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Prevalence and drug susceptibility pattern of *Neisseria gonorrhea* among symptomatic women attending gynecologic OPD in Hawassa Referral Hospital.

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ABBREVIATIONS

AMR	antimicrobial resistance
ATCC	American Type Culture Collection
CDC	center of disease control
CLSI	Clinical and Laboratory Standards Institute
EHNRI	Ethiopian Health and Nutrition Research Institute
GCON	Gonococcal infection of neonates
GNIDC	Gram negative intracellular diplococcic
GNDC	Gram negative diplococcic
HIV	Human immunodeficiency syndrome
MIC	minimal inhibitory concentration
MOH	Ministry of health
MIC50	antibiotic concentration required to inhibit half the isolates tested
PPNG	penicillinase-producing Neisseria gonorrhoeae
NCCL	National Committee for Clinical Laboratory Standards
NG	Neisseriae gonorrhea
PID	pelvic inflammatory disease
RTI	reproductive tract infection
SNNP	South Nations Nationality and People
SPSS	Statistical Package for Social Science
STD	Sexually transmitted disease
STI	Sexually transmitted infection
US	United States
WHO	World Health Organization

ABSTRACT

Background: Gonorrheae is a sexually transmitted disease caused by the bacterium *Neisseria gonorrhea* for which humans are the only natural host. It is fastidious Gram-negative cocci transmitted by human-to-human contact. Gonococcal infection highly adapted to the genital tract and often causing asymptomatic and undetected infection in females in which the highest rates are found mainly in female's of reproductive age group. There are a number of antibiotics for treatment of gonorrhea in which the preferences vary with the local situation.

Objective: To determine the prevalence and drug susceptibility pattern of *Neisseria gonorrhea* among symptomatic women attending gynecologic OPD in Hawassa Referral Hospital.

Methods: A cross-sectional study was conducted from December 2010 to February 2011 at Hawassa University Referral Hospital Hawassa, Ethiopia. During the study period, all women patients visited to gynecologic OPD with suspected of gonococcal infection were included. The inclusion criteria for the selection of 215 women of reproductive age group was based on any sign and symptoms for STD up on clinical examination of symptomatic patients at gynecologic OPD. Endocervical swab were collected by the attending physician. The Presence of gonorrhea was confirmed by culture, Gram staining and biochemical tests. Antimicrobial sensitivity test was performed using disc diffusion method and the result was interpreted accordingly.

Result: Of the total 215 patients examined, 11 (5.1%) were confirmed to have gonococcal infection. The highest prevalence was observed in age group 20-24 which is 5/11 (45.5%) and there is no statistical significant association ($p>0.05$). The susceptibility patterns were done against 11 antimicrobial agents by the agar disc diffusion technique. Low level of susceptibility to quinolones (ciprofloxacin 55%, ofloxacin 64% & lomefloxacin 64%) was reported.

Conclusion: The findings from this study indicate that gonococcal infection was moderately higher combined with high *Neisseria gonorrhea* resistance.

Key words: *Neisseria gonorrhea* (NG), women infection, Thayer-Martin medium

CHAPTER I BACKGROUND

1.1 HISTORICAL PERSPECTIVES

Reference to *Neisseria gonorrhea* infection can be found in biblical text within the Book of Leviticus and in ancient Chinese writings, making gonorrhea one of the oldest diseases known to humans. Hippocrates referred to acute gonorrhea as “strangury” obtained from the “pleasures of Venus” in the fourth and fifth centuries B.C. It was not until A.D. 130 that Galen, who mistakenly confused the purulent discharge associated with gonococcal urethritis with semen, introduced the term gonorrhea, i. e., “flow of seed” the English nicknamed gonorrhea “the clap”. However, many think the word comes from a combination of the Greek words gono and rrhea Gono which means “seed” and rrhea which means “to flow”. The flow of urine is disrupted when a person is infected with gonorrhea. “The clap” is a nickname for this disease to describe the clapping and stinging sensation that men and women both will feel during urination. (1)

The most notable account of NG infection is found in the personal diary of James Boswell, the famous biographer of Samuel Johnson. Within these pages, Boswell described more than 19 separate infections with the gonococcus, the consequences of which ultimately led to his death. Boswell also described what might possibly be the consequence of asymptomatic infection in women, in that his wife, who reportedly never exhibited symptoms of gonococcal infection, lost four of her nine pregnancies. Neisser described the causative agent of gonorrhea, *Neisseria gonorrhea*, in 1879; however, it was not until 1882 that Leistikow and Lo¨ffler finally cultivated the gonococcus. (1)

The advent of sulfonamide (in 1936) and penicillin (in 1943) antibiotic therapy for the treatment of NG infection led to a rapid decrease in gonorrhea prevalence. In the early 1960s this trend reversed with the onset of oral contraceptive methods, and NG infections reached a peak incidence of over one million reported cases in 1978 in the United States. In the late 1980s, with the onset of the HIV epidemic and a coincident widespread use of barrier contraceptives, the incidence of gonococcal infection again declined (2)

1.2. MICROBIOLOGY OF NESSERIA GONORRHOEAE

Neisseria gonorrhea is fastidious Gram-negative cocci that require nutrient supplementation to grow in laboratory cultures. Specifically, they grow on chocolate agar with carbon dioxide. These cocci are facultatively intracellular and typically appear in pairs (diplococci), in the shape of coffee beans.(3)

Neisseria gonorrhea is usually isolated on Thayer-Martin agar—an agar plate containing antibiotics (Vancomycin, Colistin, Nystatin, and SXT) and nutrients that facilitate the growth while inhibiting the growth of contaminating bacteria and fungi. Further testing to differentiate the species includes testing for Oxidase (all clinically relevant Neisseria show a positive reaction) and the carbohydrates maltose, sucrose, and glucose test in which *Neisseria gonorrhea* will only oxidize (that is, utilize) the glucose. They are distinguished from other neisseriae by their ability to grow on selective media and to utilize glucose but not maltose, sucrose, or lactose.(3)

Neisseria gonorrhea is non-motile and possesses a type IV pilus to adhere to surfaces. The type IV pilus operates mechanistically similar to a grappling hook. The pilus extends from the pole of the cell and attaches to a substrate which signals the pilus to retract, dragging the cell forward. *Neisseria gonorrhea* are able to pull 100,000 times their own weight and it has been claimed that the pili used to do so are the strongest biological motor known to date, exerting one nano newton. (4)

1.3. EPIDEMIOLOGY OF GONORRHEA

In various parts of the world provide evidence that treatable STIs are a major public health problem in all countries, but are especially so in developing countries where access to adequate diagnostic and treatment Facilities is very limited or non-existent. Even though there is little information on the incidence and prevalence of STIs in Ethiopia, the problem of STIs is generally believed to be similar to that of other developing countries. (5)

The Integrated Disease Surveillance Team of the Ministry of Health (MOH) compiled 58,623 and 27,947 STI cases from all the regions in 2002 and 2003 respectively using routine quarterly

reports. Obviously, there is under reporting of STI cases in most developing countries including Ethiopia. There is increasing evidence that a large proportion of STIs are asymptomatic and most symptomatic patients seek treatment from traditional healers, pharmacists, drug vendors shops and marketplaces, where reporting is not the standard practice. Moreover, except for the adult prevalence of HIV (4.7%) and syphilis (1.8%) there is no national estimate on the prevalence of other STIS including gonorrhea. The pattern of reporting from health institutions is not uniform some health institutions report using syndrornic approach while others use etiologic approach. In addition, there is weak STI surveillance system in the country. [5]

Moreover, most countries of the developing world lack appropriate diagnostic tools that are rapid, easy to use in the field, affordable, and of an acceptable level of reliability. So the estimate of undiagnosed gonorrhoeae prevalence is high. Young people are at an even greater disadvantage due to the absence of proper case detection and treatment, for at least two reasons. First, unmarried young people have special problems related to access to and utilization of health services. Second, they do not have the information necessary for early recognition of disease symptoms. [6]

The epidemiology of NG is highly different in developed country too. Indeed, since the beginning of the 20th century, peaks of reported cases of gonorrhea in developed countries occurred during World Wars 1 and 2, and following the "sexual liberation" of the late 1960s and early 1970s. Thereafter, there has been a sharp decline in the incidence of this disease in almost all western countries. This decline occurred first in countries of Northern Europe. For instance, in Sweden, gonorrhea incidence (reported cases) decreased from 487 per 100,000 in 1970 to 31 per 100,000 in 1987, and was below 10 per 100,000 in 1994. In parallel with this decline, there has been a steady reduction in the male:female ratio of reported cases. This early decline in incidence, as well as the reduction in the male:female ratio, can be attributed, at least in part, to improved screening programs for women and enhanced partner notification of STD cases.[7]

The estimated prevalence of NG in USA in 2004 was 294per 100,000 which increased dramatically. In 2006, the median state-specific gonorrhea test positivity among 15- to 24-year-old women screened in selected family planning clinics in USA, was 1.1% (range 0.0% to

4.8%) For women attending selected prenatal clinics the prevalence was 1.0% (range 0.0% to 3.2%) [8]

To detect prevalence of STI, and to monitor the antibiotic susceptibility of NG among women attending a gynecology outpatient department in Vientiane, Laos (2006) a total of 1125 Women aged 15 to 49 years participated in study. 41 of them have gonorrhea, the prevalence of 3.7%, and among 41, of them 20% showed resistance to ciprofloxacin, 98% to penicillin, and complete to tetracycline. The researcher Conclusion was high NG level combined with high NG resistance emphasizes that concurrent with syndromic case management, periodic evaluations of etiological diagnosis should be available to ensure adequacy of treatment algorithms and prescribed medications.[9]

The cross-sectional study which was done on asymptomatic women of child bearing age in Jordan, Patient to gynecology and family planning clinics from different areas in Jordan were tested for reproductive tract infections. The study result shows, the prevalence of N. gonorrhoeae was 2.2% and the investigator Concludeds: Based on the low prevalence of sexually transmitted infections detected in this study among Jordanian women, the need for screening programs for such infections is needed. [10]

On the other study to determine the prevalence of *Neisseria gonorrhea* and its association with other STD causing organisms, Three hundred and thirty-six women (female sex workers (FSWs) and married contacts), attending a sexually transmitted disease (STD) clinic in Mumbai, were screened, 9.7% of the women were positive for N. gonorrhoeae, 23.2% were chlamydia-positive and 5.9% had trichomoniasis. A significant association between sexual habits and prevalence of gonorrhoea was recorded. The researcher concludes that the prevalence of gonorrhoea over 1988 to 1996 remained approximately the same. [11]

To investigate the prevalence of RTIs/STIs among Married women aged 18-49 years in a rural district of Vietnam, 1012 women were interviewed and underwent a gynecological examination using cross-sectional study. Specimens were collected and laboratory diagnosis was done, Analyses on the influence of socio-economic, socio-demographic, and other determinants

possibly related to RTIs/STIs was performed. The final result showed that the prevalence of *Neisseria gonorrhea* was 0.7% which was very low compared to other STDs. (12)

In sub-Saharan Africa, the prevalence of gonorrhea among women was known-ranges from 2% to 15%, and rates of chlamydial infection range from 7% to 30% even though undiagnosed prevalence was very high.(13) Study in Papua New Guinea show that most of the time gonococcal infection was presence as mixed infection with T.vaginalis or with Chlamydia and most of the time gonorrhea may masked by these symptomatic STI which increased risk of having resistant strain. Only few studies have targeted young people to estimate the burden of STDs. Globally, however, it is estimated that every day, around half a million young people (10-24 years old), or about 1 in 20, acquire an STD, adding up to more than 150 million new infections in a year, among these females are the main. (14)

In the study to describes the prevalence and the etiology of STIs and the prevalence of cervical neoplasia among women in southern Mozambique, (2010) An age-stratified cross-sectional study was performed where 262 women aged 14 to 61 years were recruited at the antenatal clinic (59%), the family-planning clinic (7%), and from the community (34%). The prevalence of NG was 14%. A higher prevalence was observed in the reproductive age group and some of the STIs were more frequently diagnosed. It is concluded that STI control programs are a priority to reduce the STIs burden because At least one active STI was diagnosed in 79% of women. (15)

Prevalence and antimicrobial susceptibility of NG isolated from patients in various locations of Kaduna state was done in Nigeria. (2003) Out of the 1715 patients screened, 275 (16.03%) were found positive for NG infection. This study showed that the age group 15-20 years had the highest prevalence of infection (31.05%) While the highest prevalence of infection in the female patients (11.18%) was found in the age group 15-20 years. Out of the 275 gonococcal isolates, 225 (81.82%) were resistant to penicillin, 206 (74.91%) to ampicillin, 122(44.36%) to tetracycline, 34(12.36%) isolates to erythromycin, and 16(5.82%) isolates were resistant to gentamicin. All the 275 NG isolates were sensitive to Ceftriaxone, Ceproxine and Oflozacin. Generally the researcher Concluded there was high prevalence rate of NG infection and highly

resistant to most commonly used antibiotics in the treatment of gonorrhoeae in Kaduna State.[16]

In Ethiopian twenty year back the prevalence of NG was done in women attending gynecologic, obstetric and family planning clinics to correlate the serological diagnosis of gonorrhoeae with clinical evidence of pelvic infection. This is in order to define a reliable clinical diagnosis of gonorrhoeae in a country where pelvic inflammatory disease is very common but where routine laboratory culture and serological tests for gonorrhoeae are unavailable. The cross-sectional study using indirect haemagglutination test with gonococcal pilus antigen as an epidemiological tool was used in a study to screen 1851 sera for evidence of past or current gonococcal infection. Fifty nine per cent of the study group were seropositive for the gonococcal antibody test, 22% with titres greater than or equal to 1/320, indicative of current, recent or recurrent infection. The investigator concludes that asymptomatic clinic attenders must be of concern for all health workers especially those in gynecology and obstetrics and the related disciplines of family planning and neonatal pediatrics. Gonococcal antibodies were present in 54% of women with no clinical evidence of infection. Thus he said it is difficult to define a diagnostic clinical picture of NG in Ethiopian women. [17]

1.4. PATHOGENESIS OF N.GONORRHAE INFECTION

Gonorrheal pathogenesis can be grouped systematically in to; Attachment to epithelial surfaces, Invasion and activation of inflammatory activation.

A. Attachment

Neisseria gonorrhea colonizes mucosal surfaces primarily of the lower genital tract and occasionally ascends to the upper genital tract or invades and colonizes the blood. For infection to occur, the organism must adhere to the epithelium, or invade the host cells. The bacteria acquire sufficient nutrients from the host to survive and evade the host's immune response. Infection with NG stimulates an inflammatory response, resulting in a massive infiltration of polymorphs. If NG is to attach successfully to the epithelium, it must avoid being swept away by cervical secretions in women and urine in men. In addition, both the bacterial and epithelial cells are negatively charged and, therefore, would naturally repel. Pile, which are hydrophobic

surface appendages, are the primary mediators of attachment, firstly, overcoming the electrostatic forces between the bacterial and host cell and then by attachment to the mucosal surface by specific receptors. NG produces type VI pili that have multiple functions including adhesion and genetic transfer such as transformation. The host cell receptor for neisserial pili has been identified as CD46, a member of the complement resistance proteins. Secondary attachment is mediated mainly by the Opa proteins, but lipooligosaccharide (LOS) and the gonococcal porin Por have also been implicated in adhesion to the cell surface.[18,19]

The bacteria adhere to columnar epithelial cells, penetrate them, and multiply on the basement membrane. Adherence is mediated through fimbriae and opa (P.II) proteins. Although nonspecific factors such as surface charge and hydrophobicity may play a role. Fimbriae undergo both phase and antigenic variation. The bacteria attach only to microvilli of nonciliated columnar epithelial cells. Attachment to ciliated cells does not occur.[19]

B. Invasion

After the bacteria attach to the nonciliated epithelial cells of the fallopian tube, they are surrounded by the microvilli, which draw them to the surface of the mucosal cell. The bacteria enter the epithelial cells by a process called parasite-directed endocytosis. During endocytosis the membrane of the mucosal cell retracts and pinches off a membrane-bound vacuole that contains the bacteria. [20] The vacuole is transported to the base of the cell, where the bacteria are released by exocytosis into the sub epithelial tissue. The neisseriae are not destroyed within the endocytic vacuole, but it is not clear whether they actually replicate in the vacuoles as intracellular parasites. A major porin protein, P.I (Por), in the outer membrane of the bacterium is thought to be the invasion that mediates penetration of a host cell. Each NG strain expresses only one type of Por; however, there are several variations of Por that partly account for different antigenic types of the bacterium. [19]

C. Activation of inflammatory responses

During infection, bacterial lipooligosaccharide (LOS) and peptidoglycan are released by autolysis of cells. Both bacterial polysaccharides activate the host alternative complement

pathway, while LOS also stimulates the production of tumor necrosis factor (TNF) that causes cell damage. Neutrophils are immediately attracted to the site and feed on the bacteria. For unknown reasons, many gonococci are able to survive inside of the phagocytes, at least until the neutrophils themselves die and release the ingested bacteria.

Neisserial LOS has a profound effect on the virulence and pathogenesis of NG. The bacteria can express several antigenic types of LOS and can alter the type of LOS they express by some unknown mechanism. Gonococcal LOS produces mucosal damage in fallopian tube organ cultures and brings about the release of enzymes, such as proteases and phospholipases that may be important in pathogenesis. Thus, gonococcal LOS appears to have an indirect role in mediating tissue damage. Gonococcal LOS is also involved in the resistance of NG to the bactericidal activity of normal human serum. Specific LOS oligosaccharide types are known to be associated with serum-resistant phenotypes of N. gonorrhoeae. [19, 20]

1.5 Iron Acquisition

Once colonization has been established, *Neisseria gonorrhea* needs to acquire iron to survive. Unlike other organisms that produce siderophores to chelate iron from the environment, *Neisseria gonorrhea* has evolved mechanisms to acquire iron directly from human transferrin and Lactoferrin. It is postulated that, in the absence of sufficient iron, transcription of transferrin and lactoferrin receptors and transferring-binding proteins is induced in the organism. These receptors interact with human transferrin and lactoferrin, and iron is removed and transported across the bacterial cell membrane into the periplasmic space. During this process, there is a transient association with iron-binding proteins, and then, iron is transported across the cytoplasmic membrane. Other essential nutrients are transported through the porin, which is a polymer of the outer membrane proteins Por and Rmp.[19,21]

1.6 Immune Response

On successful colonisation, the organism elicits an inflammatory and antibody response in the host. N.gonorrhae is known to be ingested by macrophages, and this may be a primary mechanism by which the host eradicates infection. However, evidence that the phagocytosed organisms are killed is inconclusive. Sialylation of LOS and the expression of the Opa proteins

have been shown in vitro to enhance the ability of the organism to resist phagocytic killing. Antibody to *Neisseria gonorrhea* , both immunoglobulin G (IgG) and IgA, is produced in response to infection, both in serum and in local secretions. The function of antibody produced in response to N.gonorrhae is not known. Systemic antibody may activate the complement pathway and play a role in limiting disseminated infection. However, it is not known whether complement-mediated lysis occurs in mucosal secretions. Antibody in secretions may inhibit attachment or phagocytosis, although there is little evidence that it affords protection against subsequent infection.[20,21]

1.7 Antimicrobial Resistance

Neisseria gonorrhea has a circular DNA genome of approximately 1Mbp encoding over 2000 genes It is genetically diverse and competent for genetic exchange throughout its lifecycle. This ability has led to the acquisition and selection of resistance to most antimicrobials used for treatment of gonorrhoeae. Resistance to antimicrobials creates challenges for successful treatment of gonorrhoeae and therefore timely and accurate surveillance data on the distribution of determinants of resistance is essential to inform national treatment guidelines. Gonococci become fully resistant to antibiotics either by chromosomal mutations or by acquisition of R factors (plasmids). Two types of chromosomal mutations have been described. The first type, which is drug specific, is a single-step mutation leading to high-level resistance. The second type involves mutations at several chromosomal loci that combine to determine the level as well as the pattern of resistance. [21]

Penicillinase-producing strains of *Neisseria gonorrhea* were first described in 1976 and there are five related ß-lactamase plasmids of different sizes have been identified. Their prevalence penicillin-resistant strains have increased dramatically in world since 1984. After Plasmid-mediated resistance of N. gonorrhoeae to tetracycline was first described in 1986 and has now been reported in most parts of the world. This resistance is due to the presence of the streptococcal tetM determinant on a gonococcal conjugative plasmid. The evolution of antimicrobial resistance in *Neisseria gonorrhea* affects the control of gonorrhea. Strains with multiple chromosomal resistances to penicillin, tetracycline, erythromycin, and cefoxitin have

been identified in the United States and most other parts of the world. Sporadic high-level resistance to spectinomycin and fluoroquinolones also has been reported.[22, 23]

Neisseria gonorrhoeae usually develops resistance to antimicrobial agents within a few years of their introduction for gonorrhea therapy. Antimicrobial resistance in N. gonorrhoeae occurs as chromosomally mediated resistance to a variety of antimicrobial agents, including penicillin, tetracycline, spectinomycin, and fluoroquinolones, and high-level, plasmid-mediated resistance to penicillin and tetracycline. [24]

Because of the spread of gonococcal isolates resistant to penicillin and tetracycline, CDC has recommended that extended-spectrum cephalosporins (ceftriaxone, cefixime) and fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin) be used as primary therapies to treat uncomplicated gonorrhea. [23] At this time (2002), no failures of gonococcal infections to respond to cephalosporins have been confirmed. Failures of gonococcal infections to respond to fluoroquinolones (ciprofloxacin, ofloxacin) have been reported. In response to this increase, many states have revised recommendations for the treatment of gonorrhea. Within the class of cephalosporins, CDC recommends ceftriaxone, available as an injection and oral, the preferred treatment for all types of gonorrhea infection [24]

Between the year 2003 and 2005, the standard treatment with the antibiotic ciprofloxacin was phased out in most developed country and replaced with the newer class of antibiotics that are called cephalosporins. Now doctors are running out of options, and they are being forced to use less effective drugs to which there is no resistance. These less effective treatments are prolonged, which increases the risk of onward transmission. [23]

The Australian Gonococcal Surveillance Programme monitors the antibiotic susceptibility of NG isolated in 2008 using in vitro test method for 3,110 patients from public and private sector. Different antibiotic susceptibility patterns were seen in the various regions. Resistance to the penicillin nationally was at 44% Quinolone resistance also increase so that nationally 54% of all isolates were ciprofloxacin-resistant. All isolates remained sensitive to spectinomycin. Approximately 1.1% of isolates showed some decreased susceptibility to ceftriaxone [25]

On the other study to monitor Antimicrobial susceptibility of NG in North-East region of Romania (2004-2008) The study done from clinical cases, on 60 NG isolates to penicillin, tetracycline, amoxicillin, augmentin, clarithromycine, ciprofloxacin, and ceftriaxone, by disc diffusion method. The results showed that A high proportion of strains were resistant to tetracycline and penicillin (70 and 80% respectively); 95% of strains were sensitive to ceftriaxone, one strains was resistant and 2 of them (3.33%), intermediate resistant. For all other tested antibiotics the level of resistant strains varied from 55 to 65%.The investigeters conclusion was Penicillin, tetracycline, ciprofloxacin, clarithromycine, amoxicillin, and augmentin can not be indicated as treatment of N. gonorrhoeae infection because of high level of resistance. An active and comprehensive studies for monitoring and surveillance of antimicrobial resistance of N. gonorrhoeae needs to be established in all regions of the country, as support of new therapeutically treatment scheme [(26)]

To investigate the in vitro antimicrobial susceptibility of NG strains isolated in 2004 and 2006 in Bangui, Central African Republic; Yaoundé, Cameroon; Antananarivo, Madagascar; and Ho Chi Minh Ville and Nha Trang, Vietnam. To 5 antimicrobials (penicillin G, ceftriaxone, ciprofloxacin, spectinomycin, and tetracycline) were determined. Patterns of resistance were similar in Antananarivo, Bangui, and Yaoundé but different from those observed in Vietnam. Ciprofloxacin was highly effective in Africa, but nearly all strains in Vietnam were resistant to this drug. Overall, ceftriaxon and spectinomycin were the best antibiotics, with one strain resistant to spectinomycin in Antananarivo and one strain resistant to ceftriaxon in Ho Chi Minh Ville. The investigators conclusion was: ciprofloxacin remains highly efficient in Madagascar and central Africa, ceftriaxone and spectinomycin should be used as the first-line antimicrobial agents in treating gonorrhea in Vietnam.[(27)]

In Ethiopia antibiotics recommended for treating gonorrhea include ciprofloxacin, spectinomycin and third-generation cephalosporins such as ceftriaxone and cefixime. All of these antibiotics should be included in a susceptibility testing. Although penicillin and tetracycline are not currently being used, resistance to these antibiotics should be monitored because they are still used in other parts of the country and because the worldwide prevalence of resistance to these two antibiotics remains high.[(28)]

To determine the antimicrobial susceptibility pattern of *Neisseria gonorrhea* in Gondar, Ethiopia. (2001) A total of 142 strains of N. gonorrhoeae were isolated from 168 cultures received. The sensitivity of gonococcal isolates ranges from 98.6% to chloramphenicol to 7.7% to cotrimoxazole. Multiple drug resistance was reported in 87.5% of isolates and only four isolates were sensitive to all antibiotics. One strain of N. gonorrhoeae was resistant to as many as eight antibiotics (tetracycline, penicillin, ampicillin, kanamicin, methicilin, carbenicillin, cotrimoxazole and ceftriaxone). More than eighty five per cent of the isolated strains were penicillinase-producing Neisseria gonorrhoeae (PPNG). The investigator conclude Gonococcal resistance is a significant public health problem in Gondar region and the drugs recommended for treatment of gonococcal cases by the national sexually transmitted diseases (STDs) control programme need to be revised. In the other hand Monitoring geographical and temporal trends of antimicrobial susceptibilities provides information useful in the development of treatment guidelines and interventions for the control of infections. [(29)]

In the other study Sixty eight *Neisseria gonorrhea* strains were isolated from endocervical and urethral discharge of 233 patients attending health centers for sexually transmitted diseases (STDs) in Addis Ababa, were identified following conventional procedures and tested for susceptibility to penicillin, ampicillin, trimethoprim-sulphamethoxazole (bactrim), chloramphenicol, erythromycin and kanamycin by the agar disc diffusion technique. Penicillinase producing N. gonorrhoeae (PPNG) was identified using the chromogenic cephalosporin method and comprised 70% of the isolates. Seventy seven per cent, 73%, 64% and 17% of the isolates were found to be resistant to penicillin, ampicillin, bactrim and kanamycin, respectively. However, no resistance to erythromycin and chloramphenicol was observed. Multiple drug resistance was found to be 67%. This is a cause for concern in the control and treatment of gonococci.[(30)]

Currently most Study has revealed that STDs, especially gonorrhea is becoming more resistant to several antibiotic drugs. Scientists are worried that gonorrhea will soon become untreatable with these antibiotics. They claim that the gonorrhea is very versatile and develops resistance to antibiotics rapidly. This is the main reason that selecting the correct antibiotic will prove to be a hurdle. This leads to conclusion that if the problem won’t be attended to on time, it will become very difficult to treat the infection.[(31)]

It is known that a standard treatment regimen is expected to cure 95% or more of gonorrhoeae infections. Because of the close correlation between in vitro resistance and clinical failure, in general, an antibiotic should not be used when more than 5% of strains are resistant to it. [32]

1.8 Gonococcal Infections in Females

A. Gonococcal Cervicitis

Endocervical infection is the most common form of uncomplicated gonorrhea in women. Mucopurulent cervicitis is the diagnosis in women and may be caused by NG, C. trachomatis, and other organisms. NG primarily infects the cervical os but can also infect more peripheral areas of the cervix where columnar epithelium meets stratified squamous epithelium. Women infected with NG usually develop symptoms. However, the women who either remain asymptomatic or have only minor symptoms may delay in seeking medical attention. These symptoms may include scant discharge from the vagina that may issue forth from the inflammed cervix and dysuria (often without urgency or frequency) the physical examination may reveal a muco-purulent discharge (mucopus) issuing from the cervical os. The examiner may check for muco-purulent discharge by swabbing a sample of mucus from the endocervix and observing its color against the white background of the swab; yellow or green mucus suggests mucopus. However, only 35% of women with gonococcal cervicitis actually have a Mucopurulent discharge defined by these criteria. [21]

B. Complications of Gonococcal Cervicitis

Disseminated gonococcal infections result from gonococcal bacteremia. Asymptomatic infections of the pharynx, urethra, or cervix often serve as focal sources for bacteremia. Gonococci may ascend from the endocervical canal through the endometrium to the fallopian tubes and ultimately to the pelvic peritoneum, resulting in endometritis, salpingitis, and finally, peritonitis.[19] Women usually present with pelvic and abdominal pain, fever, chills, and cervical motion tenderness. This complex of signs and symptoms is referred to as pelvic inflammatory disease (PID). Gonococcal infection may extend deep enough to produce dyspareunia and lower abdominal or back pain. In such cases, it is imperative to consider a diagnosis of PID and to administer treatment for that disease. Ascending infection of the genital tract follows >20% of cases of gonococcal cervicitis and may result in acute endometritis

accompanied by abnormal menstrual bleeding, midline lower abdominal pain and tenderness, and dyspareunia. Spread to the fallopian tubes results in acute salpingitis, whose symptoms may be accompanied by signs of cervical motion tenderness and abnormal adnexal mass on pelvic examination. Tubal scarring leading to infertility is the most devastating sequela of salpingitis; the increased risk of ectopic pregnancy is also significant. Prompt and appropriate antibiotic therapy for gonococcal salpingitis (prior to the development of an adnexal mass) can prevent tubal infertility in nearly all cases. Bilateral tubal damage occurs in 20% of women with an adnexal mass. These women with "silent salpingitis" may report abdominal or pelvic discomfort (such as dysmenorrhea or dyspareunia) that may be attributed to other diagnoses (such as endometriosis). Spread of infection to the pelvis may result in pelvic peritonitis characterized by nausea and vomiting. Spread of gonococci or, more commonly, of chlamydiae—via the peritoneal cavity to the upper abdomen may cause perihepatitis [21]

C. Complications of Gonococcal Cervicitis

Gonorrhea in pregnancy can have serious consequences for both the mother and the infant. These women should be monitored closely for these infections throughout pregnancy. The incidence of gonorrhea in pregnancy ranges from rare to 10%, depending upon the population surveyed. Salpingitis and PID can occur during the first trimester and are associated with a high rate of fetal loss. In the second and third trimesters, the relative impermeability of the cervical mucus (under the influence of progesterone) and the obliteration of the intrauterine cavity (resulting from the attachment of the chorion to the endometrial decidua by around the twelfth week of gestation) pose physical barriers that usually prevent ascending infection. Pharyngeal infection, most often asymptomatic, may be more common during pregnancy because of altered sexual practices. Acquisition of gonococcal infection late in pregnancy can adversely affect labor and delivery as well as the well-being of the fetus. Prolonged rupture of the membranes, premature delivery, chorioamnionitis, funisitis (infection of the umbilical cord stump), and sepsis in the infant (with NG detected in the gastric aspirate of the newborn during delivery) are common complications of maternal gonococcal infection at term. Hazards to the fetus include spontaneous abortion, perinatal death, premature delivery, perinatal distress, and premature [18, 21]

A review of Lower genital tract infections among pregnant women in Ethiopia shows that it is very common among apparently healthy looking pregnant women with an overall prevalence of 40-54%. Specific pathogens that were isolated from the vagina and/or cervix of asymptomatic pregnant women include: C. albicans (14-42%), T. vaginalis (11-20%), C. trachomatis (7-31%), N. gonorrhoea (0.5-14%) and group B streptococcus (4-25%). Untreated, genital tract infections in pregnant women may result in fetal loss, preterm labour, preterm birth, premature rupture of the membranes, low birth weight, eye and lung damage in the newborn. The outer conclusion was; Routine screening for clinically important pathogens should be considered during antenatal service. There is a need to develop simple, cheap and reliable laboratory tests and better clinical algorithms for the diagnosis of reproductive tract infections among pregnant women. (33)

1.9 Statement of the Problem

Gonorrhoeae is one of the oldest sexually transmitted disease caused by the bacterium Neisseria gonorrhoeae for which humans are the only natural host. It is transmitted by human-to-human contact and is highly adapted to the genital tract, surviving poorly outside the human body. Nevertheless, it is versatile in resisting attack, for example in its ability to develop resistance to antimicrobials. And in its antigenic variability, by which it evades host defences, thus persisting and often causing asymptomatic and undetected infection. The highest rates are found mainly in female's reproductive age group. (34)

It is estimated that about half a million or 1 in 20 young people acquire STI every day, adding up to more than 150 million new infections in a year. The long-term consequences of gonococcal infections may be severe, particularly in women and cervical infection can lead to pelvic inflammatory disease, tubal scarring, infertility, ectopic pregnancy, chronic pelvic pain, and rarely, death from tuboovarian abscesses or ruptured ectopic pregnancies.(4) In addition, gonorrhoeae is associated with significant enhancement of transmission of HIV by up to 500% (35)

Many women have no symptoms, however. Physical findings in women include cervicitis with muco-purulent drainage. The cervix tends to bleed easily when rubbed with a cotton-tipped swab. Ten to 20 percent of women with gonorrhea develop ascending infection that causes

acute salpingitis with or without endometritis, also known as PID. Presentations may range from no symptoms to severe abdominal pain with a high fever. PID can negatively affect fertility, causing infertility in 15 percent of patients; 50 percent of patients who have three or more episodes of PID develop infertility. [36]

Gonorrhea in pregnancy can have serious consequences for both the mother and the infant. Therefore, early detection and eradication of the disease in the mother are extremely important. Acquisition of gonococcal infection late in pregnancy can adversely affect labor and delivery as well as the well-being of the fetus. Prolonged rupture of the membranes, premature delivery, chorioamnionitis, funisitis (infection of the umbilical cord stump), and sepsis in the infant are common complications of maternal Gonococcal infection at term. Hazards to the fetus include spontaneous abortion, perinatal death, premature delivery, perinatal distress, and premature rupture of membranes. An infection of the newborn acquired from the birth canal of gonorrhoeal mother. It may involve the cornea leading to blindness. The involvement of testicles, fallopian tubes or ovaries may result in sterility. [21, 35]

The Centers for Disease Control and Prevention (CDC) recommends that physicians maintain a low threshold for diagnosing PID because of significant negative sequelae associated with this infection. The CDC currently recommends empiric treatment of PID in women with uterine and adnexal tenderness or cervical motion tenderness if they are at risk of sexually transmitted diseases (STDs) and no other causes can be identified.[36]

Antibiotic resistance increasingly compromises effective treatment of gonorrhoeae. Inexpensive treatment regimens have been rendered ineffective while efficacious ones are often unaffordable. The temptation to use ineffective but cheaper remedies (which are ultimately more expensive because of failure to control disease spread and the cost of inevitable complications) must be resisted. It is necessary to demonstrate the benefits of appropriate treatment and for gonorrhoeae, this will require continuing, high-quality susceptibility surveillance.[37]

In general, gonococcal infection is a major global cause of illness, infertility, long term disability and death, with severe medical and psychological consequences for millions of women and children. The World Health Organization states that: "in developing countries,

STDs and their complications are amongst the top five disease categories for which adults seek health care. In women of childbearing age, STDs (excluding HIV) are second only to maternal factors as causes of disease, death and healthy life lost". [38]

1.10 Significances of the study

Gonorrhea in women is a much more frequent and serious disease than was formerly supposed. The general impression among the laity is that gonorrhea in women is limited to the prostitute and vicious classes who indulge in licentious relations. Unfortunately, this is not the case. There is perhaps more gonorrhea, in the aggregate, among virtuous and respectable wives than among professional prostitutes. It has a disproportionate impact on the health of women. In women, it is often chronic, presenting with vague or no symptoms, but may lead to severe complications such as chronic pelvic inflammatory disease, ectopic pregnancy, and infertility. Because of the lack of diagnostic and treatment facilities, limited opportunity for seeking medical care, and poor health-care-seeking behavior. The impact of gonococcal infection on ill health tends to be more severe among rural women in resource-poor settings. High rates of STDs have been documented among apparently low-risk women living in such settings. Moreover, multiple sexually transmitted infections are often common among these women, thus increasing the disease burden and risk of complications. This heavy burden of untreated STDs among women living in poor rural areas calls for a more gender-specific approach in prevention and treatment of these infections. This should include determination of the prevalence rates and pattern of resistance to gonorrhea in such communities in order to rationalize healthcare delivery. [39, 41]

Studying the prevalence and drug susceptibility pattern in countries like Ethiopia is very important, because it is more frequent in poorer countries and it causes consequences of a high incidence of complications and long-term morbidity, as well as increased HIV transmission. Since genital gonococcal infections cause no or few symptoms as many as 80% of infected women, many infections remain undetected. Especially, large proportions of young people do not seek or have special problems to access to health care services. [42]

On the other the hand danger of gonorrhea introduced after marriage is not limited to the risks to the health of the woman. When a woman thus infected bears a child the contagion of the disease may be conveyed to the eyes of the child in the process of birth. Gonorrheal pus is the most virulent of all poisons. A single drop of the pus transferred to the eye may destroy this organ in from twenty four to forty eight hours. Neonates are exposed to infected cervical exudates during delivery, so detecting, identifying and treating gonorrhea-related ophthalmia neonatorum is important because, if left untreated, it can cause perforation of the globe of the eye and blindness. Infants at risk of gonococcal conjunctivitis are those who did not receive prophylaxis for ophthalmia neonatorum, those whose mothers had no prenatal care, and those whose mothers have a history of STDs mainly asymptomatic gonorrhea should be diagnosed early for proper management. (43)

Rates of gonorrhoeae and drug resistance vary greatly among countries and in regions even in sub region of the developing world. So having prevalence's data as well as the drug susceptibility pattern within consecutive year is important especially for gonorrhea the highly drug resistant bacteria. Now a day there is no available data about the prevalence and resistance pattern of gonorrhea even if it is often incomplete or inaccurate due to; Clinical presentation not specific enough for diagnosis based solely on symptoms, Inadequate facilities, materials or personnel for laboratory-based diagnosis and Lack of proper reporting mechanisms. (42)

Because anti-gonorrhea drug resistance has worsened steadily, the available drugs have become increasingly ineffective. Therefore, there is a continuous need to ensure safeness, effectiveness and affordability of anti-gonorrhea drug. Recent report of WHO is warning that gonorrhea may soon become untreatable because of widespread improper use of drugs. The antibiotics has reduced the ability to clear up the sexually transmitted infection shows the high resistance rate of gonorrhea to different drug event though, the use of these drug persists for different reasons despite the policy change. (42, 43)

Almost there was no report on prevalence gonococcal infection among women of reproductive age group in gynecology outpatient from any part of Ethiopia, except report 20 years before which was done using indirect haemagglutination test. (17)

So this study will have its own input by assessing the prevalence of gonorrhea and current resistance patter in the region which will give baseline information. It is also known that Laboratory testing is not only for diagnosing and treating patients but also for screening in the asymptomatic population, Such activity is important for the control of disease transmission and to determine prevalence and incidence for epidemiological purposes.

1.11 Hypothesis

The prevalence of gonorrhea among women in gynecologic OPD at Hawassa Referral Hospital Hawassa, Ethiopia could be similar with prevalence in other parts of the country and with other developing countries.

1.12 Objectives

1.12.1 General Objective

To assess the prevalence of Neisseria gonorrhea and their antimicrobial susceptibility patterns among symptomatic women attending a gynecology outpatient department in Hawassa referral hospital.

1.12.2 Specific Objective

- To determine the prevalence of N.gonorrhea at gynecology OPD
- To determine the drug resistance pattern of N.gonorrhea.
- To assess the risk factors contributing to the disease.

CHAPTER II MATERIALS AND METHODS

2.1. Study design and area

This study was a prospective cross sectional study and it was conducted from Dec 2010 through Feb 2011 at the Gynecologic OPD of Hawassa Referral Hospital, Hawassa, Ethiopia.

Hawassa town is the capital city of SNNP and it is located 275 km from capital city of Ethiopia, Addis Ababa. The altitude of the town is 1697 km above sea level with mean annual temperature and rainfall of 20.9 ^{o} C and 997.6 mm respectively. The total population of Hawassa town is 130,579 with one to one male to female ratio. Hawassa Hospital was inaugurated in November, 2005; it has 850 beds and serves about 12 million people.

2.2 Source Population

During the study, all Women of reproductive age group who attended gynecology out patient at Hawassa referral hospital with suspected gonococcal infection was included.

2.3. Study population

Women of reproductive age group (15-44 years old) and with any one of the following symptoms was diagnosed for gonococcal infection:-

- pain associated with vaginal intercourse
- a painful or burning sensation when urinating
- an abnormal vaginal discharge that is a creamy yellowish, greenish or bloody in nature
- bleeding between menstrual periods
- More advanced symptoms, which indicate development of PID, include cramps and pain, bleeding between menstrual periods, vomiting, and fever

To select the target group's convenient sampling technique was employed. Thus, a careful clinical examination was conducted by physician to all patients who were attending to gynecological OPD

Exclusion criteria: women on antibiotic treatment, those who are <15and >44 year, with sever complicated disease.

Written informed consent was obtained from study participants. Demographic and other relevant data was obtained by attending physician or nurses and was transferred to the questionnaire prepared for this study (see appendix I).

2.4. Study variables

Dependent variables: - gonococcal infection and susceptibility pattern for antibiotics.

Independent variables: - age, pregnancy, marital states, ethnicity, occupation, living area, antibiotic usage, etc.

2.5. Sample size determination

Sample size was calculated based on the highest prevalence of gonorrhea estimate indicated in the epidemiology part which is 5-15% for all Africa and we take the highest 15% [13]

Expected margin of error (d) was 0.05 and confidence interval (z) will be 95%. Contingency for the unknown circumstance was 10% was taken. [47]

$$\text{Total study subjects: } n = \frac{z^2\, p\,(1-p)}{d^2}$$

$$n = \frac{(1.96)^2 * .15(1-0.15)}{(0.05)^2} = \frac{196}{(0.05)^2} + 10\% = 215$$

.

Where P = prevalence of gonococcal infection (15%)

D = degree of accuracy desired (0.05)

Z^2_1- α/2 = the standard normal deviation (1.96)

2.6 Data collection and processing

Data on socio-demographic variables and associated risk factors was collected by predesigned and pre-tested questionnaire.(Appendix I)

2.6.1 Sample collection

Two swabs were collected from patient endocervical canal by physicians or nurse's one for gram stain and the other for culture which is immediately delivered to the Microbiology Laboratory of Hawassa Referral Hospital. (Appendix II)

2.6.2 Identification of organisms

2.6.2.1 Culture and gram staining

Two swabs were taken from individual patient; one was for gram stain and other one swab inoculated on to nonselective chocolate agar and selective agar containing antimicrobial agents that inhibit the growth of commensal bacteria and fungi. (Appendix III) The antibacterial agents used in modified Thayer-Martin medium were vancomycin, colistin, trimethoprim lactate and the antifungal agent's nystatin. Some fastidious strains, such as the arginine-, hypoxanthine- and uracil-requiring strains, are more susceptible to the concentrations of vancomycin or trimethoprim used in the selective media which can grow in nonselective chocolate agar. The inoculated plates incubated at 37°C in a moist atmosphere enriched with CO_2 (5%). [4, 44]

2.6.2.2. Biochemical tests

The presumptive identification of NG rests on the isolation of an Oxidase-positive, Gram-negative diplococcus recovered from cervical sites that grows on selective media. The Oxidase test uses the tetra methyl derivative of the oxidase reagent. [32] N.gonorrhea can be differentiated from other Neisseria species, Moraxella species, Kingella species and other commensals based on its ability to grow on appropriate selective and nonselective media, produce acid from glucose only and not from maltose, lactose, sucrose and fructose. The carbohydrate utilization test was done using API NH identification kit strips (Biomerieux, France). In general all positive cultures was identified by their characteristic appearance on the media, Gram staining reaction and confirmed by the pattern of biochemical reactions using the standard method. [45]

2.6.3 Antimicrobial susceptibility

Antimicrobial susceptibility was assessed by using Kirby-Bauer disk diffusion test, according to NCCLS.[44] Gonococcal specimens were subculture from the selective primary medium to a non inhibitory medium, (chocolate agar) to obtain a pure culture of the specimen. From a pure culture 3-5 selected colonies of bacteria was taken and transferred to a tube with a straight wire and Prepare a suspension in 2.5 ml normal saline then sterile swab was used to distribute the bacteria evenly over the entire surface of chocolate agar. The susceptibility to the following antimicrobial agents (Oxoid) assessed: penicillin G (P 10 IU), tetracycline (TE 30µg), ciprofloxacin (CIP 5µg), ceftriaxone (CRO 30µg), cefixime(CFM 5µg), cefoxitin (FOX 30µg), cefpodoxim (CPD 10µg), spectinomycin (SPT 100µg). (Appendix IV)

Procedural steps for identification of NG from endocervical swabs

Small translucent convex grayish white to tan colonies, may be slightly mucoid growing on

Modified Thayer Martin Agar

Oxidase test

Negative — STOP

Positive (within 10 sec.)

Gram Stain

gram-positive bacteria (dark purple) — STOP

gram-negative bacteria (Pink to rose red)

diplococcic — biochemical identification

Bacilli/rods — STOP

fermentation of glucose only

negative

positive

(N. gonorrhoeae)

Report: Negative for Neisseria gonorrhoeae

Report: Neisseria gonorrhoeae

.

Summary of Microbiological Examination of cervical Specimen *for* N. gonorrhoeae

Day 1

- Culture Specimen on chocolate and Thayer-Martin medium incubated at 37°C in a moist atmosphere enriched with CO_2 (5%) for 24 hr
- Examine Microscopically Gram smear from swab and look for Gram negative intracellular or extracellular diplococcic

Day 2

- Examine Microscopically Gram smear from colony if there is growth and look for Gram negative extracellular diplococcic
- Examine and Report Cultures

  Examine Colonies resembling for N. gonorrhoeae

  look for growth on selective media (NG produces small raised, grey shiny colonies)
- sub culture on chocolate agar for biochemical tests

Day 3

- Perform biochemical tests

  Oxidase test

  Carbohydrate utilization using API NH kit strips.
- Perform Antimicrobial susceptibility testing.

Day 4 and Onwards

- Record the Antimicrobial susceptibility findings.
- Interpret the overall test result

The accuracy of a diagnosis of gonorrhoeae based on the isolation of oxidase-positive Gram negative cocci from a selective medium is as high as 99% from cervical sites. [45]

2.9. Reference strain

Standard reference strain of Neisseria gonorrhoeae ATCC 49226 was used as recommended by the Clinical and Laboratory Standards Institute (CLSI) for QC of susceptibility testing of gonococcal isolates. Reference strain from the EHNRI laboratory stock was used as a quality control throughout the study for culture and antimicrobial susceptibility testing. [46]

2.10. Statistical analysis

Data entry and analysis was done using computer with SPSS version 16 software. Prevalence rate was calculated for the sum of the numbers of positive cases of examined subjects and separately by age groups. Logistic regration and Fisher exact tests analysis were used to estimate adjusted odds ratios. The level of significance was set at 0.05 in order to consider a p-value <0.05 as indicator of a statistically significant difference with 95% confidence.

2.11. Ethical consideration

This M.Sc. research project proposal was approved by the Department Research and Ethical Review Committee (DMIP) and by Institutional Review Board (IRB), Faculty of Medicine; Addis Ababa University. Official permission from the study site was obtained. Written informed consent was obtained from study participants (see Appendix V).

CHAPTER III RESULTS

3.1 Description of the Study Population

One thousand three hundred forty two patients who visited the gynecological OPD of Hawassa University Hospital between December 2010 and February 2011were studied for prevalence of NG among female of reproductive age group. On their visit, they were carefully examined clinically to include the study Populations and to determine any underlying risk factors.

A total of 215(16.02%) patients were selected. The inclusion criteria for the selection of 215 women of reproductive age group was based on any sign and symptoms for STD up on clinical examination of symptomatic patients at gynecologic OPD. Of the 215 patients, there were 90 (41.9) from urban, 73 (34%) from sub urban and 52 (24.21%) from rural. The age ranged from 15 to 44 years with a mean of 25.51 years. (Table 1) Concerning the cervical swabs; 101 (47%) were mucopurulent discharge, 87(40%) were bloody and the rest 27 were whitish discharge.

Table 1 Description of the demographic data of 215 patients investigated for gonococcal infection at Hawassa University Referral Hospitals, Hawassa, Ethiopia (Dec2010- Feb 2011)

Socio-demographic characteristics	**Category**	**frequency**	**percentage**
Age- group (n=215)	15-19	50	23.3
	20-24	71	33
	25-29	34	15.8
	30-34	25	11.6
	35-39	23	10.7
	40-44	12	5.6
Address (n=215)	urban	90	41.9
	Sub-urban	73	34
	rural	52	24.1
Marital status (n=215)	Single	81	37.7
	Married	118	54.9
	Divorced	9	4.2
	Widowed	7	3.3
	Student	72	33.5

Occupation (n=215)	Gev.employees	48	22.3
	Farmers	42	19.5
	House wife	18	8.4
	Daily workers	13	6
	Merchants	11	5.1
	others	11	5.1
Religion (n=215)	Orthodox	57	26.5
	Muslim	38	17.7
	Protestant	95	44.2
	Catholic	10	4.7
	others	15	7
Ethnic group (n=215)	Sedama	72	33.5
	Amhara	42	19.5
	Oromo	38	17.7
	Welighta	33	15.3
	Gurage	11	5.1
	Tiger	10	4.2
	Others	9	4.2

3.2. Overall Prevalence of gonococcal infection

Among 215 patients, 11 (5.1%) were confirmed to have gonococcal infection. Of the 11 patients who were positive for gonococcal infection, 6(72.7%) were from rural, 3(27.2%) from sub-urban and 2 (18.1) from urban. There is no significant statistical association with living area ($p>0.05$) but, the odds of gonorrhea infection for women living in rural were 6 times the odds for women living in urban (95% CI, 1.1 - 29.5). The distribution of gonococcal infection and demographic factors are presented in table 2 and 3. Regarding marital status gonococcal infection was observed in 6/11 (54.5%) of married women and occupationally 5/11 (45.5%) were students. In this study 25 pregnant women were included and among them 1/25 (4%) was positive for NG. But this relationship was not statistically significant ($P = .75$). Out of the 11 patients who were confirmed to have gonococcal infection, the highest prevalence was observed in age group 20-24

which is 5/11 (45.5%). There is no statistically significant difference in the frequency of gonococcal infections among different age groups, occupation and living area (p>0.05). The macroscopic examination of swab shows that 8/11 (72.7) positives were from mucopurulent swab and 3/11 were from bloody swab but, there is no statically significant (P>0.05). However, the odds of developing gonococcal infection was three times higher if the discharge is muco purulent (OR = 2.877, 95% CI 1.24, 6.7)

Table 2 Prevalence of Neisseria gonorrhoeae by age group and addresses of patients investigated for gonococcal infections at Hawassa University Referral Hospitals, Hawassa, Ethiopia (Dec2010-Feb 2011)

Address		**Age group**						Total
		15-19	20-24	25-29	30-34	35-39	40-44	
Urban	no NG	22	26	18	11	7	4	88
	NG	0	1	0	0	1	0	2
	Total	22	27	18	11	8	4	90
Sub urban	no NG	15	25	10	9	8	3	70
	NG	1	1	1	0	0	0	3
	Total	16	26	11	9	8	3	73
Rural	no NG	10	15	4	5	7	5	46
	NG	2	3	1	0	0	0	6
	Total	12	18	5	5	7	5	52

Key **NG =** Neisseria gonorrhoeae no = no Neisseria gonorrhoeae

Table 3 Association between demographic characteristics and gonococcal infection of patients investigated for gonococcal infections at Hawassa University Referral Hospitals, Hawassa, Ethiopia (Dec2010-Feb 2011)

Socio-demographic characteristics	Category	NG	No NG	OR(95%CI)	Fisher's Exact Test
Age- group (n=215)	15-19	3	47	1	.894
	20-24	5	66	1.187 (.27, 5.21)	
	25-29	2	32	.979 (.155 , 6.195)	
	30-34	0	25	.000 (.000)	
	35-39	1	22	.712 (.070, 7.240)	
	40-44	0	12	.000 (.000)	
Address (n=215)	Urban	2	88	1	.064
	Sub-urban	3	70	1.886 (.307, 11.598)	
	rural	6	46	5.739 (1.114, 29.573)	
Marital status (n=215)	Single	5	76	1	.565
	Married	5	113	.673 (.188, 2.403)	
	Divorced	1	8	1.900 (.197, 18.341)	
	Widowed	0	7	.000 (.000)	
Occupation (n=215)	Student	5	67	1	.644
	Gev.employees	1	47	.285(.032, 2.520)	
	Farmers	2	40	.670(.124, 3.616)	
	House wife	2	16	1.67(.298, 9430)	
	Daily workers	1	12	1.11(.120,10.418)	
	Merchants	0	11	-	
	Others	0	11	-	
Religion (n=215)	Protestant	4	92	1	.188
	Orthodox	3	53	2.314 (.499, 10.738)	
	Muslim	2	36	1.704 (.273, 10.623	
	Catholic	2	8	7.667(1.113, 52.796)	
	Others	0	15	0	
Ethnic group (n=215)	Sedama	4	68	1	.995
	Amhara	3	39	1.308 (.278, 6.148)	
	Oromo	2	36	.944 (.165, 5.407)	
	Welighta	2	31	1.097 (.191, 6.310)	
	Gurage	0	11	0	
	Tiger	0	10	0	
	Others	0	9	0	
Macroscopic examination of swab	muco purulent	8	101	1	.042
	bloody swab	3	87	2.409 (.619, 9.378)	
	whitish	0	27	0	

3.4. Antibiotics usage and Outcome

From a total of 215 patients who gives endocervical swabs, 40 (18.5%) have taken antibiotics before sample collection in the form of therapy. It is known that women on antibiotic treatment are excluded but, these groups were women who took drugs before they came to hospital. Of the patients who received antimicrobials, only one had positive culture results and isolated as NG. While those who did not receive any antimicrobial had 10 positive culture and isolated as NG. ($p>0.05$)

3.6. Microscopic Examination

Gram staining was done twice, the first Gram stain was done directly from patient swab which was for all patients the second gram stain was done for culture positive samples from modified Thayer martin agar (MTM) or from colonies resembling NG in chocolate agar. Of the 215 endocervical specimens examined by Gram stain, 21 (9.8%) were positive for the presence of bacteria which was gram negative diplococcic (GNDC) either intra cellular or extra cellular from direct sample of patient. Among 21 gram stain positive for NG from patient swab, 19 were showing growth in the media and only two of them have no growth in further culturing.

3.7. Culture and Biochemical tests

Of the 215 endocervical specimens cultured in MTM, 16(7.4%) were culture positive. Of these, 13/16 (81.5%) showed mono-microbial growth (single bacterial type) and 3/16 (18.5%) showed Poly microbial (more than one bacterial type) growth. The biochemical tests were done for all culture positive in MTM and for colony resembling NG in chocolate agar even in the absence of growth in MTM. In general, from a total of 16 culture positive in MTM only 11 were isolated as N. gonorrhea by further biochemical tests (Oxidase test and Oxidation or utilization of carbohydrates).(table 4)

Table 4 Culture and Biochemical tests result of patients investigated for gonococcal infections at Hawassa University Referral Hospitals, Hawassa, Ethiopia (Dec2010-Feb 2011)

	Gram stain	**Growth in MTM**	**Oxidase test**	**Oxidation of glucose only in API NH kit**	**Isolated NG**
Positive	21	16	23	11	11
Negative	194	199	192	204	204
Total	215	215	215	215	215

3.5. Antimicrobial susceptibility testing

The susceptibility patterns of isolated bacteria (n=11) was done against 11 antimicrobial agents by the agar disc diffusion technique. The lowest susceptibility was reported for penicillin and Tetracycline. No resistance to Ceftriaxone and cefixime was found. Low level of susceptibility to quinolones (ciprofloxacin 55%, ofloxacin 64% & lomefloxacin 64%), recommended in the national protocol as first-line antibiotics for gonorrhea treatment was reported. There was decreased susceptibility to spectinomycin as well. The sensitivity of gonococcal isolates ranges from 100% to Ceftriaxone and cefixime to 0 % to Penicillin and Tetracycline. Most of the isolate have shown multiple drug resistance which was reported in 81.8% of isolates and no one isolates were sensitive to all antibiotics. In this finding high level of resistance (82%) to Penicillin and (55%) to Tetracycline was observed. (Table 5)

Table 5 Antimicrobial Susceptibility Patterns of N.gonorrhea Isolated from endocervical swab from patients who were visited gynecologic OPD at Hawassa University Hospitals, Hawassa, Ethiopia (Dec2010-Feb 2011)

Organism		**CRO**	**FOX**	**CIP**	**SPT**	**CFM**	**CPD**	**CTX**	**OFX**	**LOM**	**P**	**TE**
NG n=11	S	11	9	6	9	11	10	10	7	7	0	0
	I	-	2	3	2	-	-	-	2	3	2	5
	R	-	0	2	0	-	-	-	2	1	9	6
NG (n=11) *	S	100	82	55	82	100	91	91	64	64	0	0
	I	-	18	27	18	-	-	-	18	27	18	45
	R	-	0	18	0	-	-	-	18	9	82	55

S= Sensitive I=Intermediate R=Resistant

* Expressed in percent

P: Penicillin; TE: Tetracycline; CIP: Ciprofloxacin CRO: Ceftriaxone; CTX: cefotaxime; FOX: Cefoxitin; CFM: cefixime; CPD: cefpodoxime; OFX: ofloxacin; LOM: lomefloxacin; SPT: spectinomycin

CHAPTER IV

4. DISCUSSION

Gonococcal infection is a major global cause of illness, infertility, long term disability and death with severe medical and psychological consequences for millions of women and children. In women of childbearing age, STDs (excluding HIV) are second to maternal factors as causes of disease, death and healthy life lost. [38] Rates of gonorrhoea vary greatly among countries in both the developed and developing world. The distribution of gonorrhoea is affected by socio-geographical factors, which produce multiple micro epidemics, often with differing antibiotic resistance profiles. [22]

In the absence of a national gonococcus screening programme, little is known about the prevalence of gonococcal infection in women of reproductive age group in the community. To our knowledge the last gonococcal study in reproductive age group of women in Ethiopia was done before 20 years ago. [17] So Comparison with results from previous studies is therefore difficult. However there appear to be few studies on this problem in Ethiopia, [17] Nigeria, [16] Vietnam, [12] and Mozambique. [15]

In this study, the overall prevalence of Gonococcal infection among reproductive age group of women in Hawassa University Hospital is 5.1%, which is almost similar with studies in Laos 3.7% [9] and it is within the range of sub-Saharan Africa estimate report which is 2-15% [13]

Higher prevalence was reported in this study compared to other countries like Jordan 2.2% [10] Vietnam 0.7% [77] and USA and most western countries 0.5- 1.1%. [8] The higher rate in the prevalence of gonococcal infections might be indication of unawareness about the disease or it might be high rate of misdiagnosis. Another factor that can account might be due to the problem of syndromic management of the cases without differential diagnosis which can lead to increase number of untreated patient and consequently increase rate of transmition also lead to drug resistance.

On the other hand, the prevalence of gonococcal in our patients was low (5.1%) compared with that about 14% observed in southern Mozambique,[15] 9.7% in India [11] and 11% in Nigeria.[16] Epidemiological differences in the prevalence of gonococcal infections might offer an explanation for this discrepancy. Besides these, today's treatment (before or without diagnosis) is almost universal, making the rigorous exclusion of gonorrhoea, up to one-third of female gonorrhoea contacts eventually found to be negative. [49] In addition the time duration of the current and those studies have a difference of at least six year which might cause the prevalence difference. Also it is known that rates of gonorrheae and drug resistance vary greatly among countries and in regions even in sub region of the developing world, this is because the route of transmition depend on socio-demographic facters and the way the case diagnosed and treated in every region varies.

In our study patients came from rural areas appeared to have 6 fold increase risk of developing infections OR = 5.739 95% CI = 1.114, 29.573; P = 0.064, and there was no as such significant difference between rural, sub urban and urban in our study (P>.05). This is because impact of gonococcal infection on ill health tends to be more severe among rural women in resource-poor settings for that, Patients without a healthcare source are less likely to be tested, diagnosed, and treated effectively for STD. Although socio-demographic factors have great influence in the prevalence of STDs in which most studies confirmed, in our finding the distribution of gonococcal infection to most socio-demographic factors have no statistical significant association. (p>0.05) Regarding age group, there is no statistically significant difference in the frequency of gonococcal infections among different age groups but, the highest prevalence was observed in age group 20-24 which is 5/11 (45.5%) and we can say Younger patients, and certain ethnic groups were more likely to be positive. These findings are similar to those of Nigeria and USA [16, 23] women in the 15–19-year age groups and aged 20–24 were at the greatest risk of infection. Our study found that the prevalence of infection was significantly higher in rural young single women's. The possible explanation for this might be young people are at an even greater disadvantage due to the absence of proper case detection and treatment, for at least two reasons. First, unmarried young people have special problems related to access to and utilization of health services. Second, they do not have the information necessary for early recognition of disease symptoms. [6]

The physical examination of swab reveals that 8/11 (72.7%) positive patients swab was muco-purulent discharge and it is not statically significant (P>0.05). However, the odds of developing gonococcal infection was three times higher if the discharge is muco purulent (OR = 2.877, 95% CI 1.24, 6.7) and this is in agreement with Literature, Only 35% of women with gonococcal Cervicitis actually have a Mucopurulent discharge. [21]

Currently NG has become resistant to different antimicrobial agents and in some cases to nearly all agents. The antibiotic sensitivity pattern of our study confirmed that resistance exhibited by NG to the common antibiotics in use. In this particular study, the lowest susceptibility was reported for penicillin and Tetracycline. The high level of resistance to penicillin and tetracycline found in our study is not surprising as this has been widely reported throughout the world. There is no resistance to Ceftriaxone and cefixime was found. The possible explanation for this might be these drugs are expensive, not intensively used and not easily available outside the hospitals beyond this these drugs are newer compared to the others. Also it is known that third-generation cephalosporines are the only left for doctors. The lack of resistance to third-generation cephalosporines (cefixime and ceftriaxone) in our study make these drugs excellent choices as first-line treatment.

According to syndromic case management principle set by MOH, 2005 [28] the drugs (ciprofloxacin, Tetracycline and spectinomycin) prescribed for patients suspected for gonococcal infections have shown resistance. Low level of susceptibility to quinolones (ciprofloxacin, ofloxacin & lomefloxacin), recommended in the national protocol as first-line antibiotics for gonorrhea treatment was almost similar with other studies like, USA, [24] Australian [25]. This may be because of the intensive use of antimicrobial agent, easy availability and indiscriminate use of these drugs outside the hospitals, and many antibiotics are available over the counter for Self-medication. And this is also in agreement with study done in Ethiopia where most of the isolates were resistant to commonly used antibiotics. [29, 30] Although susceptibility to drugs vary across the world this finding is in agreement with most studies.

MDR was reported in 81.8% of isolates and no isolates were sensitive to all antibiotics. In this study, multiple drug resistance (MDR) was defined as resistance to three or more drugs. The cephalosporin drugs; Cefixime, Ceftriaxone and cefpodoxime were effective antibiotics for the treatment of NG which are responsible to cause endocervical infections. This is because these agents are expensive and not commonly used. Due to this fact, they showed no resistance. It appears that the clinician is left with very few choices of drugs for the treatment of gonococcal infections. We can say rate of resistance for most antibiotics tested for NG was high. This is in agreement with study done in USA, [24, 32] Australian [25] and Romanian. [26] However, the present study showed a high level of resistance to ciprofloxacin compared to the study in Central African Republic, Cameroon, and Madagascar. [27] It will be real that Scientists are worried gonorrhea will soon become untreatable with these antibiotics. This is the main reason that selecting the correct antibiotic will prove to be a hurdle. This leads to conclusion that if the problem won't be attended to on time, it will become very difficult to treat the infection. [23]

In general the presence of resistance to first line antimicrobials and possible treatment failure, the likelihood of onward transmission of the organism within the community and the development of clinical complications in the infected person are substantially increased. [48]

5. CONCLUSION AND RECOMMENDATIONS

An overall prevalence of 5.1% of gonococcal infection was observed among the study population. We can say that the prevalence of gonococcal infection at Hawassa University Hospitals was moderately higher combined with high resistance to common antibiotics. This emphasizes that syndromic case management which was adopted since 2001 provoke drug resistance, and it might have high rate of misdiagnosis which can increase overall prevalence in near future. So periodic evaluations of etiological diagnosis should be available to ensure adequacy of treatment algorithms and prescribed medications. Emerging resistance to quinolones (ciprofloxacin and ofloxacin) in Ethiopia is evident, indicating that the WHO target of greater than 95% efficacy for drugs of the first-line therapy is no longer achievable for this class. Given the known tendency for rapid resistance development in this Quinolone class and

frequent over-the-counter sale of antibiotics in Ethiopia, further rapid increase of resistance is a likely scenario. This is clearly not the first report of decreased Quinolone susceptibility in Ethiopia. Cefixime, Ceftriaxone and cefpodoxime were effective antibiotics for the treatment of gonococcal infection.

Despite low rates of gonorrhea infection, it is important to focus on high-risk populations (reproductive age group) because of the great physical and emotional costs of the disease. Identifying gonorrhea infections early in high-risk populations is the key to preventing costly sequelae and ensuring female reproductive health. Future studies should focus on identifying behavioral or environmental factors to address differences in predictors within groups. This study will used as base line data's for gonorrhea till well compiled STDs prevalence done in the region.

Based on these findings the following recommendations are made: -

- Ethiopia has been promoting syndromic approach since 2001 by adopting the WHO guidelines for management of STIs, but gonococcal infections will provoke drug resistance, which can increase overall prevalence in near future, Taking this into account treatment should be based on the result of culture and sensitivity. In order to achieve this, the capacity of microbiology laboratory should be strengthened with trained human power, budget and necessary laboratory equipments.
- This study suggests that if one could not wait the culture results in gonococcal infection, Gram stain alone is not effective to diagnose gonococcal infections. In addition to this the commonly used drugs (ciprofloxacin, penicillin, tetracycline Ofloxacin) are ineffective to treat these infections
- Future studies to assess the resistance trends in Ethiopia and to allow timely revision of treatment protocolsare highly recommended, and donors should be encouraged to fund such studies.

6. LIMITATIONS OF THE STUDY

- The study does not include gynecology wards in Hawassa University Hospitals in which gonococcal infections may be suspected. It was not possible to include due to facilities constraints.

- The study does not include health center and privet clinic due lack of experienced personnel to collect endocervical swab and lack of collecting materials like speculum and sterilization techniques.

- The study does not include other STDs which have significant association with behavioral change and their impact on HIV

- The study does not include neonate, in which gonococcal infection can result GCON, a potentially blinding condition without ocular prophylaxis, 30 to 50 percent of exposed babies.

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8. APPENDIX

8.1. APPENDIX I

Questionnaire for investigation of The Prevalence of Neisseria gonorrhea and there antimicrobial susceptibility patterns among Women Attending a Gynecology Outpatient Department in Hawassa Referral Hospital by employing a culture, biochemical test and drug susceptibility test in vivo test method. Hawassa, SNNP, Ethiopia

A data collection form for Prevalence of Nosocomial infections

a. Participant Identification

1. Serial No……... 2. Participant name …….
3. Address …… 4. Age……..
5. Ethnicity……..... 6. Marital status……
7. Occupation…… 8. Education level …….
9. History of pregnancy yes ---------- No ----------

   If yes first trimester ..... second trimester ...…… third trimester .....
10. History of recent any antibiotic treatment Yes/No
11. Date and time of cervical specimen collection …….

b. Laboratory data

1. Macroscopic examination of swab_____
2. Growth of N.gonorrhae in Thayer martin selective media……
3. Result of Gram stain……
4. Oxidase test result: positive_____Negative_____
5. Patterns of acid production from the carbohydrates:

   From Glucose only_____

   From glucose and other carbohydrates _____

   No acid produced_____

6. Antimicrobial susceptibility testing	S (mm)	I (mm)	R (mm)
Penicillin (P 10IU)	…….....	……	….……...
Tetracycline (TE 30µg)	….……	..…….	..……..
Ceftriaxone (CRO 30µg),	……	……...	……..
Cefoxitin (FOX 30µg)	…….	……...	……..
Ciprofloxacin (CIP 5µg)	...……	……	…….
Cefixime (5µg)	……	……...	……..
Spectinomycin (SPT µg100)	……	……...	……..
cefpodoxime(CPD 10µg)	……	……...	……..
Ofloxacin (µg10)	……	……...	…….
lomefloxacin (5µg)	……	……...	……..
cefotaxime(5µg)	……	……...	……..

c. Comments _____

Name of principal investigator _____

Signature _____ Date _____

8.2 APPENDIX II

Procedure for specimen collection from endocervical canal

Use a sterile vaginal speculum to examine the cervix and collect the specimen.

1 Moisten the speculum with sterile warm water, and insert it into the vagina.
Note: Do not lubricate the speculum with a gel that may be bactericidal.

2 Cleanse the cervix using a swab moistened with sterile physiological saline.

3 Pass a sterile cotton-wool swab 20–30 mm into the endocervical canal and gently rotate the swab against the endocervical wall to obtain a specimen.

4 before inserting the swab in Amies transport medium, if possible inoculate a plate of culture medium.

5 Label the specimens and deliver to the laboratory as soon as possible. Inoculated culture plates must be incubated within 30 minutes.

8.3. APPENDIX III

Laboratory procedure for Gram staining technique:

1. Labeling the slides clearly with the date and patient's name and number.
2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.
4. Fix the dried smear by using heat or chemicals (methanol).
5. Cover the fixed smear with crystal violet stain for 60 seconds.
6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.
7. Tip off all the water, and cover the smear with lugol's iodine for 60 seconds.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (30 seconds) with acetone alcohol. Wash immediately with clean water.
10. Cover the smear with neutral red or safranine stain for 1 minutes.
11. Wash off the stain with clean water.
12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.
13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Microscopic Examination

1. Place a drop of immersion oil on the stained smear.
2. Examine the smear at low power (10X) to check for proper staining and to locate areas of the smear containing many cells.
3. Then use the oil immersion objective (100X) to search in these areas for bacterial morphology types

and to count PMNs. This means that each field is at 1000X magnification for identification and counting purposes.

Cells and mucus should stain pink. Yeast stains purple. Bacteria are characterized as Gram-positive
(purple) or Gram-negative (pink), and as cocci (round), bacilli (rod-shaped), or coccobacilli (small in size with morphology in between rods and cocci).

Interpretation - Gonococcal Infection

• **Positive**: ≥1 PMN with intracellular Gram-negative diplococci of typical morphology. Extracellular
Gram-negative diplococci may also be present, and numerous PMNs are usually present.
Distinguish carefully between Gram-negative diplococci and Gram-negative rods.

• **Negative**: No intracellular Gram-negative diplococci. Extracellular Gram-negative diplococci or Gram-negative diplococci of atypical morphology may be present, but do not meet the criteria
for a presumptive diagnosis of GC (wait for culture results for final diagnosis). Mononuclear cells and PMNs may or may not be present.

8.4 APPENDIX V

Procedure for Antibiotic sensitivity test (Disk diffusion)

1. Warm, to room temperature, the appropriate number of chocolate agar plates.

   Note 1: The number of plates required per strain tested will depend on the anticipated zone inhibition diameters of the antimicrobial agents to be tested; disks should be placed on plates so that the zones of inhibition do not overlap. It may be necessary to experiment to determine the best combination and placement of disks to minimize the number of plates required.

2. Suspend isolated colonies from an overnight culture in 2.5 ml normal saline. Mix the suspension thoroughly on a vortex mixer to break up clumps of growth.

3. Adjust the turbidity until the turbidity of the suspension is equivalent to the turbidity of a 0.5 McFarland $BaSO_4$ standard. Discard this cell suspension if it is not used within 15 to 20 min after preparation, and prepare a fresh suspension for testing.

4. Moisten a sterile applicator swab in the standardized cell suspension, and express excess moisture by rotating the swab against the glass above the liquid in the tube. Inoculate the entire surface of each plate, inoculating the surface completely in three different directions to ensure uniform, confluent growth.

   Note 1: It is recommended that cotton swabs with wooden handles be used for this procedure. Synthetic swabs do not soak up sufficient suspension to inoculate the entire surface of the plate.

   Note 2: It is recommended that swabs with plastic handles not be used to inoculate plates; the handles bend and may splatter liquid out of the tube when excess suspension is being expressed, creating a biohazard.

5. Repeat step 4, using a new sterile swab, to inoculate each additional plate as needed.
6. Allow the inoculated plates to sit at room temperature for 3 to 5 min to allow the moisture from the inoculum to absorb into the medium. Inspect the inoculated plates to ensure that there is no visible liquid on the surface of the medium; the surface of the medium must be dry before the disks are applied.
7. When the surface of the medium is dry, apply disks of the selected antimicrobial agents to the surface of the medium and tamp them gently with a sterile loop or forceps to ensure that they are in complete contact with the agar surface. All disks should be applied approximately the same distance from the edge of the plate and from each other.
8. Invert the inoculated plates (lid side down), and incubate the plates at 35 C to 36 C in 5% CO_2 for 20 to 24 h.
9. Examine the disk diffusion plates from the back, viewed against a black background and illuminated with reflected light. With a caliper, measure and record the diameter of each zone of inhibition to the nearest whole millimeter.

Interpretation of results

Report the reaction of the test organism to each antibiotic as ‘sensitivity’, ‘intermediate’, or ‘resistant’, as follows:

Sensitivity (S): Zone of radius is wider than, equal to, or not more than 3mm smaller than the control.

A pathogen reported as sensitivity suggests that the infection it has caused is likely to respond to treatment of the drug to which it is susceptible is used in normal recommended dose.

Intermediate (I): Zone radius is more than 3mm smaller than the control but not less than 3mm.
A pathogen reported as being intermediately sensitive suggests that the infection it has caused is likely to respond to treatment if the drug to which it is susceptible is used in larger doses than normal.
Resistant (R): No zone of inhibition or zone radius measure 2mm or less.
A pathogen reported as resistant implies that the infection it has caused will not respond to treatment with the drug to which it is resistant irrespective of dose or site (66)

8.4 APPENDIX VI

INFORMATION SHEET FOR STUDY SUBJECTS

You are kindly invited to participate in this study, which involves about 215 women from Hawassa Referral Hospital. The title of the study is “The Prevalence of Neisseria gonorrhea and there antimicrobial susceptibility patterns among Women Attending a Gynecology Outpatient Department in Hawassa Referral Hospital, Hawassa, Ethiopia. The aim of this study is to determine prevalence of Neisseria gonorrhea and there antimicrobial susceptibility patterns among women. Women with NG infection are at high risk for PID and infertility as well as they will infect new born during delivery. Infection with this organism can cause different disease in new born and therefore this study will identify gonococcal infection of women so that those who are infected with gonorrhea will receive treatment and prophylaxis before delivery to keep their baby safe.

a. Purpose: the purpose of this research study is to assess the prevalence of Neisseria gonorrhea and there antimicrobial susceptibility patterns among women attending Guyni-OPD in Hawassa Referral Hospital, Hawassa, Ethiopia.

b. Duration: the duration of this study depend upon the availability of study subjects it can probably take about three months or more.

c. Procedures to be carried on: the procedure of sample collection is easy and straight forward; sample will be collected from cervical area using cotton swab by attending physician or midwife and then it will be analyzed in the microbiology laboratory of Hawassa Referral Hospital for the presence of NG.

d. Risk and discomfort: almost there will no be any risk associated during sample collection without little discomfort.

e. Expected benefits: from this study you are directly benefited and if you are pregnant the new born will be benefited, as screening and administering treatment and chemoprophylaxis to women

f. Confidentiality: All your personal information collected for the purpose of the present study will be kept confidential.

g. Compensation: No compensation will be provided by participating in this study.

h. Termination of the study: 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

- Keep hold information
- Decline to cooperate in the study
- To refuse provision of specimens

I would also like to inform you that this study will be approved by Department Ethical and Review Committee and ethically cleared by Institutional Review Board (IRB), Faculty of Medicine Addis Ababa University. If you have any question about the right of the study participant the address is:

Faculty of Medicine Addis Ababa University

Office of Associate Dean, Postgraduate Programs and Research

P.O. Box 9086. Addis Ababa, Ethiopia

Tel. 251-011-551-28-765

If you have question about the study the address of the principal investigator is:

Mengistu Hailemariam

Department of Microbiology, Immunology and Parasitology

Faculty of Medicine, Addis Ababa University

P.O. Box. 9086, Addis Ababa, Ethiopia Tel: 0911814453

8.7 APPENDIX VII

CONSENT FORM

Serial no…….

Card no…..……

Name of study participant: _____

I have been requested to participate in this study, which involves collecting of cervical specimen. During collection of the specimen I have told that there is no harm without little discomfort I have also read the information sheet (or it has been read to me); I have understood that this study is about prevalence NG and drug suseptability pattern among women attending Guyni-OPD Hawassa Referral Hospital, Hawassa, Ethiopia in which I will be protected from NG infection. 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 herby to approve my agreement with my signature.

Participants signature _____Date_____

Investigators signature _____ Date_____

Witness signature 1. _____Date_____

2. _____ Date_____

DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Mengistu Hailemariam, B.Sc

Signature _____

Date and place of submission _____

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   Date and place of submission _____