22.2)	
	Social Sciences & Law	3(5.9)	48(94.1)	51(27.0)	0.690
	Natural Science	1(1.9)	51(98.1)	52(27.5)	
	Business & Economics	1(2.3)	43(97.7)	44(23.3)	
Academic Year	Year I	3(6.1)	46(83.9)	49(25.9)	0.296
	Year II	3(4.9)	58(95.1)	61(32.3)	
	Year III and above	1(1.3)	78(98.7)	79(41.8)	
Age in years	<20 years	2(4.3)	44(95.7)	46(24.3)	0.678
	≥ 20 years	5(3.5)	138(96.5)	143(75.7)	

In our study the prevalence of *T. pallidum* infection among patients had more than one sexual partners were 6 (10.75%) and it was significantly associated with *T. pallidum* infection (p=0.003) .Regarding having sex after taking alcohol among seven positive for *T. pallidum* infection 6(15.0%) patients were having had sex after alcohol intake. There was significant association between *T. pallidum* infection and having had sex after taking alcohol (p=0.000).In our study among seven positive for *T. pallidum* five (17.9%) patients were not used condom after have took alcohol there was no significant association between *T. pallidum* infection and using condom after taking alcohol (p=0.684). Out of the seven *T .pallidum* infected patients two (7.4%) were chew *khat* (p=0.262) .There is no significant association between *T. pallidum* infection and *khat* chewing **(Table7.)**.

Table 7.Distribution of *T. pallidum* infection in relation to risky sexual behaviors among Jimma university main campus students suspected for sexual transmitted infection (n=189) seen at Jimma university main campus student clinic Ethiopia (April-October, 2017)

Variables		*T .palladium* infection			Fisher's Exact test
Sexual behaviors		Positive	Negative	Total	p-value
Number of sexual partners	One	1(1.00)	132(99.0)	133(70.4)	
	>1	6(10.7)	50(90.3)	56(29.6)	0.003
Ever used condom	Yes	3(2.9)	103(97.1)	104(55.0)	
	No	4(4.7)	79(95.3)	85(45.0)	0.701
Sex after taking alcohol	Yes	6(15.0)	34(85.0)	40(21.2)	0.000
	No	1(1.0)	148(99.0)	149(78.8)	
Condom use after taking alcohol	Yes	1(8.3)	11(91.7)	12(30.0)	
	No	5(17.9)	23(82.1)	28(70.00)	0.684
Chewing *khat*	Yes	2(7.4)	25(92.3)	27(14.3)	0.262
	No	5(3.1)	157(96.9)	162(85.7)	

Factors associated with sexually transmitted infection

In bivariate logistics regression analysis it was found from Socio demographic variables that gender and academic year I were candidate for multivariate logistic regression analysis ($p<0.25$).Males had high odds of having STIs COR (95%CI) =1.68 (0.73-3.94) than females, students in academic year I had high odds of having STIs COR (95%CI) =1.77(0.68-4.62) than year II and above students. From sexual behavior and substance use variables having more than one sexual partner, having had sex after taking alcohol, *khat* chewing were the candidate variables for multivariate logistic regression analysis. Students who had more than one sexual partners were eight times more likely to have STIs COR (95%CI) = 8.02(3.25-19.81) than who had one sexual partners. Students who had sexual intercourse after taking alcohol were twenty times more likely to have STIs COR (95%CI) =20.29(7.6254.04) than those didn't have intercourse after taking alcohol. Students who chew *khat* had high odds of STIs COR (95%CI) =1.92(0.69-5.30). Finally in the multivariate logistic regression analysis only patients who had sex after using alcohol was independently associated with sexually transmitted infection (p=0.000,AOR=18.79(95%CI:5.00-70.70) (**Table.8**).

Table 8. Factors associated with the selected STIs among Jimma university main campus students suspected for sexual transmitted infections (n=189) seen at Jimma university main campus student clinic, Ethiopia (April-October, 2017)

Variables	STI		COR		AOR	
	Positive n(%)	Negative n(%)	(95%CI)	p-value	(95%CI)	p-value
Gender						
Male	11(19.0)	47(81.0)	1.68(0.73-3.89)	0.225	0.74(0.23-2.34)	0.608
Female	16(12.2)	115(87.8)	1.00			
Faculty/college						
Public health & Medical science	6(14.3)	36(95.8)	1.30(0.37-4.63)	0.686		
Social science & Law	10(19.6)	41(80.4)	1.90(0.60-6.07)	0.277		
Natural science	6(11.5)	46(88.5)	1.02(0.29-3.59)	0.979		
Business & Economics	5(11.4)	39(88.6)	1.00			
Academic years						
Year I	10(20.4)	39(79.6)	1.77(0.68-4.62)	0.244	3.03(0.84-10.92)	0.089
Year II	7(11.5)	54(88.5)	0.89(0.32-2.50)	0.832		
Year III &above	10(12.7)	69(87.3)	1.00			
Age in years						
<20 years	8(17.4)	38(82.6)	1.37(0.58-3.38)	0.490		
≥20 years	19(13.3)	124(86.7)	1.00			

NSP						
One	8(6.00)	125(94.0)	1.00			
More than one	19(33.9)	37(66.1)	8.02(3.25-19.81)	0.000	2.09(0.62-7.10)	0.223
Ever used condom						
Yes	13(12.3)	93(87.7)	1.00			
NO	14(16.9)	69(83.1)	1.45(0.64-3.29)	0.371		
Sex after taking alcohol						
Yes	20(50.0)	20(50.0)	20.29(7.6254.04)	0.000	18.79(5.00-70.7)	0.000
NO	7(4.7)	142(95.3)	1.00			
Condom use after taking alcohol						
Yes	5(25.0)	15(75.0)	1.00			
NO	7(35.0)	13(65.0)	1.62(0.41-6.34)	0.492		
Chewing *khat*						
Yes	6(22.2)	21(77.8)	1.92(0.69-5.30)	0.209	1.5(0.41-5.59)	0.539
NO	21(13.0)	141(87.0)	1.00			

COR=crude odd ratio; AOR=adjusted odd ratio; CI=95% confidence interval 1=reference NSP=Number of sexual partners

CHAPTER SIX: DISCUSSION

This study provides the prevalence and associated risk factors of three main STIs among Jimma university main campus students suspected for sexual transmitted infections who attended the university main campus student clinic from April to October 2017. The overall prevalence of the three STIs was 14.3%. The prevalence of *N. gonorrohoea, T. vaginalis* and *T. pallidum* were7.4%, 4.8% and 3.7% were respectively.

In our study the most prevalent sexually transmitted infection was *N. gonorrohoea* (7.4%) which is higher than the study reported from Hawassa, Ethiopia (5.1%)(54), Port Harcourt, Rivers state, Nigeria (5%)(37)and Abuja university Nigeria (2.9%)(35). The reason might be difference in study population and difference in study area or increased resistance strains may increase the gonococcal infection rate. The study conducted in Hawassa focused on gynecology cases while and only female students in Port Harcourt, Rivers state, Nigeria.

On the other hand the prevalence of gonococcal infection in the present study was lower than compared to17.2% reported from Jimma (90),11.3% from Gambella Ethiopia(41)20.8% from Gonder (40),14%from Southern Mozambique(38) and 59% from Uganda (36). The variation in prevalence rate in those studies may be because of variation in study population university students may have more awareness on sexually transmitted infection than general population.

In our study the prevalence of gonococcal infection was higher in males 9(15.5%) than females 5(3.8%).This finding is comparable with the finding from Gambella where male 17(16.0) and female4(5.0)reported (41). This might be due to gonococal infection is more symptomatic in males than females.

In present study the second prevalent sexually transmitted infection was *T. vaginalis* (4.8%) which is similar with the finding reported from(4.7%), Abuja university Nigeria(35).The finding of this study is lower than reports from (14.2%) Gonder Ethiopia(40),(12.5%)Babcock University Ogunstate from Nigeria(80), (9%) from Brazil(78). On the other hand the prevalence of this study is higher than the study conducted in Nairobi Kenya(0.4%)(39).The observed

difference in the rate of infection could be due to variation in study population, study period and variation in age distribution, personal hygiene practice, and climatic conditions .

In the present study all students with *T. vaginalis* were females. This finding is similar with the study repotted from Abuja university Nigeria(35)and Gonder (40).This might be due to females patients are more symptomatic than males.

In this study the prevalence of *T. pallidum* infection was (3.7%).The finding of this study is higher than with the finding(0%)from Addis Ababa Ethiopia(91),(0.1%) from Jigjig a(92),(2.4%)from Abuja Nigeria(35).The variation on the prevalence rate may be due to differences in target population including ANC follow up attendants from Addis Ababa, healthy blood donors Jigjig a. On the other hand the prevalence of *T. pallidum* in this study is lower compared to other reports like (30%) from Gonder (40)(7%) from Beijing China(62),(6.5%) from Copper belt university river side campus in Zambia(79),(15.4%) from Benin Nigeria(66).The variation on the prevalence rate of infection may be due to differences in target population and sample size. General population in Gonder ,Males who have sex with males was included in Beijing china(62)whereas it was apparently healthy students in Benin Nigeria(66)

Although, the rate of STI with respect to presence of multiple sexual partners has shown some level of importance, the weight was not significant [AOR (95%CI) = 2.09(0.62-7.10)] (p= 0.223).Our study is in line with the study conducted on Jimma ART attendees (90) .Risky behavior like excessive alcohol intake may have increased the risk of having multiple sexual partners.

In our study having had sex after taking alcohol was independently associated with STI (p=0.000) and its odds ratio[AOR(95%CI)= 18.79(5.00-70.7)].This is similar with study documented on ART attendees Jimma(90).Mozambique(38).This might be due to alcohol consumption has an instating effect that may make engaged in risky sexual behaviors.

In the absence of any effective vaccine against *N. gonorrhoeae* control of gonococcal infection mainly depends on the identification and treatment of infected individuals. An early and successful antibiotic treatment of gonococcal infection is important for cure of the individual patient, prevention of complications, and reduction of transmission.

In the present study 100% resistance to penicillin and tetracycline was recorded which was analogous to 100 % resistant reported to penicillin and tetracycline in Gambella Ethiopia(41)and India(93).Such resistant may be due to the fact that emergence of penicillin resistant beta-lactamase producing strains. (85.7%) of our isolates were penicillinase producers.

In this study *N. gonorrohoea* show resistance to ciprofloxacin (21.4%) which is in agreement with 18% reported from Hawassa Ethiopia(54)(23%) from Uganda(36) and (28%) reported from Gambella Ethiopia(41).This might be because of the intensive use of antimicrobial agent, easy availability and irrational use of this drug without laboratory diagnosis.

In our study cefoxitin was found to be effective against 78.6% isolates and this in agreement with 82% susceptibility reported from Hawassa Ethiopia(54)lower than (100%) susceptibility reported from Gambella Ethiopia (41).

In this finding *N. gonorrhoeae* show (14.3%) non susceptibility to ceftriaxone which is lower than (27.8%) non susceptibility reported from Bahirdar Ethiopia(53),(33.3%)Southern Ethiopia(55), (40%)India (93),and(100%) Iran(51) the difference might be due to variation in sample size .Similarly cefixime show (14.3%) non susceptibility.Currently, extensively drug-resistant *N. gonorrhoeae* strains has been reported in Japan (6), France (7), and Spain (8) that displayed high-level resistance to cefixime and ceftriaxone and treatment failures for cefixime and ceftriaxone had reported from USA indicating that multidrug resistant, extensively drug resistant untreatable gonorrhea had been emerged (94).

6.1. Limitation of the study

- The study topic by itself assesses personal and sensitive issues related to sexuality which might have caused under reporting of risky sexual behaviors.
- Similar studies on university students in Ethiopia specific to STIs were absent to compare results**.**
- Due to resource shortage other STI status of study participants were not planned and included

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1. CONCLUSION

In general, in this study high prevalence of STI due to *N. gonorrohoea, T. vaginalis* and *T. pallidum* was found. As a key factor, having had sex after taking alcohol showed significantly associated with STIs in the studied population.

N. gonorrohoea has shown increased resistance to third generation cephalosporins like ceftriaxone and cefixime. Since antibiotic treatment is the foundations of gonorrhea control, the emergences of resistance to new generation cephalosporin diminish treatment option for the infection that can increase in the transmission of the bacteria and risk of complications.

7.2 RECOMENDATION

- Jimma University and other concerned bodies need to focus on giving health education on association between risky sexual behavior and STIs especially alcohol use.
- Surveillance for antimicrobial resistance is important for monitoring the emergence and spread of antibiotic resistance in gonococcal isolates.
- Further study is important

REFERENCES

1. CDC. Sexually Transmitted Diseases Treatment Guidelines. *MMWR Morbidity and Mortality Weekly Report* 2015;64: 1-137.
2. WHO. Global incidence and prevalence of selected curable sexually transmitted infections - 2008. WHO. 2012.
3. WHO. 10 facts on sexually transmitted infections. Geneva: . WHO. 2013.
4. Martha K. CDC Labels Antibiotic-Resistant Gonorrhea an "Urgent Threat". 2013.
5. Workowski KA, Bolan G. Sexually transmitted diseases treatment guidelines. *MMWR Recomm Rep Morb Mortal Wkly Rep Recomm Rep Cent Dis Control*. 2010;59:1–110.
6. Ohnishi M, Golparian D, Shimuta K, etal. Is *Neisseria gonorrhoeae* initiating a future era of untreatable gonorrhea?: detailed characterization of the first strain with high-level resistance to ceftriaxone. *Antimicrob Agents Chemother*. 2011;55(7):3538–45.
7. Unemo M, Golparian D, Nicholas R, Ohnishi M, Gallay A, etal. Highlevel cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: novel penA mosaic allele in a successful international clone causes treatment failure. *Antimicrob Agents Chemother*. 2012;56(3):1273–80.
8. Camara J, Serra J, Ayats J, Bastida T, Carnicer-Pont D, etal. Molecular characterization of two high-level ceftriaxone-resistant *Neisseria gonorrhoeae* isolates detected in Catalonia, Spain. *J Antimicrob Chemother*. 2012;67(8):1858–60.
9. WHO.DepartmentofReproductiveHealthandResearch. Global action plan to control the spread and impact of antimicrobial resistance in *N. gonorrhoeae*. Geneva: WHO; 2012. 2009:1–36.
10. CDC. CDC Grand Rounds: the growing threat of multidrug-resistant gonorrhea. *MMWR Morbidity and mortality weekly report*. 2013;62(6):103.
11. Satterwhite CL, Torrone E, Meites E, etal. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. *Sex Transm Dis* 2013;40:187–93.
12. Noel CJ, Diaz N, Sicheritz-Ponten T, Safarikova L, Tachezy J, etal. *Trichomonas vaginalis* vast BspA-like gene family: evidence for functional diversity from structural organisation and transcriptomics. *BMC Genomics*. 2010;11(99):1-26.

13. Sena AC, White BL, Sparling PF. Novel *Treponema pallidum* serologic tests: a paradigm shift in syphilis screening for the 21st century. *Clin Infect Dis*. 2010;51(6):700–8.
14. Kinghorn G. Syphilis and bacterial sexually transmitted infections. *8th ed 4*. 2008:1–37.
15. Murray CJ, Vos T, Lozano R, etal. Disability-adjusted life years (DALYs) for 291diseases : a systematic analysis for theGlobal Burden of Disease Study 2010. *Lancet Infect Dis*. 2012;380(9859):2197–223.
16. Health National Department of HIV & AIDS and STI strategic plan for South Africa 2007-2011.
17. WHO. Baseline Report on Global Sexually Transmitted Infection Surveillance 2012 :Geneva. World Health Organization. 2013.
18. US.CDC W. Sexually Transmitted Infections in Developing Countries. Current concepts and strategies on improving STI prevention, treatment, and control 2008.
19. WHO. Sexually transmitted infection (STI)The importance of a renewed commitment to STI prevention and control in achieving global sexual and reproductive health, 20 Avenue Appia, 1211 Geneva 27, Switzerland: World Health Organization. WHO/RHR/1302. 2013.
20. Hayes R, Watson-Jones D, Celum C, Wijgert Jvd, Wasserheit J. Treatmentof sexually transmitted infections for HIV prevention: end of the road or newbeginning. *AIDS*. 2010:24.
21. Owusu-Edusei JK, Chesson HW, TL G, etal. The estimated direct medicalcost of selected sexually transmitted infections in the United States, 2008. *SexTransm Dis*. 2013;40(3):197–201.
22. Gottlieb S, Low N, Newman L, Bolan G, Kamb M, etal. Toward global prevention of sexually transmitted infections (STIs): the need for STI vaccines. *Vaccine*. 2014;32(14):1527–35.
23. Da Ros, C.T, Schmitt Cda. Global epidemiology of sexually transmitted diseases. Asian J Andro. Jan 2008; 10(1):110–4
24. WHO. Department of Reproductive Health and Research. Global strategy for the perevention and control of sexually transmitted infections:2006-2015. *Geneva: WHO*. 2007.

25. United States.Agency for International Development:Pathfinder international. Bringing youth friendly services to scale in Ethiopia. *In USAID*. 2012:1–8.
26. Berhane Y, Mekonnen Y, Seyoum E, Gelmon L, Wilson D. HIV/AIDS in Ethiopia an epidemiological synthesis *World Bank Global HIV/AIDS Program*. 2008.
27. Federal Democratic Republic of Ethiopia. Ministry Of Health National Guidelines For The Management Of Sexually Transmitted Infections Using Syndromic Approach. 2015.
28. Gurmesa T, Fessahaye A, Sisay D. Risky sexual behavior and predisposing factors among students of jimma university, Ethiopia. *Ethiop J Health Sci*. 2012;22.
29. Mitike G, Lemma W, Berhane F. HIV/AIDS Behavioral Surveillance Survey Round one, Ethiopia 2001-2002. Addis Ababa, Ethiopia. 2002.
30. Bereket Y, Terefe G, Mulat T. Prevalence and Associated Factors of Sexually Transmitted Infections among Students of Wolaita Sodo University, Southern Ethiopia. *International Journal of Scientific & technology research*. 2013;2(2).
31. CDC. Sexually transmitted disease surveillance, 2010. Atlanta, GA: US Department of Health and Human Services. 2011:33-5.
32. WHO. Surveillance of antibiotic resistance in *Neisseria gonorrhoeae* in WHO Western Pacific and South East Asian regions. *Commun Dis Intell*. 2010;34(1):1-7.
33. Satterwhite CL, Torrone E, Meites E, etal. Sexually transmitted infectionsamong US women and men: prevalence and incidence estimates, 2008. *SexTransm Dis* 2013;40(3):187–93.
34. Shipitsyna E, Krasnoselskikh T, Zolotoverkhaya E, Savicheva A, Krotin P, Domeika M, et al. Sexual behaviours, knowledge and attitudes regarding safe sex, and prevalence of non-viral sexually transmitted infections among attendees of youth clinics inSt. Petersburg. Russia Journal of the European Academy of Dermatology and Venereology. 2013;27.
35. Thairu Y, Shehu HA, Egenti N. Profile of Sexually transmitted infections (STIs) among students of tertiary educational institution in Abuja, Nigeria. *IOSR Journal of Dental and Medical Sciences*. 2015;14(11):71-4.
36. Florence PA, Otim F, Okongo F, Ogwang M, Greco D. The prevalence and antibiotics susceptibility pattern of *Neisseria gonorrhoeae* in patients attending OPD clinics at St. Mary's Hospital Lacor Uganda. *J prev med hyg*. 2012;53:186-9.

37. KennedyT W, Ibinabo O. Prevalence of *Neisseria Gonorrhoea* among Under Graduate Female Students of University Of Port Harcpurt Using Strand Displacement and Amplification (Sda) Technique. *Journal of Dental and Medical Sciences*. 2013; 7(4): 76-9.
38. Menéndez C, Renom M. Prevalence and risk factors of sexually transmitted infections and cervical neoplasia in women from a rural area of southern Mozambique. *Infect Dis Obstet Gynecol*. 2010;11:60-93.
39. Maina AN, Kimani J, Anzala O. Prevalence and risk factors of three curable sexually transmitted infections among women in Nairobi, Kenya. *BMC Res Notes*. 2016;9:193.
40. Rozina AG, Beyene MA, Abate AB, Mengistu ES, Addisu GY. Prevalence of Selected Sexually Transmitted Infection (STI) and Associated Factors among Symptomatic Patients Attending Gondar Town Hospitals and Health Centers. Ethiop J Health Sci. 2017;27(6):589.
41. Seada A, Tsegaye S, Zewdineh S, Gebre K. *Neisseria gonorrhoeae* among suspects of sexually transmitted infection in Gambella hospital, Ethiopia: risk factors and drug resistance. *BMC Res Notes*. 2016;9:439.
42. Nadja G, Peter M, Liana R, Clark M, Jeffrey R, etal. Fitz-Hugh-Curtis syndrome: A Diagnosis to Consider in Women With Right Upper Quadrant Pain *Cleve Clin J Med* 2004;71 (3):233-9.
43. Gill DB, Atkinson JP. CD46 in Neisseria Pathogenesis.Trends In Mole. *Med* 2004;10 (9):459-65.
44. Virji M. Pathogenic Neisseriae: Surface Modulation, Pathogenesis and Infection Control. *nature review microbiology*. 2009;7:274-86.
45. Lai-King N, Martin I. The laboratory diagnosis of *Neisseria gonorrhoeae*. *Can J Infect Dis Med Microbiol*. 2005;16(1).
46. Ujevic B, Habek J, Habek D. Prevalence of Infection with *Neisseria Gonorrhoeae* or *Chlamydia Trachomatis* in Acute Mucopurulent Cervicitis. *Arh Hig Rada Toksikol*. 2009;60:197-203.
47. CDC. guidelines.Oral cephalosporin no longer a recommended treatment for gonococcal infections. *MMWR Morb Mortal Wkly Rep*. 2012;61(31):590–4.

48. Horn NN, Kresken M, Körber-Irrgang B. Antimicrobial susceptibility and molecular epidemiology of *Neisseria gonorrhoeae* in Germany. *International Journal of Medical Microbiology*. 2014; 304(2014) 586–91.
49. Ray K, Bala M, Kumari S. Antimicrobial Resistance of *Neisseria gonorrhoeae* in Selected World Health Organization Southeast Asia Region Countries:An Overview. *Sexually Transmitted Diseases*. 2005;32:178-84.
50. Cao V, Ratsima E, tri DV, Bercion R, Fonkoua MC, etal. Antimicrobial Susceptibility of *Neisseria gonorrhoeae* Strains Isolated in 2004–2006 in Bangui, Central African Republic; Yaounde´ , Cameroon; Antananarivo, Madagascar; and Ho Chi Minh Ville and Nha Trang, Vietnam. *Sexually Transmitted Diseases*. 2008;35(11):941–5.
51. Afrasiabi S, Moniri R, Samimi M, Mousavi SG. The frequency of *Neisseria gonorrhoeae* endocervical infection among female carrier and changing trends of antimicrobial susceptibility patterns in Kashan, Iran. *Iran J Microbiol*. 2014;6 (3):194 -7
52. Mir BA, Sirwar SB, Vijayaraghavan. Prevalence and antibiotic susceptibility of *Neisseria gonorrhoeae* in HIV positive and HIV negativecases at a tertiary care hospital. *Indian J Microbiol Res*. 2016;3(2):213-8.
53. Martha T, Ambachew S, Girmay M, Afework K. *Neisseria gonorrhoeae* non-susceptible to cephalosporins and quinolones in Northwest Ethiopia *BMC Infectious Diseases* 2013;13:415.
54. Mengistu H, Abebe T, Mihret A, Lambiyo T. prevalence of *Neisseria Gonorrhea* and their Antimicrobial susceptibility patterns among symptomatic women attending gynecology outpatient department in Hawassa Referral Hospital,Hawassa, Ethiopia. *Ethio J Health Sci*. 2013;23(1).
55. Anteneh A, Zeleke G, Demissie A, Setegn E. Antimicrobial resistance pattern of bacterial isolates from different clinical specimens in Southern Ethiopia: A three year retrospective study *Afr J Bacteriol Res*. 2017;9:1-8.
56. Woods CR. Syphilis in children: congenital and acquired. *Seminars in Pediatric Infectious Diseases*. 2005;16:245-57.
57. Peeling RW, Hook EW. The pathogenesis of syphilis: The great mimicker, revisited. *Journal of Pathology*. 2006;208:224-32.

58. Lafond RE, Centurion-Lara A, Gordones C, Voorhis WCV, etal. prK sequence diversity accumulates during infection of rabbits with *Treponema pallidum subsp. pallidum* Nichols strain. *Infection and Immunity*. 2006;74:1896-906.
59. Lafond RE, Centurion-Lara A, Gordones C, Rompalo AM, Voorhis WCV, etal. Sequence diversity of *Treponema pallidum subsp*. pallidum trpK in human syphilis lesions and rabbit-propogated isolates. *Journal of Bacteriology*. 2003;185:6262-8.
60. Giacani L, Lukehart S, Centurion-Lara. Syphilis. In: Barrett ADT, Stanberry LR, eds. Vaccines for biodefence and emerging and neglected diseases. *Elsevier Ltd*. 2009:1193-218.
61. WHO. Prevalence and incidence of selected sexually transmitted infections *Chlamydia trachomatis, Neisseria gonorrhoeae,* syphilis and *Trichomonas vaginalis* methods and results used by WHO to generate2005 estimates. Geneva: *World Health Organization*. 2011.
62. Zheng J, Wu Z, E K, etal. Hiv, syphilis infection, and risky Sexual behaviors among male University students who Have sex with men in beijing, china: A cross-sectional study. *AIDS Education and Prevention*. 2012;24(1):78–88.
63. Machila C, Nyirenda HT, Mulele CK. Prevalence and risk factors associated with Syphilis at the Copperbelt University Riverside campus, Kitwe Zambia. *Asian Pac J Health Sci*. 2017;4(3):59-65.
64. Ophori EA, Atanunu O, Johnny EJ, Adu M. Sero perevalance of syphilis in apparently healthy students from tertiary institution in Benin city,Nigeria. *Jpn JInfectDis*. 2010;63:437-9.
65. CDC. Association of Public Health Laboratories. Laboratory diagnostic testing for Treponema pallidum. Expert Consultation Meeting Summary Report,Atlanta, GA. January 13-15, 2009.
66. Pope V, Norris S, Johnson R. Treponema and other human host-associated spirochetes. 2007:987-1003.
67. Geisler W. The prozone phenomenon in syphilis testing. South Med J. 2004;97:327-8.
68. Levett PN, Fonseca K, Tsang RSW, etal. Canadian Public Health Laboratory Network laboratory guidelines for the use of serological tests (excluding point-of-care tests) for the diagnosis of syphilis in Canada. *Can J Infect Dis Mid Microbiol*. 2015;26.

69. Morshed M, Lee M, Jorgensen D, Isaac-Renton J. Molecular methods used in clinical laboratory: prospects and pitfalls. *FEMS Immunology and Medical Microbiology*. 2007;49:184-91.
70. UNAIDS/WHO. Working Group on Global HIV/AIDS/STI Surveillance. Guidelines for using HIV testing technologies in surveillance: selection, evaluation and implementation, 2009 update Geneva. . *WHO*. 2009.
71. Martin I, Tsang R, Sutherland K, Tilley P, Read R, etal. Molecular characterisation of syphilis in patients in Canada: Azithromycin resistance and detection of *Treponema pallidum* DNA in whole-blood samples versus ulcerative swabs. *Journal of Clinical Microbiology*. 2009;47:1668-73.
72. Martin I, Gu W, Yang Y, Tsang R. Macrolide resistance and molecular types of *Treponema pallidum* causing primary syphilis in Shangai, China. *Clinical Infectious Diseases*. 2009;49:15-21.
73. Castro R, Prieto E, Águas M, Manata M, Botas J, etal. Molecular subtyping of *Treponema pallidum subsp pallidum* in Lisbon,Portugal. *Journal of Clinical Microbiology*. 2009;47:2510-251.
74. Jamil R, Zareikar R, Kazemi A, Syousefee A, Ghazancheei. "Diagnosis of *Trichomonas vaginalis infection* using PCR method compared to culture and wet mount microscopy,". *Internal Medicine Journal*. 2006;5(1).
75. Hirt R, Noel C, Sicheritz-Ponten T, etal. *Trichomonas vaginalis* surface proteins: a view from the genome. *Trends Parasitol*. 2007;23:540-7.
76. Goodman R, Freret T, Kula T, Geller A, Talkington M, etal. Clinical isolates of *Trichomonas vaginalis* concurrently infected by strains of up to four Trichomonasvirus species (Family Totiviridae). *J Virol*. 2011;85(9):4258-70.
77. Johnston VJ, Mabey DC. Global epidemiology and control of *Trichomonas vaginalis*. *Curr Opin Infect Dis*. 2008;21(1):56-64.
78. Ambrozio CL, Nagel AS, Jeske S, Bragança GCM, Borsuk S, etal. *Trichomonas vaginalis* prevalence and risk factors for women in Southern Brazil. *Rev Inst Med Trop* Sao Paulo. 2016;58(61).
79. Iwueze MO, Ezeanyanwu LN, Okafor FC, Nwaorgu OC, Ukibe SC. prevalence of *Trichomonas vaginalis infection* among women attending hospitals/health centres in

onitsha community, onitsha north local government area of anambra state. *the bioscientist* 2014;2(1):54- 64.

80. John HS, Enitan SS, Adeyemi O, Itodo GE, Otuneme GO, etal. The Prevalence of *Trichomonas vaginalis* infection and Associated Risk Factors among Undergraduate Female Students of Babcock University, Ilishan- Remo, Ogun State, Nigeria. *International STD Research & Reviews* 2017;6(1):1-13.
81. Kingston M, Bansal D, Carlin E. `Shelf life' of *Trichomonas vaginalis*. *Int J STD AIDS*. 2003;14:28-9.
82. Nye MB, Schwebke JR, Body BA. Comparison of APTIMA *Trichomonas vaginalis* transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. *Am J Obstet Gynecol* 2009;200:181-7.
83. Peterman TA, Tian LH, Metcalf CA, Malotte CK, Paul SM, etal. Persistent, undetected *Trichomonas vaginalis* infections? *Clin Infect Dis*. 2009;48(2):259-60.
84. Hardick A, Hardick J, Wood BJ. Comparison between the Gen-Probe transcription-mediated amplification *Trichomonas vaginalis* research assay and real-time PCR for *Trichomonas vaginalis* detection using a Roche LightCycler instrument with female self-obtained vaginal swab samples and male urine samples. *J Clin Microbiol*. 2006;44:4197-9.
85. CDC. Sexually transmitted diseases treatment guidelines. MMWR. 2010;59(RR-12):58–61.
86. JimmaUniversityOfficeoftheRegistrar. Statistical data of Jimma University students. 2016/2017.
87. Cheesbrough M. District laboratory practice in tropical countries part 2.2nd ed. Cambridge: Cambridge University Press. 2006:90-7.
88. WHO. Guideline Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. 2013.
89. CLSI. Performance Standards for Antimicrobial Susceptibility Testing.26th ed. CLSI supplement M100S. Wayne, PA. Clinical and Laboratory Standards Institute. 2016.

90. Shimelis M, Andualem M, Sintayehu F. Substance use and sexually transmitted infections among anti-retroviral treatment attendees in Jimma university specialized hospital, Jimma, Ethiopia. *Ethiop J Health Science*. 2012;22(3):181-8.
91. Kinfe F, Ibrahim A, Ashebir G. Seroprevalence and Risk Factors of Sexually Transmitted Infections (HIV, HBV and Syphilis) Among Pregnant Women Provided Health Care Services, Addis Ababa, Ethiopia. *American Journal of Health Research*. 2017;5(5):154-61.
92. Yusuf M, Alemayehu B. Seroprevalence of transfusion transmitted infection among blood donors at Jijiga blood bank,, Eastern Ethiopia: retrospective 4 years study. *BMC Res Notes* 2016; 9(129).
93. Sangita R, Arpita S, Aarohi O, etal. Surveillance of Gonococcal Infections with Assessment of Their Antibiotic Susceptibility Pattern. *Advances in Infectious Diseases*. 2016;6:46-51.
94. Unemo M, Robert A. Emergence of multidrug-resistant, extensively drug-resistantanduntreatable gonorrhea. *USA Future Microbiol*. 2012;7(12):1401-22.

ANNEXES

ANNEX-III: GENERAL LABORATORY PROCEDURES

I. Laboratory procedure for collection, transport and processing of urethrogenital discharges from sample collection site.

The value and reliability of microbiological reports are directly affected by the quality of the specimen received by the laboratory and the length of time between its collection and processing. Such instructions should include:

- The amount and type of specimen required, container to use, and need for any preservative or transport medium.
- Best time to collect a specimen.
- Aseptic and safe methods of collection to avoid contamination and accidental infection.
- Labeling of the specimen container.
- Conditions in which specimens need to be kept prior to and during their transport to the laboratory.
- Arrangements for processing specimens that are urgent and those collected outside of normal working hours.
- **Gram staining procedure**
    - **Smear preparation**
- Take grease free clean slides and make an oval shaped mark at the center by using a glass marker
- Transfer a loopful of isolated culture with a sterile nichrome loop and make a smear in the pre marked area on the slide. Allow the smear to dry.
- Fix the dry smear by passing the slides 3-4 times through the flame with smear slide facing upper
    - **Staining**
- Place a slide on the staining glass rods
- Cover the smear with crystal violate stain for 30-60 seconds
- Rapidly wash the stain with clean water
- Tip off all the water and cover the smear with Grams iodine from 30-60 seconds

- Drain off the iodine
- Decolorize with acetone-alcohol for about 10 seconds
- Wash under tap water

Counter stain the smear with Safranin wait for 15 seconds and Wash with water and allow the stained smear to dry in air. Put a drop of oil on the stained smear and observe under oil-immersion lens (100x) of microscope.

II. Media preparation procedures

A. Modified Thayer Martin

1. Suspend 7.2 g of GC agar base in 100 mL of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121 ^{o}c for 15 minutes. Cool to 45 – 50 ^{o}c
4. Prepare 100 mL of a 2% hemoglobin solution and autoclave at 121^{0}C for 15 minutes.
5. Cool to 45 - 50 ^{o}c and aseptically add to the molten GC Agar
6. When the medium becomes cool add 2ml of 1% Iso Vitox and 3.0 µg/ml vancomycin, 7.5 µg/ml colistin ,2.5 units/ml nystatin ,5.0 µg/ml trimethoprim lactate(VCNT)
7. Dispense to sterile Petri dishes 20 ml amount
8. Allow the medium to solidify, Date on medium and store in refrigerator

B. Amie's transport media preparation procedures

1. Suspend 10grams of powder in 1 liter distilled water
2. Heat to boiling to dissolve the medium completely
3. Dispense 6 ml of the solution into test tubes
4. Autoclave at 15 lbs pressure at (121^{o}c) for 15 minute
5. Close capes tightly to prevent evaporation and Store at cool place

D. Antimicrobial Susceptibility test media preparation procedures

1. Suspend 7.2 g of GC agar base in 100 mL of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121^{o}c for 15 minutes. Cool to 45 – 50^{o}c
4. Prepare 100 mL of a 2% hemoglobin solution and autoclave at 121^{0}C for 15 minutes.
5. Cool to 45 - 50 ^{o}c and aseptically add to the molten GC Agar
6. When the medium becomes cool add 2ml of 1% Iso Vitox
7. Dispense to sterile Petri dishes 20 ml amount

8. Allow the medium to solidify, Date on medium and store in refrigerator

E. 0.5mcfarland Standard

1. 1.0% Barium chloride (ml) ---------- 0.05ml
2. 1.0% Sulfuric acid (ml) ---------- 9.95ml
3. Approx. cell density (1X108 CFU/mL) ------- 1.5

LABORATORY PROCEDUERS FOR *T.VAGINALIS* UROGENITAL SAMPLE PROCESSING AND MICROSCOPIC EXAMNATION

1. Insert the swab (sample) into a test tube
2. Add few drops of normal saline into the tube containing urogenital swab
3. Mix thoroughly the swab (sample) with the saline to get well homogenized sample and then discard swab.
4. Take a drop of homogenized sample using a pipette put on a clean, grease-free slide, cover with cover slip
5. Examine microscopically begin using the 10x objective noting cellular distribution and the 40x objective to identify the presence of motile *T. vaginalis* trophozoites which are oval (pyriform), larger than White blood cells, and flagellated that produce remarkable motility called "cork screw motility".

LABORATORY PROCEDURE *T.PALLIDUM*SAMPLE COLLECTION& PROCESSING

1. Collect 5ml of whole blood samples using sterile anticoagulant free test tube
2. Leave to settle for30 minutes
3. Centrifuge at 2000 rpm for 5minutes
4. Dispense 100µl(3drops) of the specimen in to circular sample well on the card
5. Interpret test results at 15minutes.do not interpret the results after 20 minutes

API NH for identification of *Neisseria gonorrhoeae*

1. Using a swab make a heavy suspension (McFarland 4) of the organism in 2ml of 0.85% sterile saline provided with the kit.

2. Into wells PEN to URE dispense about 50µ l of this suspension. Fill the tube and cupule of the last 3 tubes (LIP/ProA, PAL/GGT, BGAL/IND).
3. Cover the first seven tests with mineral oil.
4. Incubate the strip in air at 36^0C (+/- 2^0C) for 2 hours.
5. **Before adding any reagents** record the primary results using the table below.
6. Add the reagents as follows:

Note: ZYM B **is very light sensitive** and loses activity within a few days of opening. Check that the date the ampoule was opened is within the last two weeks. If you start a new ampoule, write the date on the bottle.

Well	**Reagent**
Wells 8 & 9 (LIP/ProA & PAL/GGT)	One drop of ZYM B
Well 10 (BGAL/IND)	One drop of James reagent

Reading the Strip refer to reactions for a description of how to read the reactions.

ANNEX I. INFORMATION SHEET AND CONSENT FORM

Title of the Research Project Urogenital *Neisseria gonorrhoeae, T. pallidum , T.vaginalis and* antimicrobial susceptibility of *N. gonorrhoea* among suspected Jimma university main campus: prevalence and risk factors at JUSC. South West Ethiopia

Name of Principal Investigator: Rahel Tamrat

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 symptomatic Jimma university main campus students attending JUSC who is going to participate in Research Project

Purpose of the study

The purse of this study will be to determine the prevalence of *N. gonorrhoeae, T. pallidum* and *T. vaginalis* infection and asses associated risk factors and Antimicrobial Susceptibility Pattern of *N. gonorrhoeae* among symptomatic Jimma university main campus students who attending Jimma university student clinic at 2017.

This will help to know the magnitude of the infection by these bacteria's in symptomatic students. That will help in knowing the prevalence of infection and to consider planning screening program for these infections.

Procedures to be carried on the study

If you are willing to participate in the study and sign a consent form after that the following procedures will be undertaken to obtain the Urogenital specimen and serum sample based on your sign and symptom of the disease .

- Collection of urethral discharge from male patients
- Collection of cervical and vaginal specimen from female patients
- Collection of blood specimen

Risks associated with the study

There will be no foreseeable risks to you except that you may fill discomfort while collecting cervical sample and mild pain if you give blood 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, Rahel Tamrat , on the telephone number - +251912128654. Dr Tesfaye Kassa telephone number+251931057195, Mr. Zewdneh S/Hailemariam telephone number+251913173050 and Mr. Mulatu Gashaw telephone number +251913629953. 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 …….

Assurance of Principal Investigator

ANNEX II QUESTIONNAIRES AND DATA SHEETS

QUESTIONNAIRE ON DEMOGRAPHIC AND RISKY SEXUAL BEAVOURS OF THE STUDY PARTICIPANTS

I.SOCIODEMOGRAPHIC FACTORS

1. Code _____

2. Sex 1. Male_____

2. Female_____

3. Facility/collage

1. Public Health and Medical Sciences 3. Natural Science

2. Social Sciences and Law 4. Business and Economics

4. Academic years

1. Year I 3. Year III and above

2. Year II

5. Age in years

1. <20 years 2. ≥ 20 years

II. SEXUAL BEHAVER AND AWATENESS TO WARDS STIs

1. Have you ever used condom?

1. Yes 2. No

2. Number of sexual partners?

1. One 2. More than one

3. Have you had sex with sex workers?

1. Yes 2. No

4. Have you ever had sex after using alcohol?

1. Yes 2. No

5. If your answer is yes, did you use Condom after having alcohol?

1. Yes 2. No

6. Do you chew *khat*(chat)

1. Yes 2. No

INFORMATION SHEET CONSENT FORM AMHARIC VERSION

የጥናት መረጃ መስጫ ቅፅ

የተመራማሪ ስም፡ ራሄል ታምራት

ተቋም፡ የጅማ ዩኒርሲቲ ጤና ሣይንስ ተቋም የህክምና ላብራቶሪ ሣይንስ ትምህርትክፍል፡፡

ስፖንሰር፡ ጅማ ዩኒቨርሲቲ

የጥናትርዕስ፡በጅማዩኒቨርስቲ ተማሪዎች በግበረስጋ ግኑኝነት አማካኝነት የሚተላለፉ በሽታዎች ስርጭትእና በሽታዉን የሚያመጡ ተያያዥ ምክንያቶች፡፡ ጅማ ደቡብ ምዕራብ ኢትዮጵያ፡፡

የጥናቱዓላማ፡ ይህ ጥናት የጨብጥ፣ ቂጥኝ፣ እና የትሪኮሚናስ ቫጃናሊስ ስርጭት እና በሽታዉን የሚያመጡተያያዥ ምክንያቶች እና የጨብጥ በሽታ ተዋስያን እና ፀረ ተዋስያን መድሀኒት መቋቋም ፀባይ ማወቅ በጅማ ዩኒቨርስቲ ተማሪዎች የበሽታዉ ምልክት ከሚታይባቸዉ መካከል ጥናቱ በሚካሄድበት ወቅት በዩኒቨርሲቲዉክሊኒክ በሚገኙ ተማሪዎች ላይ ማጥናት፡፡

<u>የጥናቱ ቅደም ተከተል</u>

በመጀመሪያ ጥናቱ ላይ ለመሣተፍ ያንቺ/ተ/ን ሙሉ ፈቃድ ይጠይቃል፡፡ ለጥናቱ ተብለዉ ለተዘጋጁ ጥያቄዎች አጠር ያለ ምላሽ ትሠጣለህ/ሽ/፡፡

<u>በመቀጠልም</u>

-ለሴቶች ከማህፀን በር እና ከብልት ላይ ናሙና ይወሰዳል

-ለወንዶች ከብልት ላይ ናሙና ይወሰዳል

-በመቀጠልም የደም ናሙና ይወሰዳል

<u>ሥጋትና ጉዳት</u>

ምርምሩ ጥያቄና ለምርመራ ከማህፀን በር ላይ እና ከብልት ላይ ናሙና መውሠድን የሚጠይቅ ስለሆነ ናሙናው በሚወሰድበት ጊዜ ያለመመቸት እና ትንሽ ህመም የደም

ናሙና ሲወሰድ ከመቻሉ ውጭ ምንም አይነት የጤና ችግር አያጋጥምህ/ሽ/ም ለዚህም አስፈላጊዉ ሁሉ ጥንቃቄ ይደረጋል፡፡

የጥናቱ ጥቅም፡ ጥናቱ ላይ በመሳተፍህ/ሽ/ የተለየ ጥቅም አይኖረውም፡፡ ነገር ግን የላቦራቶሪዉ የምርመራ ውጤት ካሣየሽው የህመም ምልክት ጋር በማቀናጀት በመርማሪው የጤና ባለሞያ አስፈላጊዉን ህክምና እንዲደርግልህ/ሽ/ ይረዳል፡፡

ሚስጥራዊነት፡ ለጥናቱ የምትሠጠ/ጭ/ው ማንኛውም መረጃ በሚስጥር እንደሚያዝ እናረጋግጣለን፡፡ የላቦራቶሪ ውጤትህ/ሽ/ም ከመረመረህ/ሽ/ የጤና ባለሞያ ውጭ ለማንም ይፋ አይሆንም፡፡ አድራሻህ/ሽ/ን ወይም ስምሽ/ህ/ን ያለመስጠት ባለ ሙሉ መብትነህ/ሽ/፡፡

የጥናቱተሣታፊመብቶች፡በጥናቱ ላይ የምትሣተፈ(ፊ) ውባንተ(ቲ) ሙሉ ፈቃደኝነት ብቻ እንደሆነ እያሣወቅንበጥናቱ ላይ ያለመሣተፍ ወደ ጥናቱም ከገባህ(ሽ)በኋላም በፈለገ(ህ)ሽ ሠዓት አቋርጠ የመውጣትም መብትእንዳለህ(ሽ) ልገልፅልህ(ሽ) እወዳለሁ፡፡ በጥናቱም ላይ በመሣተፍ ማንም ተፅዕኖ አያሳድርብህ(ሽ)ም በጥናቱም ላይ ያለህ(ሽ)ን ጥያቄ ሁሉ የምትጠይቅህ(ሽ)ን የጤና ባለሞያ የመጠየቅና የመረዳት ባለ ሙሉ መብትነህ(ሽ)፡፡

ለተጨማሪ መረጃ፡ ማነጋገር ብትፈልግ/ጊ/ ማንኛውም አይነት ጥያቄ ቢኖርህ/ሽ/ አሁን ወይም ሌላ ጊዜ የሚከተለትን ሠዎች በሚከተለው አድራሻ ማግኘት ትችላለህ/ ትችያለሽ/፡፡

ራሂል ታምራት	ስልክ. ቁ. +251912128654	rech.tam@gmail.com
ዶ/ር ተስፋዬ ካሳ	ስልክ. ቁ. +251931057195	
አቶ ዘዉድነህ ሳህለማርያም	ስልክ. ቁ. +251913173050	
አቶ ሙላት ጋሻዉ	ስልክ. ቁ. +251913629953	

i) **ማህበራዊ መረጃዎች**

1.ኮድ----------

2.**ፆታ**

1.ወንድ 2.ሴት

3. **ፋካልቲ/ኮሌጅ**

1. **የህብረተሰብ ጤና /ህክምና** 2. **የህብረተሰብ ሳይንስ እና ህግ**

3. **የተፈጥሮ ሳይንስ** 4. **ንግድ እና ምጣኔ ሀብት**

4. **የትምህርት አመት**

1. **አንደኛ አመት** 2. **ሁለተኛ አመት**

3. **ሶስተኛ አመት እና ከዚያ በላይ**

5. **እድሜ**

1. <20 **አመት** 2. ≥20**አመት**

ii) **የፆታዊ ሩካቤ ባህሪ እና ግንዛቤ**

1. **በወሲብ ጊዜ ኮንደም ተጠቅመህ/ሽ ታውቃለህ/ሽ**

1. **አዎ** 2. **አይደለም**

2. **ከምን ያህል ሰዉ ጋር ወሲብ ፈጽመህ/ሻል**

1.**ከአንድ ሰዉጋር** 2. **ከአንድ ሰዉ በላይ**

3. **ከሴተኛ አዳሪ ጋር ወሲብ ፈጽመህል**

1. **አዎ** 2. **አይደለም**

4. **አዎ ከሆነ አልኮል ከጠጣህ/ሽ ቡኃላ ወሲብ ፈጽመህ/ሽ ታውቃለህ/ሽ**

1. **አዎ** 2. **አይደለም**

5. **አልኮል ከጠጣህ/ሽ/ ቡኃላ ኮንደም ትጠቀማለህ/ሽ/**

1. **አዎ** 2. **አይደለም**

6.**ጫት ትቀማለህ/ሽ**

1. **አዎ** 2. **አይደለም**

INFORMATION SHEET AND CONSENT FORM (AFFAN OROMO)

Mata duree Qorannichaa

QorannichaaQorannoo dhibeen Niiseeriyaa Gonooree, faanto,trikoomonas vaginaali fi niiseeriyaa gonooreeye ; hariiroo waantota dhukkubaa kanaaf saaxilaan fi qoriichaaNiiseeriyaa Gonooree kan bara 2007E.C kiliinikaa baraatoota jimma Yuuniveersiitiitti mooraa gudichaatti yalamaan.

Maqaa qorataa: Raahel Taamirat

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.

Kayyoo qorannoo

Kaayyoon Unki kun qophaa'eef inni guddaan hirmaattootni qorannoo mata duree Qorannoo dhibeen Niiseeriyaa Gonooree, faanto,trikoomonas vaginaali fi niiseeriyaa gonooreeye qoriichaa isaa kiliinikaa baraatoota jimma Yuuniveersiitiitti keessatti tajaajilaman irratti qabu, Kibba Lixa Itoophiyaatti,geggeffamu irrati namootni hirmaatan fedhii isaanii kan ittiin mirkanneffatanii dha. Gareen qorannoo kana gaggeessu kan of keessattuu qabatu, qorataa 1ffaa fi gorsitoota lama Yuuniveersiitii Jimmaa irraa

Adeemsa qoraanichaa irratti godhamuu

Qoraano kanaa irratti hirmaachufi yoo fedhii qabaatan mallato dhukkubichaa irratti hunda'uun namunaa qoraanichaaf barbaachisuu nuuf lachuun unkaa qophaa'ee nuuf gutuun nufii mallatesitun namunoota armaan gadii haaloota armaani gadiitin nufii laatu

- dhaangala'aa ujumoo fincaani dhiraa keessa ba'uu sassabu
- dubartoota irraa cervikaali fi buqushaa irra namunaa fudhaachufi

. namunaa dhigaa waraabu

Miidhama

Qorannoo kanatti hirmaachuun miidhamni gama fayyaan mul'atuu fi isin irraa ga'uu danda'u tokko illee hin jiru, dhukkubiin xiqoon yoo dhigaa laatan isiiniti dhaga'amuu danda'aa

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 1ffaa, **Raahel Taamiirat** lakk. Bilbila kanaan booda gaafachuu ni dandeessu- Lakk. Bilbilaa **+251-9 12-12-86-54, rech.tam@gmail.com** ykn **Dok.Tasfaayee Kaasaa Lakk. Bilbilaa: +251-931-05-71-95 ,ObboZowdineh S/Maariyam Lakk. Bilbilaa: +251-913-17-30-50 fi Muulaat Gaashaaw Lakk. Bilbilaa +251-913-62 -99-53 .**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

I.SOCIODEMOGRAPHIC FACTORS

1. koodii _____

2. saala 1. dhiira_____

2. dhalaa_____

3. dhaabbata/collejii

1 Koollejjii Saayinsii Meedikaalaa fi Fayyaa Hawaasaa. 3. Saayinsii uumamaa

2.saayinsii hawaasaa fi seeraa 4. Daldalaa fi dinagdee

4. waggaa

1. waggaa I 3.waggaa III fi isaa ol

2. waggaa II

5. umurii waggaadhaan

1. <20 2.≥20

II. BEHAVIOURAL FACTORS

1. kondomii fayyadamtee beektaa?

1.eeyyee 2. lakkii

2. nama meeqaa waliin wal quunnamte?

1. tokko 2. Tokko ol

3. dubartoota mana bunaa waliin wal-quunnamtee beektaa?

1. eeyyee 2.lakki

4 ergaa alkolii fayyadamtee bodaa wal quunnamtee goote beektaa?

1. eeyyee 2. lakki

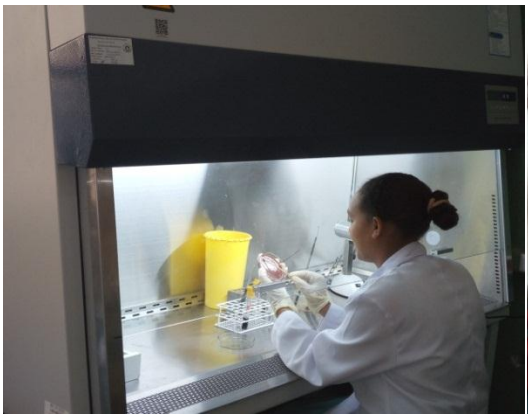
5. Yooeeyye ttaee alkoolii ergaa dhugdee bodaa kondomii fayyadamtee beektaa?

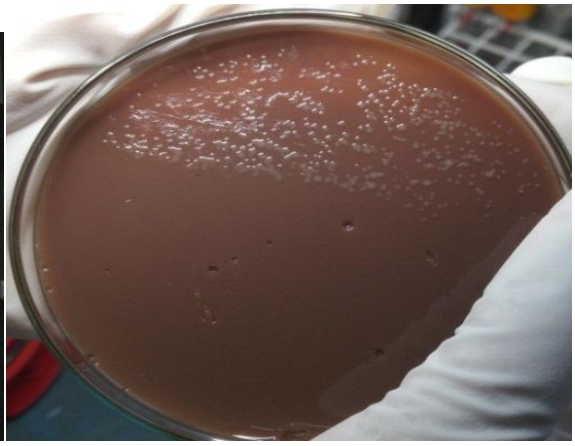
1. eeyyee 2.lakki

6.Chaattii enqqaammtaa?

1. eeyyee 2.lakki

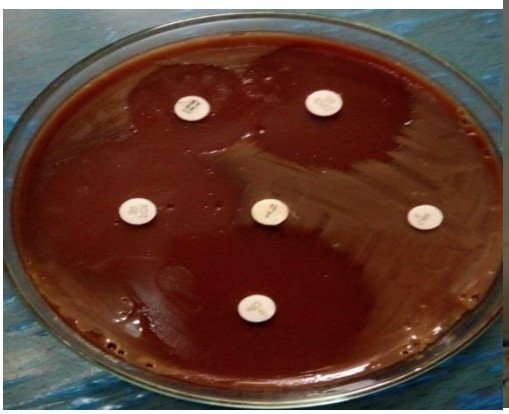
PHOTOGRAPHS





A) Inoculating urogenital swab from Amie's transport media to MTM medium

B) Colony of *N. gonorrhoeae* on MTM medium



C) Antimicrobial susceptibility of *N. gonorrhoeae* on GC Agar

D) Microscopic examination of *T. vaginalis*

E) Immunochromatographic test for *T. pallidum*

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: _____/_____/_____

APPROVAL SHEET OF THESIS

As a member of the board of examiners of the master of science thesis open defense examination, I certify that I have read, evaluate the thesis prepared by Rahel Tamrat, and examined the candidates as well. I recommended that the thesis be accepted by fulfilling the thesis requirements for the degree of Master of Science in Medical Microbiology.

1. Internal Examiner_____

   Date_____ Signature_____

2. External Examiner_____

   Date_____ Signature_____