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Magnitude and drug resistance patterns of *Neisseria gonorrhoeae* among sexually transmitted infection treated patients in selected health centers of Addis Ababa , Ethiopia.

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School Of Graduate Student

This is to certify that the thesis prepared by Senait Tadege entitled **" Magnitude and drug resistance patterns of *Neisseria gonorrhoeae* among sexually transmitted infection treating patients in selected health centers of Addis Ababa , Ethiopia"** submitted in partial fulfillment of the requirements for the degree of masters in clinical laboratory sciences (Diagnostic and public health microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Advisor _____ Signature _____Date _____

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Chairman of the Department or Graduate Program Coordinator

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List of Abbreviations

AAHB	Addis Ababa Health Bureau
AAU	Addis Ababa university
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
AOR	Adjusted odd ratio
ART	Anti-retroviral therapy
AST	Antimicrobial susceptibility test
ATCC	American type culture collection
BV	Bacterial vaginosis
CI	Confidence interval
CLSI	Clinical and laboratory standard institute
COR	Crude odd ratio
DRERC	Departmental Research and Ethics Review Committee
EPHI	Ethiopian Public Health Institute
GC	Gonococcal
HC	Health center
MDR	Multi drug resistant
HIV	Human Immunodeficiency Virus
MIC	Minimum inhibitory concentration
MSM	Male sex with male
MTM	Modified Thayer Martin
NOI	Newborn Ophthalmic infection
NRERC	National Research Ethics Review Committee
OPD	Outpatient department
PI	Principal Investigator
PID	Pelvic inflammatory disease
SDA	Saubrod dextrose agar
SMLT	School of medical laboratory technology

SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
STI	Sexually transmitted infection

Abstract

Background: Gonococcus is a major public health challenge currently, due to the high frequency of infections accompanied by a declining of treatment options. *N.gonorrhoeae* has repeatedly demonstrated its extraordinary capacity to develop resistance to all antimicrobials introduced for treatment of gonorrhea.

Objective: To determine magnitude and drug resistant patterns of *Neisseria gonorrhoeae* amoung STI treated patients in health centers of Addis Ababa , Ethiopia

Methods: A prospective cross-sectional study was done from March-October 2017 at six public health centers of Addis Ababa, Ethiopia. Urethral and Cervical swabs were transported to EPHI microbiology laboratory. Standard microbiological procedures were followed for isolating and identification of *N. gonorrhoeae*. Antimicrobial sensitivity test was performed using E-test following 2016 CLSI guidelines for interpretation of zones of inhibition. Data entry, transforming and analysis was done using SPSS version 21.

Results: Out of 176 study population 64 were males within 20-60 years of age and 112 were females within 18 to 44 years of age .About 32 (18.2%) *N. gonorrhoeae* were isolated and from these78.1% were from males. The highest frequency (34.4%) of GC infection occurred among 20-24 age group. Frequent alcohol users were seven times in risk than non alcohol user. None of antimicrobial drug were 100% susceptible to the isolate.

Conclusion and recommendation: the prevalence of gonococcal infection among STI patients relatively high in health centers. Ceftriaxone, Ciprofloxacin and Spectinomycin increasing non susceptibility rate. Establishing a national surveillance program might be clarify more of the treatment response and might be needed guideline review.

Key word: *Neisseria gonorrhoeae*, Non-susceptible, Ethiopia.

1. Introduction

1.1 Background

N. gonorrhoeae is an intracellular gram negative diplococci and human pathogen that causes the sexually transmitted disease (STD) which is gonorrhea. It does not infect other animals and does not survive outside the human body. Gonorrhea is transmitted through sexual contact with an infected person. This includes oral, anal, and vaginal sex. It can also spread from a mother to a child during birth. Initially the organism attaches to the columnar mucosal cells. Then, it penetrates and proliferates inside the cells. This results in local inflammatory response or systemic manifestations [1, 2].

N. gonorrhoeae causes infections principally of the urethra in men and the endocervix in women, although it may also infect extra genital mucosal sites, including the oropharynx and anorectum. Ocular infections also occur in neonates that cause blindness. Genital infection in men usually presents with a urethral discharge, but silent infections are common in women. Genital tract gonorrhea gives rise to serious complications. These include upper reproductive tract infections in women such as pelvic inflammatory disease (PID) with possible consequence serious complication [3]. Also disseminated gonococcal infection may occur in both sexes. It's typically manifests as arthritis, tenosynovitis, and dermatitis [4,5].

In CDC , report estimate 357 million new STI was reported among adults15-49 years of age in 2012, Also *Neisseria gonorrhoea* infects over 78 million individuals worldwide in each year. During 2012, the global incidence rate of gonococcal among men and females was 24 cases/1000 and 19 cases/1000 respectively [1]. In 2014, a total of 350,062 gonorrhea cases were reported, and the national gonorrhea rate increased to 110.7 cases per 100,000 population in USA. This result expected to increase in developing countries because of limited public health infrastructure and limited access to health care. STI survey conducted in Ethiopian since 2014 *N. gonorrhea* was the leading pathogen that caused urethral discharge in males as compared to females with vaginal discharge and bacterial vaginosis was the common cause of vaginal discharge in females [6].

The other most important features of *N. gonorrhoeae*, in the context of antimicrobial resistance pattern, are its phenotypic and genotypic variability which enables it to evade the host response. Phenotypic variability occurs through differential expression of existing parts of the genome. Genotypic variation is achieved by incorporation of new genetic material, which can be acquired

either by conjugation or transformation. It is because of this feature that *N. gonorrhoeae* has acquired penicillinase producing plasmids. Another important feature of *N. gonorrhoeae* is its antigenic variability. This helps the bacterium to survive in its limited host, *i.e.*, humans. Antigenic variability of *N. gonorrhoeae* is partially due to its ability to acquire genetic material from related organisms [7].

Currently syndromic case management for gonococcal infection is a single intramuscular injection of 250mg ceftriaxone or spectinomycin2g IM stat accompanied by either azithromycin, 1 g orally, or doxycycline, 100 mg orally twice daily for seven days. High prevalence of *N. gonorrhea* strains with resistance to most antimicrobials including sulfonamides, penicillins, earlier cephalosporins, tetracyclines, macrolides, and fluoroquinolones is common. The recent occurrence of failures to treat gonorrhea with the extended-spectrum cephalosporins in different country. cefixime and ceftriaxone and the emergence of gonococcal strains exhibiting high-level clinical resistance, combined with resistance to nearly all other available therapeutic antimicrobials, have caused great concern, as evidenced by publications in the medical literature and the lay press and by development of global, regional, and national action-response plans[8,9].

1.2 Statements of the problem

Infections caused by *Neisseria gonorrhea* are one of the most widely disseminated STIs world wide . The high rate of asymptomatic infections and the consequent under- diagnosis explains, along with the widespread problem of underreporting, the difficulty in assessing the true incidence. Prompt and appropriate antimicrobial treatment is important to eliminate the pathogen and restrict its transmission [3].

N.gonorrhea infections can lead to serious complications in females reproductive organ, such as pelvic inflammatory disease (PID). PID is one of the most common complications of a sexually transmitted disease in women. It can lead to irreversible damage to the uterus, ovaries, fallopian tubes, or other parts of the female reproductive system, and is the primary preventable cause of infertility in women. Normally, the cervix prevents bacteria that enter the vagina from spreading to the internal reproductive organs. If the cervix is exposed to a sexually transmitted disease such as gonorrhea the cervix it becomes infected and less able to prevent the spread of organisms to the internal organs. PID occurs when the disease-causing organisms travel from the cervix to the upper genital tract. Untreated gonorrhea cause about 90% of all cases of PID.A serious complication of gonococcal infection lead to the worst cases cervical, prostate or bladder cancer .Also extension of mucosal infection to relating areas may give rise to infertility in men[10 ,11].

Neisseria gonorrhoeae has an impacts on pregnant women and their new born. As WHO report the prevalence of *N. gonorrhoeae* during pregnancy ranged from 1.5 percent in West and Central Africa to 4.9 percent in East and Southern Africa. It is a significant cause of first-trimester abortion and Newborn ophthalmic infection. First trimester abortion upend because of gonococal infection. Newborn ophthalmic infection is acquired during passage through an infected birth canal during delivery than the latter may lead to corneal perforation and blindness [12,13].

Homosexual relationship and extra genital intercourse contribute the possibility of occurring extra genital infection. Oropharyngeal and anorectal gonococcal infections may be acquired by persons practicing receptive oral or anal intercourse or by contamination from cervical secretions. The pharynx is the most common site of gonococcal infection in men who have sex with men. Gonococcal pharyngitis is most commonly acquired during orogenital contact [14, 15].

Disseminated gonococcal infection occurs 0.5%-3% of cases, and it is thought to play a major role in the phatogenesis of gonococcal arteritis. Patients with DGI may present with 60% dermatitis

arthritis syndrome or 40% with a local septic arthritis [16].Skin lesions appear initially as small vesicles then become abscesses and develop a hemorrhagic base Gonococcal arthritis is asymmetric and migratory, can involve any joint, knees, wrists, ankles, and finger joints are most commonly affected. Tenosynovitis most often occurs in the hands, presenting as erythema and local tenderness along a tendon sheath [4, 5]..

Gonococcal infection also increases HIV transmission in both sexes. Laceration on mucosal cell make easy for virus to inter to host .Asymptomatic infections by *N. gonorrhoeae* largely contribute to the persistence and transmission of disease in a community. Therefore, to control *N. gonorrhoeae* infections and its impact on HIV transmition, it is essential to treat them with most effective drugs [9].

The most gonococcal antimicrobial resistances have been originated from the WHO Western Pacific Region (WPR). Antimicrobials that are used for gonococcal treatment such as penicillin resistance, spectinomycin resistance and fluoroquinolone resistance (with the latter being a well-documented example), all appear to have originated and demonstrated high resistance prevalence in this region and subsequently spread worldwide, clearly illustrating that gonococcal AMR is a global concern [2].

Currently, most countries besides penicillin, spectinomycine, and floroqunolone, they use extended-spectrum cephalosporins (ESCs) for gonococcal treatment. Treatment failures with cefixime (oral) and ceftriaxone (injectable) have been verified in Japan, Australia, several European countries, South Africa and Canada [3].

In Ethiopia, *N. gonorrhea* decreasing the susceptibility of different drug in different area. Even though STI patient treated in our contry by syndromic treatment (without confirmation of *N. gonorrhoeae*) and it might be increase the rate of non-susceptible pattern.There is no representative data on rate of non-susceptible pattern on current drug and magnitude of *N. Gonorrhoeae*e specially in Addis Ababa health center.

1.3 Significant of the study

The aims of this study are:

The magnitude of *N.gonorrhoeae* showed relative increasment among reproductive age group in most of the study report. This study used to show the current prevalence of gonococcal infection and possible risk factor of gonococcal infection around study area

Mostly symptomatics patient go to health center rather than referral hospital and antibiotic is given to STI symptomatic patients without conformation of gonococal infection or laboratory test. This study used to show the current status of drug resistant condition at health center level.

This study could also add information on the magnitude of Gonocoocal infection and their antimicrobial resistance among female participants which was lacking in previous study of EPHI.

It proceed information on drug resistant pattern of *Neisseria gonorrhoeae* and used as initial information for further study on the effectiveness of ceftriaxone and Spectromicine treatment in our country.

2. Literature Review

2.1 Magnitude of *Neisserria gonorrhea*

In 2012, the World Health Organization report global and regional STI estimates based on literature reviews of prevalence data among low risk of general populations. Among women aged 15–49 years, the estimated global prevalence of gonorrhoea 0.8% (0.6–1.0%), among men estimated gonorrhoeae 0.6% (0.4–0.9%).The overall global prevalence estimated was 78 million of gonorrhoea infection (53–110 million).The highest estimated prevalence rates were in the Western Pacific Region[3].

In USA, the Centers for Disease Control and Prevention (CDC) estimates that more than 820,000 people get new gonorrheal infections each year, In the U.S., according to their data the highest reported rates of *N. gonorrhoea* infection was among sexually active teenagers and young adults. The society of high number Infected individual are African Americans [6].

A study conducted in Bacolod city, Philippines, showed a total of 88 isolates were taken during the period of 1 January 2015 to 30 June 2017. The highest incidence of gonorrhea infection was in the group aged 20–24 years (34.09%). Among these patients, nine (10.23%) were considered pediatric (18 years old or younger) and 79 (89.77%) were considered adults. A 12-year-old was the youngest and a 72-year-old was the oldest patient from whom the organisms were isolated. Gonococcal infections were also noted to be the most prevalent in the group aged 20–24 years (34.09%) followed by the group aged 25–29 years[23]

In 2012 world health organization was reported the prevalence of *N. gonorrhoeae* in fourty six country of African Region based on reviewsa different study. Out of an estimated number of population 384.4 million adults between the ages of 15-49 the prevalence of *N.gonorrhoeae* was 8.2 million [3].

A cross sectional study was conducted at health centers in Addis Ababa from August 2013-August 2014 by Ethiopian Public Health Institute (EPHI) bacteriology department .The study participant was male STI patients. All urethral discharge specimens were cultured on Modified Thayer Marthin media and suspected *gonococcal* colonies were confirmed using biochemical tests followed by API-NH. The magnitude of *Neisseria gonorrhoeae* infection among them was 69.3%. Out of 599 participants around 40% patients were in the age group of 25-29 years, and about one third of the patients 33% were 20-24 years old[17]

In 2011, a crossectional study were conducted among symptomatic women attending gynecology outpatient department in Hawassa referral hospital, result showed , the total 215 cases examined, 11 (5.1%) were confirmed to have gonococcal infection. Out of the 11 patients GC confirmed patients 45.5% were from urban area. Their leaving area had a statistical association with gonococal infection. Gonococcal infection was higher in married women and increased in students. In that study, 4.0% of pregnant women was positive for *N. gonorrhoeae*. Also the magnitude of GC infection was high in 20-24 age group. [18].

In Gambella hospital outpatient department among 186 participants, 106 (57 %) were males and their age ranged from 15 to 65 years the mean age being 28.9 ± 8.3 years. From those 11.3 % were confirmed to have gonococcal infection. The highest frequency of GC infection occurred among 25-29 year-olds. Also the prevalence of GC infection in males was much higher than females accounting for 76.2% and 23.8% respectively. Also the prevalence of infection was higher among rural residents (17.3 vs. 9.0 % urban), married person (12.5 vs. 9.5 % unmarried), employees (15 vs. 11.1 % merchants) and higher educated (13.3 vs. 10.5 % illiterate and 8.1 % primary school) [19].

A retrospective study conducted in Northwest Ethiopia, Bahir Dar, a genital specimen analysis report from bacteriology laboratory register between September 2006 to June 2012, there were 29 clinical strains of *N. gonorrhoeae* isolate found among 352 genital specimens were analyzed in ARHRL, Bahir Dar Center by using bacterial culture and biochemical test. The mean age of the participants was 28.1 years and equal number of females and males were GC confirmed in that study [20].

2.2 Magnitude of drug resistant pattern of *Neisseria gonorrhea*

In 2017, report of WHO Global Gonococcal Antimicrobial Surveillance Program (GASP) a data from 2009-2014 showed continued widespread resistance to penicillin, tetracycline, and ciprofloxacin; increasing resistant to azithromycin;and emergence of decrease susceptibility and resistant to cephalosporin (ESCs). Increase the resistant isolate to ESCs in each year (2009-2014)[3].

In USA, there was a Gonococcal Isolate Surveillance Project at 27 state in 2014. Total of 5,093 isolates were collected of these 19.2% to ciprofloxacin, and 16.2% to penicillin. The prevalence of Ceftriaxone resistant increased from 0.1% in 2006 to 1.4% in both 2010 and 2011, decreased to

0.4% in 2013, and increased to 0.8% in 2014. Ceftriaxone resistant increased to 0.1% in 2008 to 0.4% in 2011 but slightly decreased to 0.1% in 2013 and 2014. The percentage of isolates resistant to ciprofloxacin, penicillin, or all two antimicrobials, was greater in isolates [21].

In Italy, a study on trend of ciprofloxacin resistance in *Neisseria gonorrhoeae*. A total of 599 *Neisseria gonorrhoeae* strains collected in 2 periods, 2003 to 2005 and 2007 to 2008, were screened for ciprofloxacin susceptibility by E-test and molecular method. The percentage of ciprofloxacin-resistant strains increased from 42 (2003– 2005) to 58 (2007–2008) in the second period, increased non susceptible strains (MIC value> 32 μg/mL) .Also the moloculartechiniqe confirmed gene mutations in 88% of the strains. [22].

In other study which was conducted to monitor the antibiotic susceptibility of *N. gonorrhoeae* in Bacolod City, Philippines. A total of 88 isolates were taken during the period of 1 January 2015 to 30 June 2017. Testing for the isolates' susceptibility was carried out by Kirby-Bauer disc diffusion by using cefixime (30g), ceftriaxone (30g), ciprofloxacin (5g), penicillin G(10units), spectinomycin (100g), and tetracycline (30g) antibiotics. The susceptibility pattern to those antibiotics was ceftriaxone 100%, cefixime 82.6%, spectinomycin 92.1%, ciprofloxacin 4.9%, tetracycline5.1%, and penicillin G with 0% [23].

In other study, Antimicrobial sensitivity pattern of *Neisseria gonorrhoeae*among commercial sex workers in Rajshahi city, Bangladesh, showed that all *N. gonorrhoeae*strains were highly or intermediately resistant against tetracycline, penicillin, ciprofloxacin, ceftriaxone. High-level resistance was found in three isolates against tetracycline, erythromycin and ciprofloxacin. Eythromycin was resistant against three isolates but was highly active against one isolate. Only cefixime was potentially active against all isolates indicating that cefixime is currently the choice of drug for gonorrhea treatment [24].

In China, 334 *N. gonorrhoeae* isolates were collected consecutively from symptomatic men attending the Nanjing STD Clinic between April 2011 and December 2012. The drug were assessed using agar plate dilution. About 98.8% (330/334) of *N. gonorrhoeae* isolates were resistant to ciprofloxacin, 97.9% (327/334) to tetracycline and 67.7% (226/334) to penicillin. All isolates were susceptible to ceftriaxone (MIC ≤0.25 mg/L) and spectinomycin (MIC ≤32 mg/L)[25].

In sub-Saharan Africa, the gonococcal treatment is based on syndromic approach using single dose floroquinolone treatment. It is hypothesized that resistance to fluoroquinolones is low in Africa, but there has been limited systematic data collection and analysis. A multicounty antimicrobial resistance study on gonoccocal strains isolated in 2004-2006, indicated low rates of fluoroquinolone resistance with 0% ,1.3and 4.0% % in Bangui, Central African Republic, in Yaoundé, Cameroon, and in Antananarivo, Madagascar respectively [26].Beside a data from several other countries in sub-Saharan Africa suggest increasing levels of fluoroquinolone resistance. A study done In 2004 in South Africa showed 7% of isolates in the Pretoria region, 8% in the Western Cape and 17% in Johannesburg were resistant to this class of antibiotics, where as in 2007, 27% of Cape Town isolates and 32% of Johannesburg isolates from identical populations were resistant to ciprofloxacin [27].

According to the result of Hawassa regional lab study , susceptibility patterns of isolated bacteria (n=11) was done against 11 antimicrobial agents by the agar disc diffusion technique. The sensitivity of gonococcal isolates ranges from 100% to Ceftriaxone and cefixime to 0 % to Penicillin and Tetracycline. The lowest susceptibility was observed for penicillin and Tetracycline. No resistance was found to Ceftriaxone and cefixime. However, low level of susceptibility to quinolone (ciprofloxacin 55.0%).There was decreased susceptibility to spectinomycin as well (82%).Most of the isolates haven't shown multiple drug resistance 9/11 (81.8%) and none of the isolates were sensitive to all antibiotics and high level of resistance (82%) to Penicillin and (55%) to Tetracycline was observed[18].

Retrospective study in Northen Ethiopia, showed that out of 29 isolated *N. gonorrhoeae,* 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. Twenty percent of the isolates were found to be non-susceptible to both ceftriaxone and ciprofloxacin. Non-susceptibility to an injectable cephalosporin and any two of quinolones, penicillins or tetracyclines was observed in 27.8% of the isolates. The percentage of *N. gonorrhoeae* which were non-susceptible to tetracycline or penicillin G was high. The percentage of fluoroquinolone or cephalosporine non-susceptible strains showed an increasing trend. Generally in that study *N. gonorrhoeae* isolates from genital specimens was non-susceptible to an injectable cephalosporin (Cefoxitine) and any two of quinolones, penicillins or tetracyclines [20].

A study conducted on STI suspected patients of Gambella hospital from March to July 2015, showed that 11.3 % of the STI suspected patients were confirmed to have *N. gonorrhoeae*. All *N. gonorrhoeae* isolates were susceptible to ceftriaxone and cefoxitin. But, the strain were 100 % resistant to penicillin and tetracycline. Alarming rate (28.6 %) of resistance was also seen against ciprofloxacin. Moreover, intermediate resistance 4.8 % of the isolate for spectinomycin and 14.3 % for ciprofloxacin. That study show Ceftriaxone and cefoxitin can be considered as excellent first-line treatment options [19].

Currently in Ethiopia, There is no surveillance report or data which estimate the prevalence and AST pattern of *N.gonorrhoeae infection.* It is because of the most health organization report using syndrormic approach.

3. Objective

3.1 General objective

- To determine the magnitude and drug resistant patterns of *Neisseria gonorrhoeae* among sexually transmitted infection treating patients in selected health centers of Addis Ababa.

3.2 Specific objectives

- To determine magnitude of *Neisseria gonorrhoeae* among male and female STI treated patients.
- To determine antimicrobial susceptibility pattern of *N.gonorrhoeae* isolate.
- To determine the prevalence of candidiasis and bacterial vaginosis infection among female STI treating patients.
- To assess associated risk factors for *Neisseria gonorrhoeae.*

4. Hypothesis

There is no difference in the magnitude and AST patterns of *N. gonorrhea* between this study and Gambella study.

5. Materials and Methods

5.1 Study area

The study was conducted in Addis Ababa which is the capital city of Ethiopia, in six different health center which is found under four subcities(Figure-1). Adisketema ,Kuasmeda, Abinet, Kassanchis , Arada and Kirkos health center were selected based on high prevalence of *N. gonnorrhoeae* during EPHI 2013- 2014 G.C study on STI diseases and susceptibility. The rest two sector selected criteria was because of nearness to the high prevalent area.

Kassanchis health center is found in Addis Ababa city in Kirkose Sub City Woreda 08 which locally named Kassanchis. The health center starts its service on 1935 EC. Currently the HC has a total of 150 staff members and around 114 up to 118 clients visit per day to access these different services from Kirkos sub-city woreda-08 Addis Ababa and other area.

Addis ketema health center (Mihraf) is found in Addis Ababa city in Addis ketema Sub City Woreda 04 which locally named Mesalemia. The health center starts its service on 1943 EC. The HC has a total of 160 staff members and around 108 up to 113 clients visit per day to access these different services from Addis ketema sub-city woreda-04 Addis Ababa and other area. Addis Ketema health center.

Kirkos health center which is found in kirkos sub-city woreda 11 Which is locally named Genet Hotel. It also cover wereda 10 population totally 57,087 in number. It start a service on on 1966 EC.The HC has a total of139 staff members and around 180 up to 200 clients visit per day to access these different services from kirkos sub-city Addis Ababa and other area.

Arada health center is a is found in Arada sub-city around the area of Degach Wube or Wube Bereha .Which is locally named Minilick Squire .It start a service on 1941 G.C. The HC has a total of 160 staff members and around 110 up to 120 clients visit per day to access these different services from Arada sub-city, Addis Ababa and other area.

All health center provide almost similar medical services like Laboratory, OPD, minor Surgery, ART, Delivery, Antenatal care, Emergency, VCT and TB clinic. All are under the city administration of Addis Ababa Health Bureau.

Figure 1: Map of Addis Ababa sub city (Adapted from https//www.image. AA city administration.

5.2 Study design and period

A Prospective, cross- sectional study was conducted to assess magnitude and drug resistance patterns of *Neisseria gonorrhea* among STI symptomatic patients in selected health centers and the study period was from March-October 2017.

5.3 Population

5.3.1 Source of population (Target population)

The source population was all health center patients who visit during the study period.

5.3.2 Study population

The study population was all men with urethritis complain of a urethral discharge and women with cervicitis complain of lower abnormal pain or increased vaginal discharge during the study period and full filling the inclusion criteria.

5.4 Inclusion and Exclusion criteria

5.4.1 Inclusion criteria

All STI patients who come to selected health centers at the time of the study period and volunteer to participate.

5.4.2 Exclusion criteria

Those patients who start antibiotic treatment during data collection time

5.5 Study Variable

5.5.1 Dependent variables

- Burden of *N. gonorrhoeae*
- Antimicrobial susceptibility testing(AST) pattern

5.5.2 Independent variables

- Age
- Sex
- Alcohol user
- Drug user
- Co-infection with HIV
- Co-infection with candida

5.6 Measurement and Data collection

5.6.1 Sample size

Sample size was calculated based on the prevalence indicated in the other study which was conducted in Gambella hospital, Ethiopia. In that study 11.3 % of the STI suspected patients were confirmed to have *N. gonorrhoeae*[19].

Expected margin of error (d) is 0.05 and confidence interval (z) is 95%.

$$n = \frac{(Z\alpha/2)2 * (1-p)}{d2}$$

$$(1.96)2 * 0.11\ (1-0.11)/\ (0.05)2 = 150$$

Contingency for the unknown circumstance is 10%.

Therefore the sample size become = 150 with contingency for the unknown circumstance (10%) =166. Finally we have collected 176 samples.

5.6.2 Sampling Method

A convenient sampling methods for selection of the study groups.

5.6.3 Data collection procedure

First the questionnaire was developed in English and then translated to Amharic by legal translator and check the consistency of translation by other legal translators. In each health center the volunteer nurse/midwives assigned to prepare participant patient and to collect a sample.

The study participants selected according to their symptoms of urethritis in men typically include urethral discharge, penile itching or tingling. In female, a symptoms include a mucopurulent discharge and lower abdominal pain [28].The participants have been informed about the study by the assigned midwives or nurses. After signed on written consent form the data was collected using a structured questionnaire designed to obtain socio-demographic data and other related information such as educational level, marital status, current HIV result and history of drug and alcohol user was taken from study participants.

Urethral discharge swabs from men were collected by gently massaging or milking the urethra down ward after cleaning by normal saline and using sterile cotton swabs. In females, after cleaning the vaginal area with saline the cervical swabs were collected from the endocervical canal using well sterilized vaginal speculum. The questionnaire was administered by the assigned midwives and nurses.

5.6.4 Laboratory analysis

Cervical and urethral discharge with multiple swab samples (Three for females and Two for males) was collected by nurse or midwife following standard procedures and streak one swab of all genital specimens onto modified Thayer Martin media immediately after collection, the second swab for gram stain smear and the remaining third swab of the females' sample inoculated on Sabroad dextrose agar media. Thayer Martin media was the primary isolated media for *N. gonorrhoeae* and it contains antimicrobial agents (i.e. Vancomycin, Colistin, and Nystatin) which inhibit the growth of other bacteria and fungi. SDA media used to grow/isolate fungal element like candida species which cause genital candidiasis. The inoculated MTM plates were put using candle jar. The

inoculated medias were transported to EPHI clinical bacteriology and mycology laboratory within maximum of 3 hours after collection. The MTM media incubated at 37°C in 5% CO_2 and SDA media at 37°C. Gonococci form smooth, round grey/brown colonies on primary isolation medium (Figure-3). The plates was read 24 up to 72 hours .Growth colonies were identified biochemically following sub culturing to Thayer Martin medium media until pure colonies were obtained.

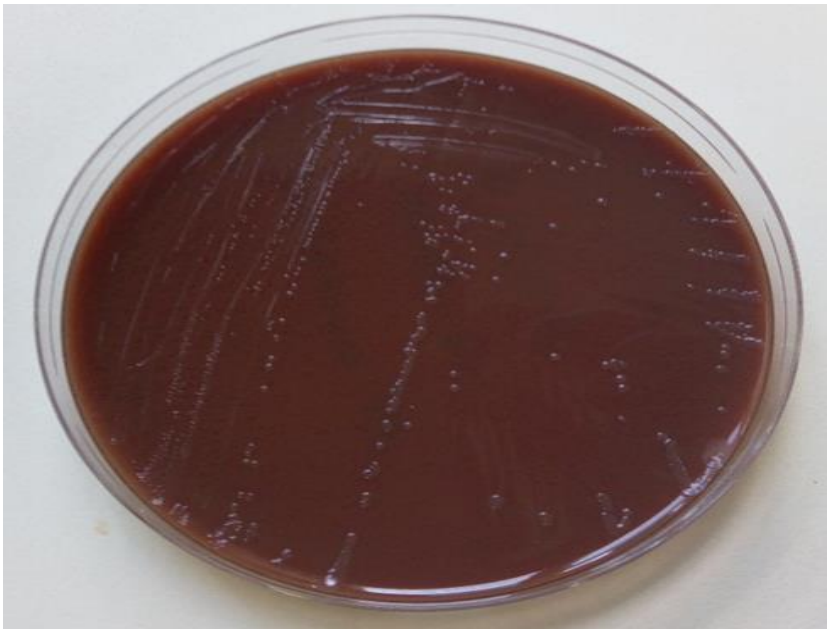

Figure 3: Appearance of *Neisseria gonorrhea* colonies in modified Thayer Martin media after hours of incubation 24-72 hours.

5.6.4.1 Gram stain and Biochemical test

Neisseria gonorrhea is an intracellular gram negative diplococcic (Figure-4).Gram stain used to differentiate *Neisseria gonorrhea* from those gram negative rod which can grow on Thayer Martin media. Oxidase test was done for gram negative diplococcic and used to differentiate certain coccobacilli, including Kingella denitrificans, might appear to be Gram-negative diplococci in gram-stained smears. If microorganisms are oxidase positive (eg : *N. gonorrhea*), the Oxidase test paper color was changes white to blue within 5 seconds then superoxol (30% H_2O_2) test, it used to identify oxidase positive organism which were strong reactive with superoxole. A presumptive identification of *N. gonorrhea* isolates recovered from a genital specimen on selective medium can be made with a gram stain, oxidase and catalase test [29]. If any growth of organism on SDA

media within 48-72 hour of incubation, germ tube test was used to differentiate *Candida albicans* from other yeast.

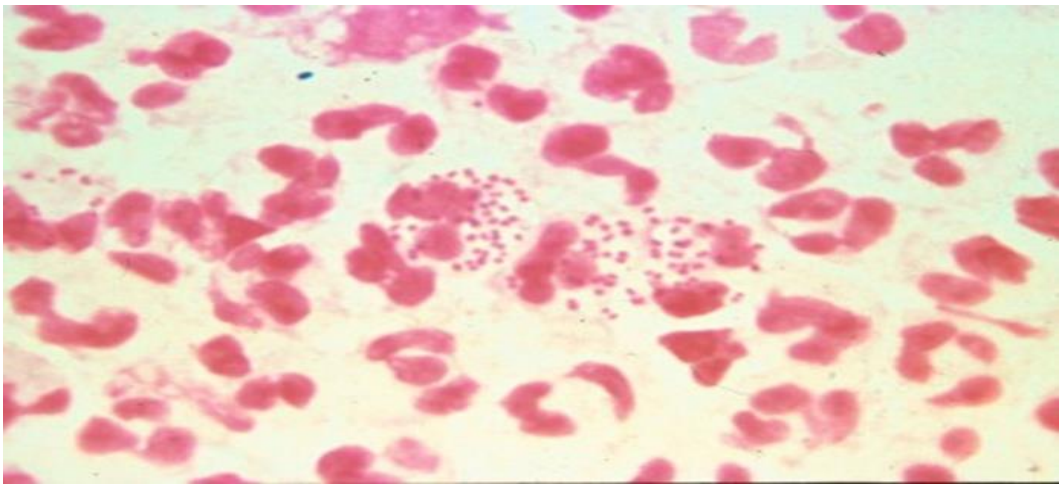

Figure 4: Gram stain structure of intracellular gram negative diplococcic (Adapted fromhttps ://www.std.uw.edu/go/syndrome)

Also Gram stain from swab used to show the presence of "clue cells (Figure 5). It indicate bacterial vaginosis (BV). It is the most common cause of vaginal discharge in women. BV occurs when *Lactobacillus spp*, the predominant species in healthy vaginal flora are replaced by anaerobic bacteria, such as *Gardenella vaginalis*, *Mobiluncus curtisii*, *M. mulieris*, other anaerobic bacteria and/or *Mycoplasma hominis*. According to some study analysis there was an association between BV and NG infection. Women with BV had 1.9 times increased risk for *Neisseria gonorrhea* [30,31].

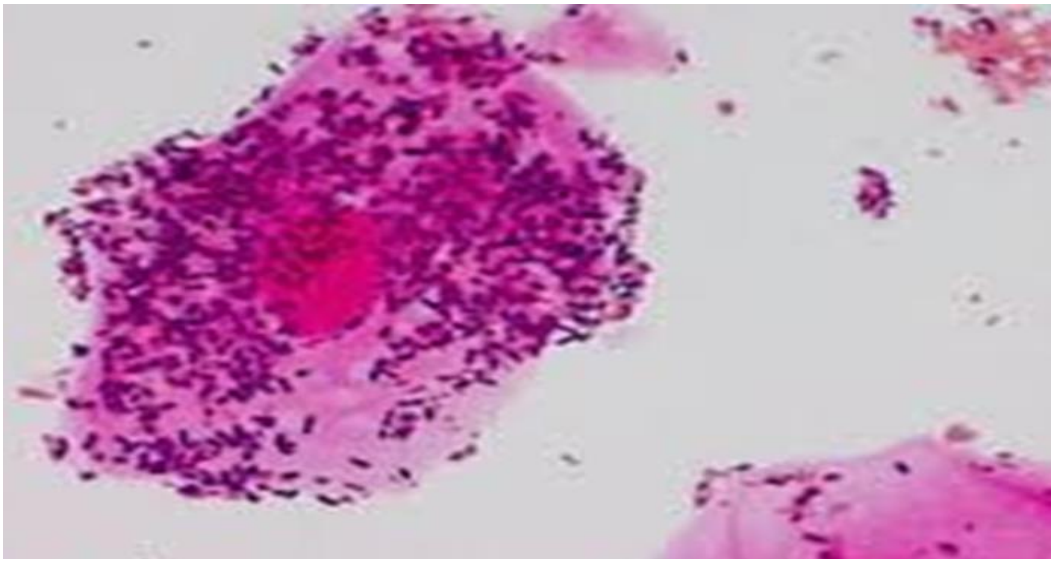

Figure 5: Gram stainof cervical discharge which had clue cell (Adapted from https://www.std.uw.edu/go/syndrome)

5.6.4.2 Antimicrobial Susceptibility test

Antimicrobial Susceptibility Testing was performed according to Clinical and Laboratory Standard Institute 2016 guideline of CLSI [32]. Direct suspension of pure colony in a sterile 0.85% normal saline, equivalent to 0.5 McFarland standard was done. Sterile swab was used to distribute the bacteria evenly over the entire surface of GC agar. E-test strip plated on the GC agar within 3-15 min then incubated at 37°C in 5% CO_2incubator. The Minimum inhibitory concentration (MIC) value was read within 20-24 hour of incubation (Figure-5). The tested antibiotics Penicillin (P), Ciprofloxacin (CIP), Ceftriaxone (CRO), Cefoxitin (FOX) and Spectinomycin (SPT) were selected based on the national guideline to treat infections by Syndromic management package for the management of sexually transmitted infections [8].

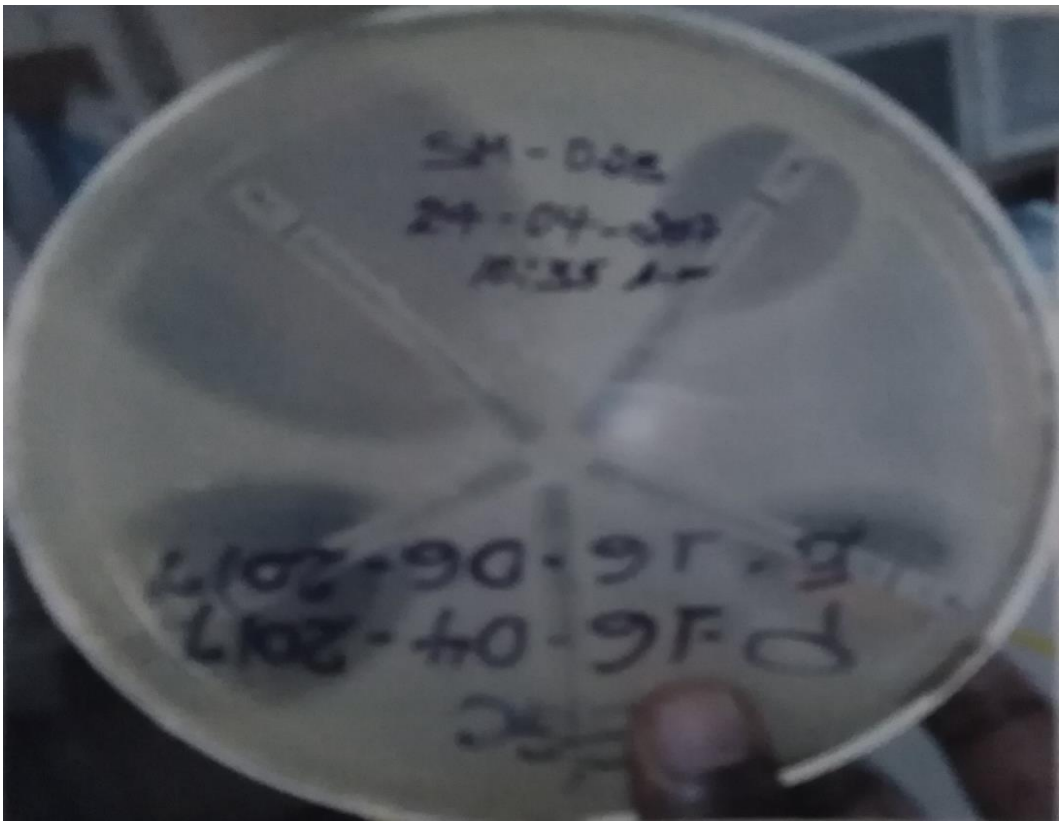

Figure 6: Antimicrobial susceptibility test of *N. gonorrhoeae* isolates by E-test.

5.7 Quality Assurance

EPHI clinical bacteriology laboratory participates in national accreditation program. A reagents quality checked based on their quality assurance schedules. They participate in external quality assurance program. The quality of data was maintained in pre-analytical, analytical and post analytical phases of the research process.

Pre analytic:-All specimen collected based on standard operational procedure and stored in proper condition. Temperature of incubator and refrigerator was monitored daily. Before use of any reagents and culture media any physical change like cracks, excess moisture, color, hemolysis, dehydration, and contamination was assessed and expiration date was also checked. Prevent moisture from penetrating into or forming within the package or storage container. E-test strips must be kept dry with active desiccant.

The quality reagents were checked using SOP of EPHI clinical Bacteriology Laboratory.

1 Gram Stain

- *Staphylococcus aureus*, ATCC 25923: Gram positive
- E. coli, ATCC 25922:Gram negative

2. Oxidase
   - *Pseudomonas aeruginosa*, ATCC 27853: Oxidase positive
   - *Staphylococcus aureus* ATCC 25923: Oxidase negative

3. Superoxol
   - *S. aureus*ATCC 25923–Positive (bubbles)
   - *S. pyogenes* ATCC 19615 – Negative (No bubbles)

4. Thayer Martin culture media and 1% GC agar were checked using ATCC 49226 *N. gonorhoeae*

   - *Neisseria gonorrhoeae* ATCC 49226 Expected results: Growth.

5. E-test

   - *Neisseria gonorrhoeae* ATCC 49226 Expected results: MIC value for each antibiotics.

Analytic:First checked the reagents were stored at appropriate temperatures and condition

- A sterile wooden applicator stick used to pick a colony from overnight growth media to rub it onto the oxidase filter paper.
- AST test was done by pure colony of isolates.
- Checked a E- test gradient strip package temperature before use
- Use McFarland turbidity standard during preparation of suspended isolates
- Check that the inoculated agar surface is completely dry before applying E-test gradient strips
- Remove the E-test gradient strip from the packaging using forceps or other manual applicator.
- All tests were performed using standard operational procedures.

Post-analytical:

- The result interpreted based on SOP and all finding recorded.
- The identified isolate kept at -80 by using trypticase soya broth media with 20% glycerol.
- All used media discarded after autoclaving and all contaminated materials discarded after disinfecting with 0.5% sodium hypochlorite.

5.8 Operational definition

Drug: anything such as a substance emotion or action which one is addicted (khat, Shisha, Injecting /sucking substance and cigarettes).

Multi drug resistance: resistance to at least two chemically different drugs

Sero-statuse: serological statues of HIV infection

Symptomatic: it described by patients in their complaint or history of the present illness.

5.9 Data analysis and interpretation

The completed questionnaires was checked for completeness, consistency and coded by the principal investigator. Any error identified was corrected immediately. Data entry and analysis was done using SPSS version 21. Frequency of variables was determined and descriptive findings were presented using tables and graphs. Crude odds ratio (COR) and adjusted odds ratio (AOR) with 95 % confidence interval (CI) were calculated. P value was calculated to identify statistical significance .To assess the associations between dependent variable which was *N. gonorrhoeae* infection and independent which were demographic and behavioral variables we used binary logistic regression. For electronic records, password protected databases was created and maintained throughout the study period and back up was picked using compact discs.

5.10 Ethical considerations

The study was conducted after securing ethical clearance from the Departmental Research and Ethics Review Committee of Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Clinical Laboratory Sciences. The samples were collected as part of routine investigation of patients at health center. Confidentiality of data was secured throughout the study by locking hard copies and password protection of electronic data.

5.11 Result dissemination

The findings of this study will be presented to the department of Medical Laboratory Sciences for public defense. The result will also be communicated to a clinician whom treat the patient. Effort will be made to publish the findings in peer reviewed journals.

6. Results

6.1 Socio-demographic characteristics of the study participant

During the study period a total of one hundred seventy six STI suspected patients were seen in six health centers in Addis Ababa, Ethiopia. During study period the high number of participant found in Addis ketema health center which was 46 participants, the lower was Abinet health center which was 17 participants as Figure-6 showed. From those participants sixty four (36.4%) were males and their age ranged from 20 to 60 years and Females age range was 18-44.The other socio-demographic characterstics listed on Table-1

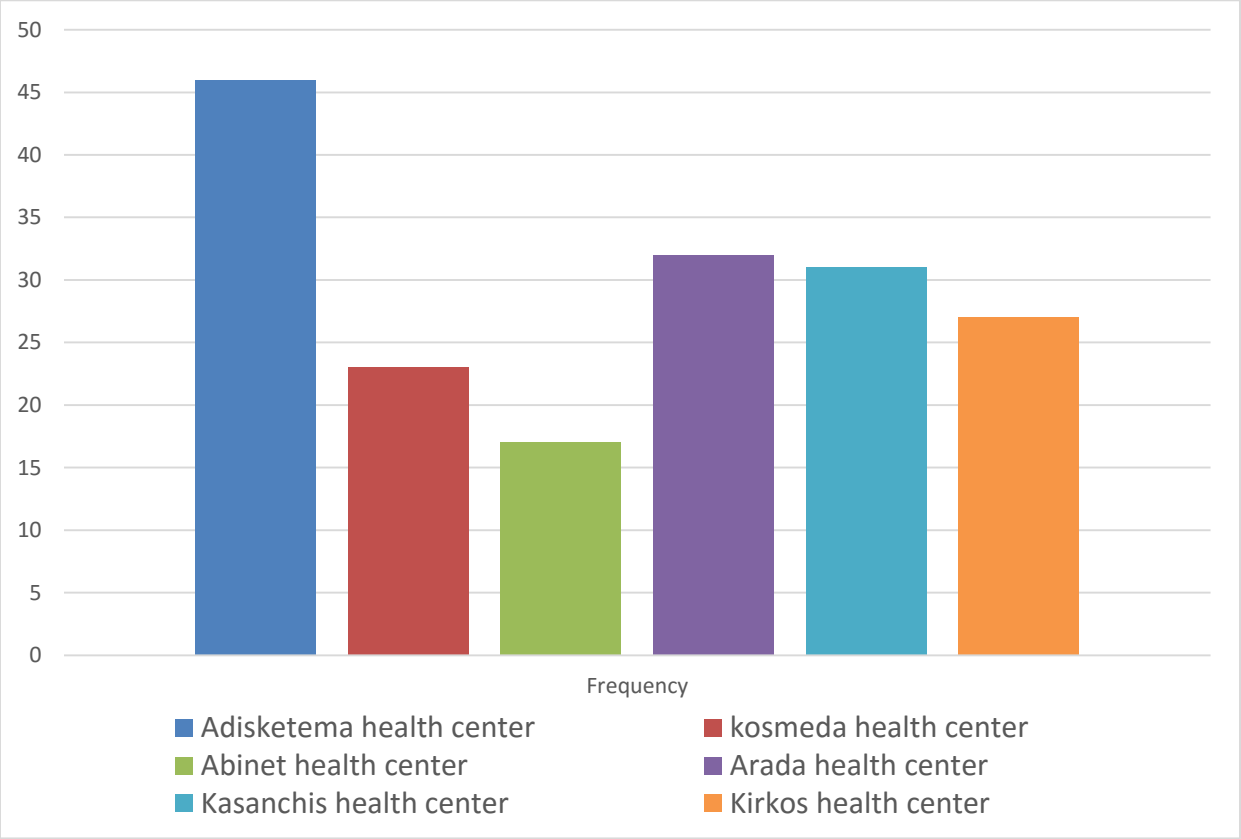

Figure 7: Distribution of (n=176) study population in health facilities (March-October 2017).

Table 1:-Socio-demographic characteristics of the study population in six health centers Addis Ababa, Ethiopia 2017(Mar-Oct 2017) (n=176)

Socio-demographic characteristics		Frequency	Percentage
Health center	Addisketema	46	26.1
	Kuasmeda	23	13.1
	Abinet	17	9.7
	Arada	32	18.2
	Kazanchis	31	17.6
	Kirkos	27	15.3
Age group	15-19	7	4
	20-24	52	29.5
	25-29	53	30.1
	30-34	35	19.9
	35-39	15	8.5
	>39	14	8
Sex	Male	64	36.4
	Female	112	63.6
Address(Sub-city)	Addisketema	69	39.2
	Lideta	17	9.6
	Arada	32	18.2
	Kirkos	58	33
Marital status	Married	82	46.5
	Single	79	44.9
	Divorced	12	6.9
	Widowed	3	1.7
Educational level	Primary	47	26.7
	Secondary	82	46.6
	Higher	31	17.6
	Illiterate	16	9.1
Sexual relationship	Live in partner	78	44.3
	Regular	56	31.8
	Casual	23	13.1
	Multiple	19	10.8
Occupation	Employee	57	32.4
	Student	47	26.7
	Laborer	33	18.7
	Jobless	39	22.2

6.2 Magnitude of *Neisseria gonorrhea* among study population

Out of 176 participants whose urethral or endocervical swabs were investigated, 32 (18.2 %) were confirmed to have gonococcal infection. GC confirmed number of females and males was 7(21.9%) and 25(78.1%) respectively. High frequency of GC infection occurred among 20-24 (34.4%) years of age .

6.3 Association of *N. gonorrhoeae* and other independent factor

The association of *N. gonorrhoeae* with other risk factors shown in Table-2. Out of 176 study participant 32 of them had confirmed gonococcal infection and the magnitude of GC infection in males was higher than female. It was 78.1 % (25/32) and 21.9% (7/32) respectively. The odds ratio of having culture positive in men was nine times higher than in women (p = 0.001, AOR = 9.62, (95 % CI 3.14, 29.44).The magnitude of infection in marital status were higher in marred 15(46.9%) . In terms of educational status majority of infected persons 43.7% (14/32) were in Secondary level. Besides 82.9% (146/176) had known HIV result and 25% (8/32) were GC positive with confirmed HIV status,46.9% (15/32) participants had GC but HIV negative and 28.1% (9/32) were GC confirmed of unknown HIV serostatus.

The number of drug users were 20% (35/176) of total and from those 43.8% (14/32) were gonococcal confirmed. The odds ratio of GC confirmed drug users were four times higher than non-drug users (COR = 4.56, p = 0.001). Total number of alcohol user were 31.8% (56/176) from those 68.8% (22/32) were GC confirmed alcohol users. The odds ratio of alcohol user was 7.12 times greater than non alcohol user (p = 0.001, AOR = 7.12, (95 % CI 3.07, 16.5)GC confirmed.. Bacterial vaginosis was detected by the presence of “clue cell” in 35(31.2%) females and 57.1% (4/7) among gonococcal infection females. Magnitude of yeast cell among female study participants were 27(24.1%) and 3(42.8%) were both GC and candidiasis infected females.

Table 2:- Association of risk factors and gonococcal infection in study population of Six health center Addis Ababa, Ethiopia.(Mar-Oct 2017) (n=176)

Variable	Category	Frequency Number	GC confirmed n=32	COR	P value	AOR	P value
Age group	15-19	7	1(3.1%)				
	20-24	52	11(34.4%)				
	25-29	53	9(28.1%)				
	30-34	35	8(25%)				
	35-39	15	3(20%)				
	>39	14	0(0%)				
Sex	Male	64	25(78.1%)	9.62	0.001	6.16	0.001
	Female	112	7(21.9%0				
Educational level	Primary	47	11(34.4%)				
	Secondary	82	14(43.8%)				
	Higher	31	5(15.6%)				
	Illiterate	16	2(6.2%)				
Occupation	Employee	57	9(28.1%)				
	Student	47	8(25%)				
	Labor	33	5(15.6%)				
	Jobless	39	10(31.3%)				
Marital status	Married	82	15(46.9%)				
	Single	79	14(43.7%)				
	Divorce	12	3(9.4%)				
	Widowed	3	0%				
Sexual relationship	Within marriage	78	12(37.5%)				
	Regular	56	10(31.2%)				
	Causal	23	3(9.4%)				
	Multiple	19	7(21.9%)				
Alcohol user	Yes	56	22(68.8%)	7.12	0.001	4.02	0.003
	No	120	10(31.2%)				
Drug user	yes	35	14(43.8%)	4.56	0.001		
	No	141	18(56.2%)				
HIV(Serostatuse)	positive	24	8(25%)				
	negative	122	15(46.9%)				
	Unknown	30	9(28.1%)				
C. albicans	Positive	27	3(42.9) (n=7)				
	negative	85	4(57.1%)				
Bacterial vaginosis	positive	35	4(57.1%) (n=7)				
	negative	77	3(42.9%)				

6.4 Antimicrobial Susceptibility Testing

The susceptibility patterns of 32 gonococcal isolates were done against five antimicrobial agents by MIC using E-test gradients strips (Table-3). The susceptible of *Neisseria gonorrhoeae* isolates were 90.6% to Ceftriaxone, 84.4% to cefoxitin, 68.8% to Spectinomycin, 3.1% to penicillin and 53.1% to Ciprofloxacin. An intermediate level of resistant was 9.4% to Ciprofloxacin, 28.1% to spectinomycin and 9.4% to Cefoxitin. The antibiotic resistant percentile of the isolate was 37.5%, 3.1%, 96.9% to Ciprofloxacin, Spectinomycin and Penicillin respectively. Minimum inhibitory concentration of ceftriaxone and spectinomycin against gonococcal isolates as seen in Figure-7 and Figure-8.and multidrug resistant of *N. gonorrhoeae* isolates seen in Table:4

Table 3:-Antimicrobial susceptibility pattern of *Neisseria gonorrheae* isolates from STI treating patients in selected health centers of Addis Ababa, Ethiopia(Mar-Oct 2017)(n=32)

Name of isolate	**% Susceptibility**				
N. gonorrhoeae N=32	**Ciprofloxacin**	**Ceftriaxone**	**Spectinomycin**	**Penicillin**	**Cefoxitin**
	53.1%	90.6%	68.8%	3.1%	84.4%

MIC Ranges for Resistance and Intermediate Susceptibility as defined by CLSI Guidelines:

Ceftraxone : Susceptible $\leq$ 0.25

Cefoxitine: Susceptible: $\leq$ 2 ug/mL, Intermediate : 4 ug/mL, Resistant $\geq$ 8 ug/mL

Ciprofloxacin: Susceptible: $\leq$ 0.06 Intermediate 0.12-0.5 ug/mL, Resistant $\geq$ 1 ug/mL

Spectinomycin: Susceptible $\leq$ 32 Intermediate 64 ug/mL, Resistant $\geq$ 128 ug/mL

Penicilline : Susceptible $\leq$ 0.06 Intermediate 0.12-1 ug/mL, Resistant $\geq$ 2 ug/mL

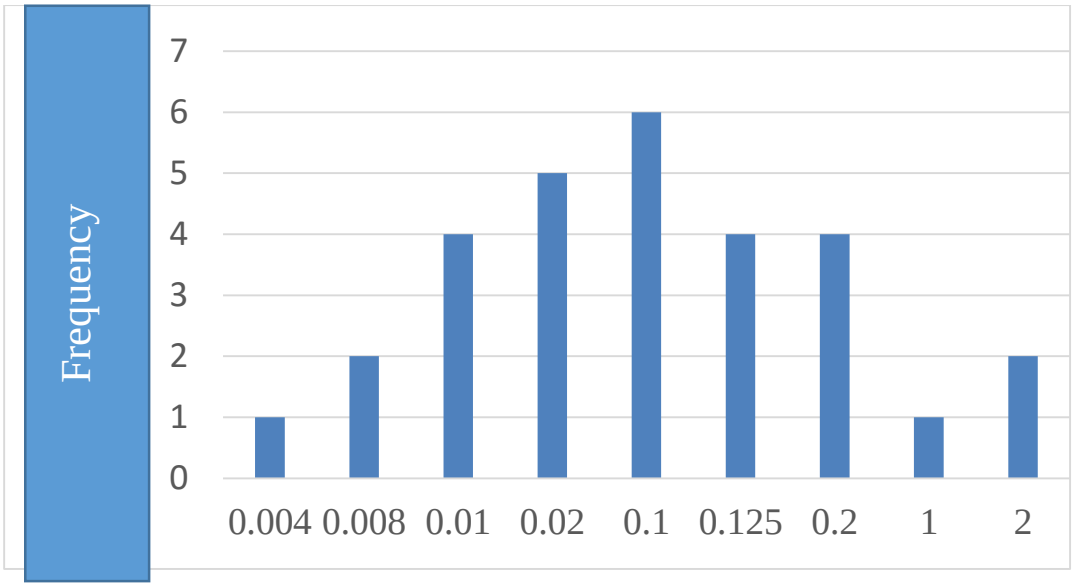

Figure 8: Minimum inhibitory concentration of Ceftriaxone among n=32 *Neisseria gonorrhoeae* isolates.

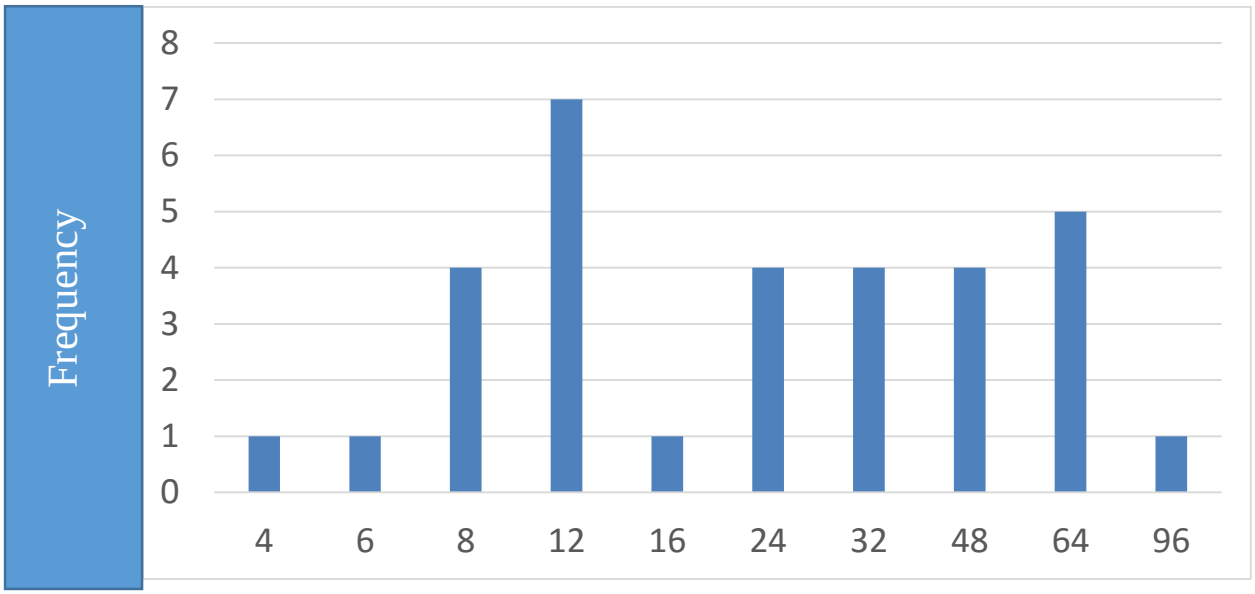

Figure 9: Minimum inhibitory concentration of Spectinomycin among n=32 *Neisseria gonorrhoeae* isolates.

Table 4:- MDR pattern of *N. gonorrhea* isolates, in selected health centers of Addis Ababa, Ethiopia March-October 2017 GC(n=32)

Name of the isolate	Resistant for antimicrobial agent					
	R0	R1	R2	R3	R4	R5
***N. gonorrhea* N=32**	0	13	9	8	1	1

R0= Susceptible for all drug, R1= Resistance for one drug, R2= Resistance for two drug, R3=Resistance for three drug, R4= Resistance for four drug and R5= Resistance for five drug.

Multidrug resistant was existed in59.4%*Neisseria gonorrhoeae* isolates. None of the isolate were susceptible to all drugs.

7. Discussion

In this study the magnitude of GC was 18.2%. This finding was higher than (11.3%) in *Gambella* study [19], 5.1% in *Hawassa* study [18] and Similar studies conducted abroad showed relatively less prevalence (8.4%) in *Tanzania* [33] and 1.2% in *Bangiladish* [34].This relative increased magnitude of infection in our study might be due to the presence of high riskgroup like who had multiple sex partners.In addition our study was conducted in Health centers rather than referral health facilities. At the same time this finding is less than other studies conducted in EPHI, Addis Ababa which was 53.1% [17], 31.7% in *Jimma* [35], 59% in *Uganda*, 80% in *Malawi* [36]. This difference may be due to selecting target population like only on male in EPHI and *Malawi* study. Also the target population of *Jimma*study was commercial sex workers.

Magnitude of *N. gonorrhoeae* in males was higher 78.1 % (The odds ratio of having GC infection in men was nine times higher than in women (p = 0.001, AOR = 9.62, (95 % CI 3.14, 29.44)). This result was agree with 76.2% male in *Gambella* study [19].It might be due to male patient develop a symptomwith in short period of time when they infect with this organism. But this finding disagree with Ayider study, were females (57.5%) and males (42.5%)[47].This variation might be due to they used systematic sampling techiniqe(lottery method).

Relatively higher frequency of *N. gonorrhoeae* infection occurred among 20-24 (34.4%) years of age group and followed by 28.1% in 25-29 age group these result similar to other study among females in aged 15–19 years and 20–24 years had the highest rates of gonococcal infection in USA[37], in *Gambella* study high prevalence among 25-29(15.3%) age group followed by 20-24 (18.9%), in Jimma study high prevalence among 25-34[35].All these study agrees that the magnitude of *N. gonorrhoeae* is relatively high reproductive age specially among20-29 age groups.

Our study result showed GC infection higher in multiple sexual relationship than other group of classification. From those19 participant who had multiple sex partiner 7(36.8%) of them had gonococcal infection confirmed. These result agree with 31.7% in *Jimma* study [35].Both result showed us relatively high gonococcal infections among a person who had multiple sexpartner.

From those gonococcal confirmed 46.9%(15) of them were married and it was higher than other group. This finding was agree with Gambella study [19] but disagree with *Hawassa* study [18]. What we consider from those results are the magnitude of GC infectionin marital status vary from study to study.

The majority of infected participants 43.8% (14/32) found in Secondary level (Also 59.4% of gonococcal confirmed in secondary and abave). This result agree with 66.7% of secondary and above educational level in *Gambella* study [19]. But this result disagree with *Jimma* study [35] and *Indian* study [40].Variation between with other country might be due to socio cultural difference.In Cherie A et al., studyin Addis Ababa study, a studentsin secondary education had more risk exposure because of their age interest to do sex and less awareness about STI [38] however this has to be investigated further

Most GC confirmed participants31.3%(10/32) were jobless. This finding agree with *USA* study [39] but disagree with *Indian* study [40].USA study conclude economical dependency offemalesexposed to sexually transmitted infection. What we consider in this results are jobless population has more in risk than the other group.Also may be this factor has different outcome among different socio cultural characteristics.

In this study alcohol user GC confirmed number was 68.8% (22/32). The odds ratio of alcohol user was 7.12 times greater than non-alcohol user (p = 0.001, AOR = 7.12, (95 % CI 3.07, 16.5).In Vander A. *et al*., 2000 study the influence of alcohol can weaken judgment, compromise the power of balanced decision making about sex and increase risky sexual behavior [41] Similarly, a study conducted in Addis Ababa showed that those who consumed alcohol were two times more likely to have risky sexual practice than those who did not use alcohol[42].These all study confirms using alcohol has statistically significant association with the transmission of STI.

A drug userswere 43.8% (14/32) from GC confirmed.The odds ratio of GC confirmed drug users were 4.56 times higher than non-drug users (p = 0.001) this result agree with Gambella study[19] and Bereket Y. *et al*., 2013 study[48]. This is due to the fact that they are more motivated to take risks of unsafe sex while under the influence of drug. Also 25% (8/32) were GC positive with confirmed HIV status and there was no association with two infection. This result disagree with Jimma study [35] and *Bangiladish* study[43].The variation might be due to information base in our data.

The magnitude of bacterial vaginosis among female study participants were 31.2%(35/112).This result similar to *Bangladesh* studywhich is 29.2%[43], and 57.1% (4/7) BV positive GC confirmed females. This resultslightly similar to 35% in STI attending patients in Debeko Kstudy [42].Magnitude of yeast cell among female study participants were 27(24.1%) and 3 (42.8%) both

GC and candidiasis infected females.These result lower than *Bangladesh* study which was 53.6% [43].In our study there is no significant association between BV and candidiasis with gonococcal infection.The difference is might be due to sample size.

In our study, 90.6% of *N. gonorrhoeae* isolatesusceptible to ceftriaxone. This result similar to Allen Vin *Canada*,2011 [44], 92.5% in *India*[40]. But lower than 100% in *Gambella*[19] and *Hawasa*[18].This variation may be due to the time of study.Gambella and Awassastudy were conducted before the treatment guidelinewas changedciprofloxacinto ceftriaxone. This finding showed us*N. gonorrhoeae*rising in resistance trend to this drug.

In this study, *Neisseria gonorrhoeae* isolates showed non susceptible rate (46.8%) to ciprofloxacin.which is higher than 42.9% in *Gambela* [19], 45% in *Hawasa* [18] and in other country 20 % in *Nepal* [45], 21.4 % in *Brazil* [46]. But lower than 78% in *Philippines* [23], 57.5% resistant in India[40].This different rate of resistance seen might be due to easily accessibility of this antibiotic and health policy to use this drug for treating this disease.

In this study, the isolates showed 96.9% resistant to penicillin, This result higher than 86.6 % in *Gonder* [49] and 70% in *India* [40].At the same time our result lower than 100% in Gambella [19] and 100% in *Hawasa* [18]. The variation in rate of penicillin resistance *N. gonorrheae* isolates in the same country might be an alarming of the different strain of *N. gonorrhoeae* and some of them susceptible to out dated drug penicillin.

In this study the susceptibility of the isolate to spectinomycin was 68.7% and 28.1% of *N. gonorrhoeae* isolate showed intermidate resistant. This result less than 95.2 % in Gambella[19], 82% in Hawassa[18], 91.2%in Thailand, 85% in India [40] and 92.1% in *Philippines*[23]. The variation due to study time and it showed that this organisms were resists spectinomycin with in last two years.

In this study multidrug resistant was existed in 59.4% *Neisseria gonorrhoeae* isolate. None of the isolate were susceptible to all drug.This result higher than 27.8% in Bahirdar [20]. The finding showed that the decreasing of treatment option of gonococcal infections.

8. Strength and Limitation

8.1 Strength of the study

- Quantitative /MIC was done using E-test
- The study was try to show the probability of candidiasis and bacterial vaginitis infection among STI patients.

8.2 Limitation of the study

- This study overed limited health centers so we can't speak about Addis Ababa condition.

9. Conclusion

This study used to conclude that the prevalence of gonococcal infection among STI patients relatively high in health center than referral hospital, reproductive age groups are more in risk than others for gonococcal infection.

The magnitude of *N. gonorrhoeae*is high in male than in female, using alcohol had a significant association to acquiring gonococcal infection.

This study also confirm Ceftriaxone, Ciprofloxacin and Spectinomycin increasing non susceptibility rate and as drugs for the treatment of gonorrhea is doubtful as all the strains were either less sensitive or resistant to these drugs.Beside that antibiotic resistance and multidrug resistant *N.gonorrhoeae* is increasing in the health center.

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10. Recommendation

The high isolation frequency of *N. gonorrhoeae* among reproductive age group indicate that there is insufficient awareness in this group of society about this disease hence the respective health facilities should strengthen health information and education.

In this study, there is resistance to the commonly used antibiotics such as Ceftriaxone and Spectromycine which calls for performing susceptibility testing before administration of any of these antibiotics Specially for second time exposers with in short period of time

In this study there is reduction of susceptibility of *N. gonorrhoeae* to ceftriaxone and spectinomycin which are recommended by Ethiopian STI treatment guidelineshence caution should be taken on the use of these antibiotics and regular.

Large scale studies are required to know the burden of N. gonorrhea and antimicrobial resistance patterns along with other etiologies and molecular techniques.

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12. ANNEX

ANNEX 1: Participant information sheet

Addis Ababa University, Collage of health science, School of Allied Health Science, Department of medical laboratory science
E-mail: SMLT@ethionet.et
Tel. +251 112-75-51-70

ENGLISH VERSION PARTICIPANT INFORMATION SHEET

1. **Study title:**

Magnitude and drug resistant patterns of *Neisseriagonorrhoeae* among STI treating patients in selected health centers of Addis Ababa , Ethiopia

2. **Invitation paragraph:**

You have been invited to take part in this research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask question if there is anything that is not clear or if you would like more information.

3. **Introduction of the disease**

Neisseria gonorrhea is human pathogen that causes the sexually transmitted disease (STD) which is gonorrhea. Gonorrhea is transmitted through sexual contact with an infected person. This includes oral, anal, and vaginal sex. It can also spread from a mother to a child during birth. Initially the organism attaches to the columnar mucosal cells. Then, it penetrates and proliferates inside the cells. This results in local inflammatory response or systemic manifestations. It causes infections principally of the urethra in men and the endocervix in women, although it may also infect extragenital mucosal sites, including the oropharynx and anorectum. Ocular infections also occur, and in neonates can cause blindness.

4. The purpose of the study

The aim of this study is to determine Magnitude and drug resistant patterns of *Neisseria gonorrhoeae* among a STItreating patients in selected health centers, Addis Ababa Ethiopia.

5. Why you have been chosen

You are invited to participate in this study as suspected gonococcal infected based on your symptoms.

6. Participant right

Participation in the study is voluntary, and refusal to participate involves no penalty or loss of benefits to which you are otherwise entitled. The study participants have a right to with hold information, decline to cooperate in the study and refuse running of specimens.

7. Duration

The duration of this study depend upon the availability of study subjects. It might take about three months or more.

8. Study procedures

For this study to be successful we need your participation. If youare voluntary to participate in this study, you are expected to understand and sign the informedconsent. Then, socio demographic condition related to gonococcal infection which are important for this study will be taken. Samples will be collected by experience nurse and/or midwives.Collected samples will be transported to EPHI microbiology laboratory within 3hours and will be analyzed for the presence of *Neisseragonoriaea* and drug susceptibility test by using standard operating procedures (SOPs). In addition, history of HIV co-infection will take from study participants' medical records.

9. Risk

The risk associated with the specimen collection is minimal because the collection of these specimens will follow a standard procedures by experienced nurse.

10. Expected benefits

We will inform the drug resistant laboratory results to STI focal person in charge at Health center for the better management of gonorrhea.

11. Confidentiality

All your personal information collected for the purpose of this study will be kept confidential.

12. Payment

No payment will be provided by participating in this study.

13. Approval

This research project has got ethical clearance from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Science and Addis Ababa Health Bureau Institutional Review Board

14. Whom to contact

If you have any question or description about this study, you can communicate on the following address:

1. Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences

Tel: +251-112-75-51-70

Fax: +251-112-75-46-69

E-mail: SMLT@ethionet.et

P.o.Box: 1176, Addis Ababa, Ethiopia

2. The address of investigator: SenaitTadege

Mobile: +251-112-75-51-70

E-mail: senaittadege99@gmail.com

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ANNEX II: The consent form

English version

Serial no…….

Card no…..……

Name of study participant: _____

I have been requested to participate about this study, which plans to determine magnitude and drug resistant patterns of *Neisseria gonorrhoeae* among STItreating patients in selected health center, Addis Ababa Ethiopia.

I have been informed this study which involves collecting of cervical/urethral discharge specimen. During collection of the specimen I have been told that there is no harm except little discomfort and I have also read the information sheet or it has been read to me.

I have been also informed that all information contained with in the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care access.

It is therefore with full understanding of the situations that I agreed to give the informed consent voluntarily to the researcher to use the specimen taken from cervical/urethral for the investigation. I also agreed to give my HIV result from my medical record. Moreover, I have had the opportunity to ask questions about the project and I have received clarification to my satisfaction. I was also told that results would be reported timely to the requesting physicians for the appropriate treatment and management of the gonorrhea infection.

I agree that I am contributing to the treatment of my fellows by participating in this project. I have asked some questions and clarification has been given to me. I have given my consent freely to participate in the study, and I approve my agreement with my signature.

Participants' signature: _____Date_____

Principal Investigator's signature: _____Date _____

Witness _____Date _____

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ANNEX III: Questioner

English version of the questionnaire

The title of this study is magnitude and drug resistant patterns of *Neisseria gonorrhoeae* among STI treating patients in selected health centers of Addis Ababa , Ethiopia.

Interview

I thank gratefully for your agreement to participate in this study. Now I am going to take interview with you and the interview is about general socio demographic characteristics and clinical data. All of the answers you provide in this study will be kept confidential. The information you give to us is very essential for this study. Therefore, I politely ask you to give me the right response.

	A. Back ground information	
1.	Study ID:	
2.	Participant Card No:	
3.	Address:	Region: _____ Sub city:_____ _____ Kebele: _____ Tel: _____
4.	Full name of the Participant:	Age Sex
5.	Marital status	I). Single- Never married iii). Divorced/Separated ii). Married iv) .Widowed
6.	Occupation	i). Employer ii).Student iii).Labor iv). Jobless

7.	Educational level	I). Primary II). Secondary III). Higher education IV). Illiterate
8.	Current HIV status	I). Negative II). Positive III). Unknown
9	Drug user	I). Yes II). No
10	Alcohol user	I). Yes II). No
11	Sexual relationship	I). Within marriage II).Regular relationship III). Causal IV). Multiple partner

Date : _____

Time of specimen collection: _____.

Thank you very much for taking the time to complete our study!

Name of principal investigator :_____

Signature: _____ Date : _____

AMHARIC VERSION OF QUESTIONNAIRE

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ANNEX IV: Standard operational procedure

All standard operational procedures of each tests taken from EPHI laboratory quality policy manual.

A.SOP for MTM media preparation

Purpose: - This procedure provides instructions to prepare Modified Thayer Martin(MTM).

Clinical Utility: - The GC Medium Base, is used with various additives in isolating and cultivating *Neisseria gonorrhoeae* and other fastidious microorganisms.

Principle:- Thayer Martin Agar (Modified) is a solid medium used commonly for the primary isolation of *Neisseria gonorrhoeae* from mixed specimens. The agar can also be utilized for primary isolation of *Neisseria meningitidis* from mixed specimens. The agar is classified as a selective enrichment agar. Enrichments added to this medium include both **X and V factors**. The modified formulation of the Thayer Martin agar includes **more agar** to help **prevent swarming Proteus**. The agar contains antibiotics to inhibit the growth of normal flora, non-pathogenic *Neisseria species* and most other organisms. ***Neisseria gonorrhoea*****e, *Neisseria meningitidis*, and *Neisseria lactamica*** will grow on the agar. *Neisseria lactamica* is usually nonpathogenic. The antibiotics in the agar include: vancomycin to inhibit gram-positive organisms, nystatin to inhibit the growth of fungi, colistin to inhibit most gram-negative rods, and trimethoprim helps to prevent Proteus from swarming.

Materials and Supplies

- ✓ GC agar Base (BBL)
- ✓ Distilled water
- ✓ Flask
- ✓ Sterile graduated cylinder
- ✓ Sterile Petri dishes with 100 mm diameter
- ✓ VCNT
- ✓ Vitox
- ✓ Sheep blood

Equipment

- ✓ Balance
- ✓ Agar dispenser

- ✓ Distiller
- ✓ Bunsen burner
- ✓ Autoclave
- ✓ Hot plate
- ✓ Spatula
- ✓ Refrigerator
- ✓ PH meter
- ✓ Water bath

Procedure

1. Weigh 36 grams of the medium on a clean paper and transfer to one liter of distilled water
2. Mix thoroughly, heat with frequent agitation until the medium dissolves completely
3. When it becomes cool adjust the PH of the broth to 7.2± 0.2
4. Sterilize by autoclaving 15 lbs, 121oC for 15 minutes
5. After sterilization , cool the medium to 50oC
6. Add 50 ml defibrinated sheep blood mix with gentle rotation
7. Heat the medium in water bath at 80 – 85oC for 10 – 15 minutes, mix the GC agar base and the blood by gentle agitation periodically until the medium becomes chocolate in color
8. When the medium becomes cool add Vitox and VCNT
9. Dispense to sterile Petri dishes 20 ml amount
10. Allow the medium to solidify, Date on medium and store in refrigerator
11. Weigh 36 grams of the medium on a clean paper and transfer to 500 ml of distilled water
12. Weigh 10 gram of Crystal hemoglobin in 500 ml distilled water
13. Make sure the crystal haemoglobin is dissolved completely (you can use magnetic stirrer

Result- N/A

Expected Cultural Response: Cultural response on Chocolate Agar incubated at 35 ± 2oC aerobically or under 5% CO2, as appropriate, and examined for growth after 18 – 24 hours.
Expected Growth

- *Neisseria gonorrhoeae* ATCC 49226 Expected results: Growth

- *Proteus mirabilis* ATCC 43071 Expected results: Inhibition (partial)
- Staphylococcus epidermidis ATCC 12228 Expected results: Inhibition (partial)

Limitations

1. GC agar medium bases are intended for use with supplementation.Although certain diagnostic tests may be performed directly on the medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.

2. Improper specimen collection, environment, temperature, CO2 level, moisture and pH can adversely affect the growth and viability of the organisms.

3. Inactivation or deterioration of antibiotics in selective media may allow growth of contaminant

4. GC agar medium bases have sufficient buffering capacity to offset the very low pH of the small amount of nutritive enrichments added. However, the pH of some media may have to be adjusted with 1% NaOH after the addition of these enrichments

B.SOP for Preparation of SDA

Purpose:

Uses for the selective cultivation of yeasts, molds and aciduric bacteria.The medium is often used with antibiotics for the isolation of pathogenic fungi from material containing large numbers of other fungi or bacteria.

Principle:

Peptone (Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue) provide the nitrogen and vitamin source required for organism growth in SDA. Dextrose is added as the energy and carbon source. Agar is the solidifying agent.Chloramphenicol and/or tetracycline may be added as broad spectrum antimicrobials to inhibit the growth of a wide range of gram-positive and gram-negative bacteria. Gentamicin is added to further inhibit the growth of gram-negative bacteria.

Procedure:

1. Combine all ingredients in ~900 ml of deionized water.
2. Adjust to pH 5.6 with hydrochloric acid and adjust final volume to 1 liter.
3. Heat to boiling to dissolve the medium completely.
4. Autoclave at 121ºC for 15 minutes.
5. Cool to ~45 to 50°C and pour into petri dishes.

Sabouraud agar plates can be inoculated by streaking, as with standard bacteriological media, or by exposing the medium to ambient air. Typically, molds are incubated at room temperature (22 to 25°C) and yeasts are incubated at 28 to 30°C or 37°Cfor 48-72 hour.

C. Discharge Sample collection and inoculation of culture plate

Purpose: to identify *Neisseria gonorrhea* from cervical and ureteral discharge sample.

Principle:Endocervical sample is most preferable sample for specimens for females and urethral discharge sample in men during genital infection.

Material and equipment

- Autoclave
- Sterile vaginal speculum
- Couch
- Sterile cotton applicator
- Glove
- Normal saline
- Glass slide
- MTM media
- Incubator

I, In men: Urethral swab

- Collect specimen at least 2 hours after urination as voiding decreases the amount of exudates.
- Retract the prepuce, clean the tip of the meatus with normal saline and collect the pus directly onto a glass slide or sterile swab in case of frank urethral discharge.
- If no urethral discharge is seen, milk / strip the urethra from the root of the penis to the glans and collect the discharge as above.

II, In women

a) Endocervical swab: Cervical specimens are collected for a women who started sexual relation.

Procedure

- A sterile vaginal speculum moistened with warm water
- The women will be lie on her back and legs spread and feet placed in stirrups.

- A speculum will be inserted into her vagina than the ectocervix is visualized. This tool is used to gently spread apart her vagina.
- A sterile cotton applicator is inserted into the endocervical canal and rotated. This is done to collect a discharge.
- Once a sufficient sample is collected, the applicatore is removed.

b) Vaginal swab : For Prepubertal / Virgin,sample in this age group take from the vagina and not the cervix.No antiseptics or lubricants should be applied.

- A vaginal swab is done to collect a sample from the lower part of the girl vagina.
- The girl will be lie on her back, legs spread and feet placed on stirrups.
- A sterile cotton applicator will be insert into her lower vagina or just near the entrance of the vagina.
- The swab will be rotate gently and then remain still for a few seconds before it is removed. This is done make sure enough secretions have been collected for the test.

B, Sample inoculation procedure on culture media (MTM).

1. Roll swab directly on MTM medium in a large "Z" pattern to provide adequate transfer of organisms.
2. Label culture plate with code and collection date on media side of plate, not the lid which may be separated from the specimen itself.
3. Place culture in a candle can as soon as possible (within 15 minutes). Be sure to relight the candle each time the can is opened. Place can in 35-37 degrees Centigrade incubator if available. If incubator is not available, plates may stay at room temperature only if transport is to occur the same day.

Samples will be rejected if they are:

- Unlabeled - All specimens **must** have a unique patient identifier.
- Insufficient in Quantity - No specimen received, no specimen on plate or insufficient specimen to perform testing.
- Improperly Preserved - Specimens must be received on MTM culture plates.
- Damaged - Specimen broken or damaged in transit.

D. Sop for Gram stain

Purpose: This procedure provides instructions to perform gram stain.

Principle: Gram positive bacteria have thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall) which stains purple while Gram-negative bacteria have a thinner layer (10% of cell wall),which stains pink.

Clinical Utility: The gram stain is used to classify bacteria on the basis of their forms, sizes, cellular morphologies, and gram reactions. It is a critical test for rapid presumptive diagnosis.

Materials: Reagents Supplies

- ✓ Crystal violet
- ✓ Lugol's iodine
- ✓ Acetone alcohol
- ✓ Safranin
- ✓ Disposable plastic loops
- ✓ Glass microscope slides
- ✓ Slide warmer, dry heat block, absolute methanol

Quality control

- Gram positive: *S.aureu s*(ATCC 25923)
- Gram negative: *E.coli*(ATCC 25922)

Procedure:

1.A glass microscope slide was labeled with the laboratory number.

2. Samples taken from colony(culture), one drop of saline on a slide was placed and picked one colony using loop and mixed with saline on the slide.

3. Placed air dried smears in a coplin jar with methanol for one minute, drained slides and allowed to dry before staining.

4. The prepared slide was flooded with crystal violet for one minute

5. The slides was rinsed gently with tap water

6. The slide was flooded with Gram's iodine for one minute

7. The slide was rinsed gently with tap water

8. The slide was decolorized by acetone-alcohol for 5 seconds and rinsed with tap water.

9. The slide was flooded with Safranin for one minute

10. The slide was rinsed gently with tap water

11. The slide was drained in an upright position. The slide was blotted and placed on a slide warmer or heating block to completely dry.

12. Scanned 20-40 fields using oil immersion.

Result interpretation:

- Gram-positive bacteria and yeast stained blue to purple
- Gram-negative bacteria stained pink to red.

Limitations:

- Recovery of organisms not observed on direct gram stains should prompt a review ofboth the smear and the culture.
- Application of excessive heat during fixation of smear may affect the morphologicappearance of host cells and microorganisms.
- Treatment with antimicrobial agents may cause gram positive bacterial to appear gramnegative.
- The gram stain is not an infallible tool for diagnosis, identification, or phylogeny; however, it is extremely limited use in environmental microbiology.

E. SOP for Oxidase test

Purpose

- The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme.
- The test is used as an aid for the differentiation of *Neisseria, Moraxella, Campylobacter* and*Pasteurella* species (oxidase positive).
- It is also used to differentiate pseudomonads from related species.

Principle of Oxidase Test

Cytochrome containing organisms produce an intracellular oxidase enzyme. This oxidase enzyme catalyzes the oxidation of cytochrome c. Organisms which contain cytochrome c as part of their respiratory chain are oxidase-positive and turn the reagent blue/purple. Organisms lacking cytochrome c as part of their respiratory chain do not oxidize the reagent, leaving it colorless within the limits of the test, and are oxidase-negative.

Oxidase positive bacteria possess cytochrome oxidase or indophenol oxidase (an iron containing haemoprotein). Both of these catalyse the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen). The test reagent, N'-tetramethyl-p-phenylenediaminedihydrochloride acts as an artificial electron acceptor for the enzyme oxidase. The oxidised reagent forms the coloured compound indophenol blue.

The cytochrome system is usually only present in aerobic organisms which are capable of utilising oxygen as the final hydrogen receptor. The end product of this metabolism is either water or hydrogen peroxide (broken down by catalase).

I, Test tube method

Materials and reagent

- Test tube
- Test tube rack
- Applicator stick
- Kovacs Oxidase Reagent
    - 1% tetra-methyl-*p*-phenylenediaminedihydrochloride, in water

Quality control

- *Pseudomonas aeruginosa*, ATCC 27853: Oxidase positive
- *Staphylococcus aureus* ATCC 25923: Oxidase negative

Procedure

1. Grow a fresh culture (18 to 24 hours) of bacteria in 4.5 ml of nutrient broth (or standard media that does not contain a high concentration of sugar).
2. Add 0.2 ml of 1% α-naphthol, then add 0.3 ml of 1% p-aminodimethylaniline oxalate (Gaby and Hadley reagents).
3. Shake vigorously to ensure mixing and thorough oxygenation of the culture.
4. Observe for color changes.
5. Microorganisms are oxidase positive when the color changes to blue within 15 to 30 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 2 to 3 minutes. Microorganisms are oxidase negative if the color does not change.

Result interpretation

Positive Result

- Development of a deep purple-blue/blue colour indicates oxidase production within 5-10 seconds.

Negative Result

- No purple-blue colour/No colour change.

II, Filter paper method

1. Grow the isolate(s) to be tested for 18-24 hours on a MTM at 35-37°C with ~5% CO_2 (or in a candle-jar).
2. On a nonporous surface (i.e., Petri dish or glass plate), wet a strip of filter paper with a few drops of Kovac's oxidase reagent.
3. Let the filter paper strip air dry before use.
4. Use a disposable plastic loop, a platinum inoculating loop, or a wooden applicator stick to pick a portion of a colony from overnight growth of colony and rub it onto the treated filter paper.
    - Do not use a nichrome(iron) loop, as it may produce a false-positive reaction.
5. Observe the filter paper for color change white to purple(blue).
6. Perform steps 3 and 4 with a positive and negative QC strain to ensure that the oxidase reagent is working properly.

Reading the oxidase test results

- Positive reactions will develop within 10 seconds in the form of a purple color where the bacteria were applied to the treated filter paper. Delayed reactions are unlikely with *N. meningitidis*.
- Negative reactions will not produce a color change on the treated filter paper.

F. Sop for Catalase test

Principle

Bacteria that synthesize the enzyme catalase hydrolyze hydrogen peroxide into water and gaseous oxygen, which results in the liberation of gas bubbles. The test is useful in initial characterization of most bacteria.

Purpose: The catalase test separates staphylococci (positive) from streptococci and enterococci (negative). For spore forming organisms, *Bacillus* spp. are catalase positive, and *Clostridium* spp. are catalase negative. *Neisseria gonorrhoeae* produces an enhanced elaboration of bubbles not seen with other members of the genus due to superoxol.

The superoxol is a simple test that uses 30% hydrogen peroxide (H_2O_2) as a reagent. Reactions of superoxol with *N. gonorrhoeae* are typically "explosive" (4+, very strong), compared with weaker (2+) reactions with most non-gonococcal*Neisseria species*, and a negative reaction with *K. denitrificans*. In contrast, the catalase test is performed with 3% hydrogen peroxide and yields much weaker results.

Materials

a. Catalase reagent: 3% Hydrogen Peroxide (H_2O_2)
   - Dilute 30% H_2O_2, 1:10 in deionized water, store at 2-8^{o}C
   - Reagent may be stored for up to six months

b. Superoxolreagent for*Neisseria*
   - 30% Hydrogen Peroxide (H_2O_2), store at 2-8^{o}C.

c. Glass slide, sterile wooden sticks or inoculating loops

Quality Control (QC)Organisms and Expected Results:

*S. aureus*ATCC 25923–Positive (bubbles)

S. pyogenes ATCC 19615 – Negative (No bubbles)

QC Frequency: Perform QC on each new lot or shipment.

Procedure

a. Touch the center of a well-isolated colony; transfer to a clean glass slide.
   - Take enough of the colony such that it is visibleon the slide.
   - If colony is from BAP, use care not to pick up blood.

b. Place one drop of hydrogen peroxide reagent on slide; bubbles should form immediately.

- Do not reverse the order of adding the reagent to the colony; false negative result can occur.
- Do not mix reagent and the colony.
- Hold over dark background to see bubbles clearly.

c. Discard slide into sharps container.

Interpretation

Positive: shows immediate appearance of bubbles.

Negative: shows no bubbles or a few bubbles after 20 seconds.

Reporting

For *Neisseria* spp*:* record as Superoxol positive or Superoxol negative.

For other bacteria: record as Catalase test positive or Catalase test negative.

Procedural Notes

a. Caution: 30% Hydrogen Peroxide (H_2O_2) is extremely caustic to skin. If contact occurs, wash immediately with 70% ethyl alcohol not water.
b. 30% Hydrogen Peroxide (H_2O_2) reagent can be used for all tests, but it is more hazardous.
c. Red blood cells contain catalase. To avoid false positive results, do not pick up blood agar with colony. If colony does not easily pick up or grow well, repeat the test from Chocolate Agar, which does not interfere with the assay.
d. Selecting colonies with some metal bacteriological loop materials will yield false positive results; platinum loops do not yield false positive results.
e. Testing colonies that are older than 24 hours may yield false negative results.

G. SOP for Germ tube test

Purpose:Germ Tube Test is a screening test which is used to differentiate *Candida albicans* from other yeast.

Principle of Germ Tube Test

Formation of germ tube is associated with increased synthesis of protein and ribonucleic acid. Germ Tube solutions contains tryptic soy broth and fetal bovine serum, essential nutrients for protein synthesis. It is lyophilized for stability. Germ tube is one of the virulence factors of *Candida albicans.* This is a rapid test for the presumptive identification of *Candida albicans*

Materials required

- Sheep serum or pooled human serum
- Test tube
- Wooden applicator stick
- Microscopic glass slide
- Cover slip
- Pasteur pipette
- Micropipette
- Incubator

Procedure of Germ Tube Test

1. Put 0.5 ml of sheep or human serum into a small tube. Note: Fetal bovine serum can also be used instead of human serum.
2. Using a Pasteur pipette, touch a colony of yeast and gently emulsify it in the serum. Note: Too large of an inoculum will inhibit germ tube formation.
3. Incubated the tube at 37°C for 2 to 4 hours.
4. Transfer a drop of the serum to a slide for examination.
5. Coverslip and examine microscopically under low and high power objectives.

Results and Interpretation

Positive Test: A short hyphal (filamentous) extension arising laterally from a yeast cell, with no constriction at the point of origin. Germ tube is half the width and 3 to 4 times the length of the yeast cell and there is no presence of nucleus. Examples: *Candida albicans*and *Candida dubliniensis*

Negative Test: No hyphal (filamentous) extension arising from a yeast cell or a short hyphal extension constricted at the point of origin. Examples: *Candida tropicalis, C. glabrata*and other yeasts.

Quality Control

Positive Control: Candida albicans (ATCC 10231)

Negative Control:*C. tropicalis* (ATCC 13803), *C. glabrata* (ATCC 2001)

Limitations

1. *C. tropicalis* may form early pseudohyphae which may be falsely interpreted as germ tubes.
2. The yeast formerly named Candida stellatoidea also produces germ tubes; however, it has been combined with *C. albicans* and no longer exists as separate species.
3. This test is only part of the overall scheme for identification of yeasts. Further testing is required for definite identification.

H. SOP for E-test

Purpose: E-test (Epsilometer test) is a quantitative technique for determining the antimicrobial susceptibility of Gram negative and Gram-positive aerobic bacteria

Principle:

The E-test gradient technology is based on a combination of the concepts of dilution and diffusion principles for susceptibility testing. It is applied to an inoculated agar surface, there is immediate and effective transfer of the preformed antibiotic gradient on the plastic carrier surface into the agar matrix. A stable, continuous and exponential gradient of antibiotic concentrations is formed directly underneath the strip. After incubation, whereby bacterial growth becomes visible, a symmetrical inhibition ellipse center along the strip is seen. The MIC value is read from the scale in terms of μg/mL where the pointed end of the ellipse intersects the strip.

Materials:

- Agar plates (150 mm) with the appropriate susceptibility test media
- Inoculum suspension media
- Swabs (sterile, non-toxic and not too tightly spun), test tubes, and scissors
- 0.5 McFarland turbidity standards
- Incubator (35 ± 2 °C), anaerobic jar or chamber or CO2 enriched chamber
- Quality control organisms
- E-test gradient strip
- 2-3ml of 0.85% Normal saline
- Test tube
- Test isolate(pure colonies)

Procedure:

- Removed a test strip package from a refrigerator/freezer and put at room temperature before opening (approx. for 15 minutes)

- Prepare a mixureof isolated colonies from an overnight agar plate in a suitable suspension medium to achieve the specified inoculum turbidity by comparing to a McFarland turbidity standard.
- Inoculate the suspended isolate to appropriate agar plates (90 mm or 150 mm) by using sterile polister applicator stick.
- After 3-15 min put a E test strip on inoculated media than incubate at optimum temperature
- Read the MIC after 20-24 hour of incubation.

Result interpretation:

Read the MIC value: where the pointed end of the inhibition ellipse intersects the side of the strip

- Only when an even lawn of growth is distinctly visible

Do not read (Repeat the test):

- If the culture appears mixed
- If the growth is too light or too heavy

Quality control (QC): *Neisseria gonorrhoeae* ATCC 49226 Expected results:

- MIC that fall below the lower QC limit should be rounded up to the next upper two-fold show -QC compliance.
- MIC results that above the upper limit show non-QC compliance.

13. Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the candidate: Senait Tadege (BSc)

Signature_____

Place: Addis Ababa University, School of Allied Health Science, Department of Medical laboratory Science, Ethiopia

Date of submission_____/_____/_____

This thesis has been submitted with my approval as university advisor.

Name of advisors: Mr. Kassu Desta (MSc, PhD fellow) Signature _____

Mr Surafel Fantaw (MSc) Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_____/_____