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DEPARTMENT OF MEDICAL LABORATORY SCIENCES

BACTERIAL VAGINOSIS AND VULOVVAGINAL CANDIDIASIS AMONG WOMEN COMPLAINING GENITAL TRACT INFECTIONS AT ST. PAUL HOSPITAL MILLENNIUM MEDICAL COLLEGE AND FAMILY GUIDANCE ASSOCIATION OF ETHIOPIAN MODEL CLINIC, ADDIS ABABA, ETHIOPIA, 2016.

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List of Abbreviations

AAU	Addis Ababa University
ATCC	American Type Culture Collection
BV	Bacteria Vaginosis
CAMP	Chrstine Atkines Munch Peterson
CLSI	Clinical and Laboratory Standards Institute
DERC	Departmental Research and Ethical Review Committee
EPHI	Ethiopian Public Health Institution
ETB	Ethiopian Birr
FGT	Female Genital Tract
FGAEMC	Family Guidance Association Ethiopia Model Clinic
HIV	Human Immune Deficiency virus
HSV-2	Herpes Simplex Virus type 2
GBS	*Group B Streptococcus*
MIC	Minimum Inhibitory Concentrations
MOH	Ministry of Health
OPD	Outpatient Department
OTC	Over-The-Counter
PID	Pelvic Inflammatory Disease
IRB	Institutional Review Board
RTI	Reproductive tract infections
SPSS	Statistical Package for Social Science
STD	Sexual Transmitted Disease
SPHMMC	St. Paul's Hospital Millennium Medical College
USA	United States of America
VVC	Vulvovaginal Candidiasis

Operational definitions

Bacterial vaginosis: Which is characterized by a reduction in the numbers of lactobacilli and an increase in the concentration of Gardnerella vaginalis and resident anaerobic bacteria,which is diagnosis by Nugent score method.

Vaginitis: It is a condition that involves inflammation of the vulva and vaginal wall due to bacteria vaginosis and vuluva candidiasis

Pathogenic bacteria: Bacteria isolate from vaginal swab

Abstract

Background: Bacterial vaginosis and vulvovaginal candidiasis are major health problems associated with gynecologic complications

Objective: To determine the prevalence bacterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection at SPHMMC and FGAEMC Addis Ababa, Ethiopia.

Methods: A health facility based cross sectional study was conducted from February,2016 to July, 2016. Demographic variables were collected using a structured questionnaire. Two vaginal swab specimens were collected from each participant. For diagnosis of bacterial vaginosis ,gram stain Nugent score was used. All fungal and bacterial pathogens were isolated and characterized by employing conventional techniques. Antimicrobial susceptibility profile of bacterial isolates was Performed using disc diffusion technique as per the standard Kirby-Bauer method. The results were analyzed using SPSS version 20.

Result: The overall vaginal infection was 70% **.**The overall prevalence of bacterial vagnosis was 102(48.6%).Bacterial vaginosis was statistically significant associated with odor (p=0.007), frequency vaginal bathing (p=0.045) and frequency of changing of pants (p=0.01). The overall prevalence of vulvovaginal candiasis was 87(41.4%).Vulvovaginal candidiasis was statistically significantly associated with illiterate patients (p=0.021), number of life time male sex partner (p=0.037), number of sex partner in the last 12 months (p=0.001) and with previous bacterial vaginosis (p= 0.004). A total 151 bacterial isolates were recovered, of which 105(69.5%) of the isolates were gram negative and 46 (30.5%) isolates were gram positive bacteria. The overall drug resistance rates of gram negative bacterial isolates ranged from 14.3% to Amikacin 77.3% to Tetracycline. The overall drug resistance rates of gram positive bacterial isolates ranged from 0% to vancomycin and 67.4% to penicillin.

Conclusion and recommendation: Both bacterial vaginosis and vulvovaginal candidiasis was high and hence many such types of studies are recommended to be conducted across the country.

Keywords: Bacterial Vaginosis and Fungi isolate

1. Introduction

1.1. Back ground

Vaginitis is an inflammation of the vagina that can result in discharge, itching, pain and unpleasant odor.The most common infectious vaginitis are bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis [1].

Bacterial vaginosis (BV) is described as a shift in the balance of the vaginal microflora characterized by an increase in the vaginal pH, a reduction in lactobacilli and an overgrowth of a mixture of facultatively and obligately anaerobic bacteria [2-4]. The bacterial pathogen associated with vagina infection are *Neisseria gonorrhoea, E. coli, Streptococcus pyogenes, S. aureus, Treponema pallidum, E. faecalis, Clostridium perfringens, Proteus mirabilis, Klebsiella aerogenes* etc [5].

Besides bacterial pathogens, Candida spp. are the most common cause of fungal infections, leading to a range of life-threatening invasive to non-life-threatening Mucocutaneous diseases. Among Candida spp. *Candida albicans* is the most common infectious agent. It is a commensal that colonizes skin, the gastrointestinal and the reproductive tracts. mucocutaneous infections have been classified as oropharyngeal, esophageal and vulvovaginal candidiasis. Among the many causes of vaginitis, vulvovaginal candidiasis is the second most common after bacterial vaginosis [6, 7]. Itching, white discharge, edema, and erythema of the vulva have been identified as the major clinical signs and symptoms of this infection [8].

Bacterial vaginosis and vulvovaginal candidiasis are affected by hygiene behaviors, number of life time sex partner, number of sex partner in the last twelve month, history of abortion and certain sociodemographic characteristics [8,9]

A diagnosis of yeast vaginitis and bacteria bacterial vaginosis are made by the combination of clinical presentation, physical examination findings, and observation of yeast or clue cells on the wet preparation[9,10]. The diagnosis is also supported by the “whiff test and Gram stain of vaginal discharge also called Nugent score which is the golden standard for diagnosis of BV [11]. A culture may be done in cases of recurrent bacteria and yeast vaginitis or for concerns of less common varieties of bacteria or yeast that may be more resistant to standard therapy[10]

1.2. Statement of the Problem

Reproductive tract infections are one of the most serious public health issues in both developed and developing countries [12]. Around 150,000,000 cases of reproductive tract infections in South East Asia and 65,000,000 cases in African countries occur annually [13].

Vaginitis is one of the most common infections in women. Many studies have shown that nearly 5-10 million females every year seek gynaecologic advice for vaginitis [14]. Bacterial vaginitis can occur in any age group but it is more prevalent in females of reproductive age group worldwide [9].The prevalence of bacterial vaginosis differs widely from country to country in the same region and even in similar population groups it has been estimated to be in the range of 8% and 75% [15]. The prevalence of BV in African has been estimated to be in the range of 20% to 50% [6]. Although bacterial vaginosis is considered to be a trivial disease it is a morbid disease in terms of loss of working days and treatment cost [14].

For many years bacterial vaginosis has been given little attention. In recent years, however, the association of bacterial vaginosis with ascending genital tract infection in one hand and sexually transmitted diseases in another has made the infection a major a global problem [16]. As excellently reviewed by Verstraelen et al [16], women with bacterial vaginosis are at increased risks for STDs, HIV, gonorrhea, trichomoniasis, and herpes simplex virus type 2 (HSV-2).Bacterial vaginosis can cause Pelvic inflammatory disease (PID), postoperative infections, spontaneous abortion, preterm birth, and postpartum endometritis. Additionally, late foetal loss and spontaneous preterm birth have also been identified as infections related to bacterial vaginosis and, during pregnancy. Furthermore, it has been documented that bacterial vaginosis propagates viral replication [17-19], and vaginal shedding of the HIV-1 and HSV-2 viruses [20] thereby further enhancing the spread of these viruses.

Vaginal *E. coli* may cause symptomatic infections such as vaginitis or tubo-ovarian abscess and is associated with life-threatening neonatal sepsis [21]. *Streptococcus agalactia*e or Group B streptococcus (GBS) is also a well-defined pathogen in with causing intr-amniotic infection, endomyometritis and neonatal infections [22-24].To prevent the association of bacterial vaginosis with ascending genital tract and sexually transmitted disease, evaluation of the

prevalence and species distribution of bacteria implicated in causing bacterial vaginosis appears to be one of the highest priorities.

On the other hand vulvovaginal candidiasis is the second most common after bacterial vaginosis [6, 7]. In 75% of women, a diagnosis of vulvovaginal candidiasis is made at least once during their childbearing years [8].and 40– 50% will have recurrent episodes [25]. The pathogenesis and prognosis of candida infections are affected by the host immune status and also differ greatly according to disease presentations. Therefore, diagnosis, management and treatment choices vary and need to be considered in the overall setting of the affected human host.

Although the association of vaginitis with ascending genital tract infection and sexually transmitted diseases a major a global problem, In Ethiopia, some studies on the prevalence of bacterial vaginosis and vulvovaginal candidiasis have been conducted [26-28].Additionally it has been neither the focus of intensive study nor of active control programs. Therefore, the purpose of the study is -determining prevalence of bacterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection.

1.3. Significance of the study

Vaginitis is a very common but neglected disease so this study provides recent information on prevalence of bacteria vaginosis and vulva candidiasis. The information generated from this study will provide a baseline data for future epidemiological studies. The results obtained from this study will help the concerned bodies to formulate guidelines for choosing effective laboratory diagnosis and antibiotic therapy. It also used to increase knowledge of the society on prevalence of bacteria and fungus pathogen among women complaining genital tract infection for various prevention and control measures.

2. Literature Review

A study conducted on prevalence of bacterial vaginosis and vulvovaginal Candidiasis mixed infection in Southeastern America by Charles, *et al*., 2011 [29], showed that a prevalence of bacterial vaginosis (BV) and Vulvovaginal candidiasis was 72.5% and 15.7% respectively. Among women with BV, 33.1% were colonized with yeast. The prevalence of BV/vulvovaginal candidiasis mixed infections among young women was observed to be 4.4%. Similar study carried out by Joscelyn N *et al* 2014 [30], on Prevalence of Bacterial Vaginosis and Candida among Postmenopausal Women conducted in the United States also exhibited the prevalence of BV was higher and increased with age while the prevalence of Candida was low .

Another study in USA by Mintz *et al.*, 2013[31], on prevalence of *Non-Albicans Candida* Infections in Women with recurrent Vulvovaginal symptomatology, A total of 103 women were tested by genital fungus culture and Candida-specific polymerase chain reaction (PCR). Among all isolated *NAC species*, 28.6% (6/21) were determined to be *C. glabrata*, 23.8% (5/21) *C. krusei,* 23.8% (5/21) *C. parapsilosis*, and 23.8% (5/21) other Candida species. Approximately 30 % of women with recurrent vulvovaginal symptomatology have detectable Candida strains.

Further studied in USA Zhou et al., 2007 [32],added information on differences in the composition of vaginal microbial communities found in healthy Caucasian and black women that identify Communities dominated by roughly equal numbers of more than one species of Lactobacillus were rare in black women, but common in Caucasian women. This finding was supported by Achkar.et al 2010 [33], which found that a significantly higher incidence in African-American than in white American women or women of other races.

Cross sectional study in Baghdad Al-Yermouk Teaching Hospital, in 2011 showed that the Gram positive bacteria are more predominant than Gram negative bacteria in vaginal infections. The highest prevalence isolate is *Escherichia coli* followed by *Staphylococcus aureus* and *Candida albicans.* The highest isolate is *Escherichia coli* 28(15%) and it was highly sensitive to cefotaxime (90%), while it was resistant to chloromphenical (20%). On the other side *Staphylococcus aureus* which was rack the second among the isolates 25 (13.44%) was mostly sensitive to cloxacillin (85%). The majority of Gram negative isolates were resistant to the co-trimoxazale except *Klebsiella spp.* [34].Studies in clinical settings in Bangladesh and India in

2005, reported that only 30 and 60% of women complaining of vaginal discharge had a laboratory confirmed RTI. As a result, the syndromic approach for the treatment of vaginal discharge leads to inappropriate treatment in a high proportion of cases [35].

A study which was conducted in India on prevalence of bacterial vaginal infections in pre and postmenopausal women by Lakshmi *et al*., 2012 .High vaginal swab samples was collected and the microorganisms were identified. *Escherichia coli, Staphylococcus aureus and Candida spp*. were isolated from 15.2%, 8.7%, 19.6% premenopausal women and 14.8%, 9.3%, 13% postmenopausal women respectively [36].

A etiology and risk factors of recurrent vaginitis & its association with various contraceptive methods study was conducted in India by Thulkar *et al*.,[37] ,2010 assessed that Tubal ligation (38.8%) and non-contraceptives (34.0%) were the most common methods used by recurrent vaginitis patients. Bacterial vaginosis (53.8%) and mixed infection (36.8%) were commonly seen infections. Bacterial vaginosis was not observed in OC pill users. This study also show that male condom use provided protection against recurrent vaginitis and its use should be promoted with other contraceptive methods in high risk cases.

Another study in India by Narayankhedkar.*et al*., 2015 [12], on Clinicoetiological Characterization of Infectious Vaginitis amongst Women of Reproductive Age Group ,The presenting symptoms were vaginal discharge 106 (96.4%), vulval itching/irritation19(17.3%), malodor 5 (4.5%), paininabdomen3(2.7%), and dysuria1(0.9%).The commonest etiology detected was Candida in 33 (30%) cases, of which 18 (54.5%) were *C.albicans* and 15 (45.5%) *non-albicans Candida*(NAC)infections. The NAC isolates were *C.glabrata* (n = 10), *C.tropicalis* (n=3), and *C.krusei* (n=2). Bacterial vaginosis observed in 19 (17.3%). A statistically significant association between Candida infection and presence of curdy-white discharge (p = 0.001) and vulvalitching/irritation (p = 0.007) was noted. Another study in india by Bhalla.*et al.* 2007on Prevalence of bacterial vaginosis among women Bacterial vaginosis was diagnosed in 70 (32.8%) subjects. A high percentage though asymptomatic (31.2%) were found to have bacterial vaginosis. Highest prevalence was seen in urban slum (38.6%) followed by rural (28.8%) and urban middle class community (25.4%) [38].

Another cross-sectional study carried out in Iran Turk in 2014 assessed the Prevalence of genital tract infections in pregnant women .*Candida albicans* (35.76%), *Escherichia coli* (17.97%), and *Streptococcus spp*.(13.06%) were the most observed infections, with a higher prevalence rate of reproductive tract infections during the second half of pregnancy compared to the first half [13].

A prospective study was conducted involving 65 women who consecutively attended Gynecological ward in Maternity and Child Hospital in Ramadi for complaints of genital malodour and/or abnormal vaginal discharge by AL-Alwani *et al*.,2008 period Bacterial vaginosis was diagnosed in 30 (46.2%) women, vulvovaginal yeast fungi infection in 12 (18.5%) women, other aetiology in 16 (24.6%) and in Seven (10.8%) women showing sterile vaginal discharg[39].Another study here in Iraqi by Al-Obadi I et al. reported a prevalence of 38% *Candida albicans* infection in 50% of women complaining of vaginal discharge and 15% of diabetic women without vaginal discharge [40].

According to study conducted in Lybia by Khamees *et al.*, 2012 on Characterization of vaginal discharge among women complaining of genital tract infection .Bacterial vaginitis is the most common cause of abnormal vaginal discharge and constituted 79.5% of total cases, with (*Staphylococcus aureus* (21.8%), *Escherichia coli* (14.2%) and *Klebsiella species* 13.6%) followed by candidal infections 13.6%. A comparatively Bacterial vaginosis (due to *Gardenerlla vaginalis)* (2.2%) was last seen as a cause of vaginal discharge [5].

Study conducted in West-Cameroon by Kouamouo *et al* on Female genital tract infections and engines of antibiotic resistance in fast growing populations indicated that major etiologies includes *E.coli* (34.9%), *Enterobacter spp.* (23.8%), *Staphylococcus spp*.(14.8%),*Citrobacter spp.* and *Streptococcus Spp*.(9% each), *Proteus spp*.(5.3%) and *Klebsiella spp*.(3.2%).Antibiotic susceptibility testing disclosed high and moderate resistance rates against common (sulfamides and penicillins) and uncommon (nitrofurans and rifamycines) conventional antimicrobials families, respectively [41] .

Another study conducted by Ibrahim et al., 2009 in Nigeria found that *Candida albicans* infection is the commonest cause of pathologic vaginal discharge and it is common in the unmarried and in those within the reproductive age group [42]. Similar in South-Eastern Nigeria

by Alo *et al* showed that women within the age 36-40 years and 26-30 years had the highest prevalence of *C. albicans* (33.33%) and co-infections (43.00%) respectively [43].

Similar study conducted in Kenya Thika district Hospital by Menza *et al*. exhibited the percentage distribution of vaginal candidiasis within age group was highest in the age range 26 - 35 years with 56(60%) patients and in the 3rd trimester of pregnancy with 64(68.09%) patients[44].

A study conducted on prevalence of bacterial and *Candida albicans* infection amongst women attending Irrua Specialist Teaching Hospital, Irrua in Nigeria by Isibor, *et al*. showed that from the seventy-five specimens analyzed, 56 (74.7%) isolates were from symptomatic patients while 25 (44.6%) were from asymptomatic patients. *C. albicans* was the most isolated pathogen with 35 (47.7%) isolates, followed by *Staphylococcus aureus* with 25 (29.8%), E. coli, 11 (13.1%), *Klebsiella spp,* 5 (6.0%) *Enterococcus faecalis*, 4(4.8%), *Proteus spp 3* (3.6%) and Pseudomonas aeruginosa, 1(1.2%)[45].

Study conducted by Lawrence *et al*. on The prevalence of bacterial vaginosis in both self-reported symptomatic and asymptomatic female students of the Michael Okpara University of Agriculture,Umudike in Nigeria show that Out of 200 samples examined,148(74%) had one form of microbial organism or the other, ranging from bacteria to fungi; bacteria making up to 104 of the isolates while 44 isolates were of fungal infection. The frequency of isolation of organism was *E.coli* 68(46.0%), *Yeast* 44(29.7%) and *Staphylococcus aureus* 36(24.3%). The most effective antibiotic against *E.coli* isolates was ciprofloxacin,52(76.5%) while tetracycline 2(3.0%) was the least effective. *Staphylococcus aureus* isolate was most sensitive to ciprofloxacin 33(91.7%) whereas they were resistant to cotrimoxazole and nalidixic acid, 0(0%) each [46]. A study conducted by Uneke C.*et al.* in Nigerian on non gonococcal and non-Chlamydia microbial isolates from high vaginal swabs showed that the individual aged 30-40 significantly more infected with bacteria and *c.albicans* [47].

A cross-sectional study in Nigeria on Genital Tract Infection: Prevalence and Causes in Women attending Aminu Kano Teaching Hospital Kano by Yarʺzever et al., 2013 The study showed that the total number of 210 (42.00%) subjects were found to be infected, with 121 (24.2%) having candidiasis, 13 (2.60%), 48 (9.60 %) having staphylococcal infection, 9 (1.80 %) having

streptococcal infections and 19 (3.80%) having other infections related to coliform-like organisms respectively. None of the subjects had gonorrhea. It was concluded that sexual activities and low standard of hygiene are among factors aiding the spread of genital tract infection [48].

Further study among African women wearing tight clothes reported a higher prevalence of *Candida albicans* in Vulvovaginal candidiasis than those wearing loose clothing. Where regular users of tight clothings had 88.2% of *Candida albicans* and occasional and non-wearers had 68.6% of *Candida albicans* [49].Similar study conducted in Nigeria by Akpan *et al*., 2011 [50], showed that a high incidence of vulvovaginal candidiasis (76.8%) with its associated symptoms were observed among women who regularly wore nylon tight and other synthetic pants than those who regularly wore cotton tight/cotton underwear/pants (42.9%). It can be concluded that women who predominantly wear nylon tight and other synthetic underwear/pants are at a higher risk of vulvovaginal candidacies.

In Ethiopia study conducted by Ayenalem *et al.*, 2010 on Lactic Acid Bacterial Vaginosis among Outpatients in Addis Ababa, BV frequency among these study women was 32%. Only 15% were symptomatic as detected by the presence of malodor or abnormal discharge. The majority (44%) was in the age group of 18-30 years and less than a quarter of them had BV [26]. Mengistie *et al.*, 2014 also assessed on the prevalence of bacterial vaginosis among pregnant women attending antenatal care in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. The prevalence of bacterial vaginosis is 19.4% using Gram stain Nugent scoring system. In addition, prevalence of bacterial vaginosis was 31.6% and 15.9% among symptomatic and asymptomatic pregnant women respectively [27]. On other study conducted by Mulu *et al.,* 2015 in Ethiopia showed over all 15.4% have vaginal infection. In this study the most common identified vaginal infections were candidiasis (8.3 %) and bacterial vaginosis (2.8 %). The isolation rate of *N. gonorrhoeae* and *group B Streptococcus colonization* was 4 (1 %) and 6 (1.2 %), respectively. Norfloxacin (75.6 %), ciprofloxacin (79.6 %) and gentamicin (77.6 %) revealed high level of sensitivity whereas high resistance rates were observed for amoxicillin (82.2 %), tetracycline (63.3 %) and cotrimoxazole (62.2 %) [28].

3. Objective

3.1. General objective

- To determine the prevalence of bacterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection at St. Paul's Hospital Millennium Medical College Gynecology OPD and Ethiopia Family Guidance Model Clinic ,Addis Ababa, Ethiopia, 2016.

3.2. Specific objectives

- To determine the prevalence of bacterial vaginosis among women complaining genital tract infection
- To determine the prevalence of vulovvaginal candidiasis among women complaining genital tract infection
- To determine the antibiotic susceptibility pattern of bacterial isolates
- To assess association of clinical manifestation and risk against bacteria vaginosis and vulvo vaginal candidiasis.

4. Materials and Methods

4.1. Study Area Description

The study was conducted at St. Paul Hospital Millennium Medical College and Family Guidance Association model clinic. St. Paul Hospital Millennium Medical College is located in Gulele sub-city. It has 1486 professional and supporting staff, provides health services for about 700 patients daily. The hospital has 13 department and 340 beds offering different specialized services. Gynecology department has 5 OPD clinic and many people are referred from all over the country to this hospital.

Family Guidance Association of Ethiopian, Model Clinic is non-government, not-for-profit organization which provided reproductive health services. It is found in Arada sub-city .The clinic has 20 professional and supporting staff, provides health services for about 120 patients daily. It has 4 OPD clinics and many heath facilities refer patients for laboratory diagnosis to this clinic.

4.2. Study Design

Health facility based cross sectional study was conducted at St. Paul Hospital Millennium Medical College and Family Guidance Association of Ethiopian, Model Clinic from February to July, 2016.

4.3. Population

4.3.1. Source Population

All women who visit St. Paul Hospital Millennium Medical College gynecology OPD and Family Guidance Association of Ethiopian, Model Clinic from February to July, 2016.

4.3.2. Study Population

Women who complaining symptomatic genital tract infection at St. Paul Hospital Millennium Medical College gynecology OPD and Family Guidance Association of Ethiopian and who fulfill the inclusion criteria during the study Period.

4.4. Sample Size and Sampling Technique

A consecutive sampling technique was used. All women with symptoms of genital tract infection were included in the study until the required sample size is obtained. Since the study is based on a single population proportion, the sample size was calculated using prevalence of (15.4%) from study conducted by Mulu et al. [28] as follows;

$$n = \frac{Z^2_{1-\alpha/2}\, P\,(1-P)}{d^2}$$

Where; n is the sample size to be determined, the value of Z for 95% power is 1.96, p is the prevalence, 1-p is (84.6%) and d is margin of allowable error (0.05). Using the above formula and considering 95% confidence interval n =201

By Assuming 10 % non-response rate, the sample size was: n=201+10 %=201+21= 222.

4.5. Study variables

4.5.1. Dependent Variables

- Bacteria vaginosis
- Bacterial isolate
- Fungal isolate
- Antibiotic susceptibility pattern

4.5.2. Independent Variables

- Socio demographic factors: age, marital status, educational
- Clinical finding: abnormal vaginal discharge, vaginal itching and vaginal burning and odder,
- vaginal bathing ,
- number of life time male sex partner, no of sex partner in the 12 months,
- History of abortion and Previous BV/GTI

4.6. Inclusion and Exclusion Criteria

4.6.1. Inclusion criteria

Women who showed symptom of vaginal infection and willingness to participate in the study, presumptive diagnosis of vaginal infection and no history of antibacterial and antifungal therapy within two weeks prior to their attendance were the inclusion criteria.

4.6.2. Exclusion Criteria

Those women with genital malignancy and who douched their vagina with chemicals were excluded from the study.

4.7. Data Collection

4.7.1. Socio-demographic, Clinical data and Risk factor

Socio demographic data (age, marital status, educational) and Clinical finding: abnormal vaginal discharge, vaginal itching and vaginal burning, odder and vaginal hygiene, number of life time sex partner ,number of sex partner in the last twelve month were obtained using pre –designed structural questioner by nurses and supervision by principal investigator. The requisition form filled up by physicians was used as standard preform to document socio-demographic and clinical information.

4.7.2. Specimen Collection

Upon admission to the study, physicians performed clinical examination of each participant and recorded signs of vaginal abnormalities. During the examination two high vaginal swabs were collected aseptically from study participants using sterile rayon tipped applicator stick swabs with experienced nurses then label and transport with transport medium to the Microbiology Laboratory of the Ethiopian Public Health Institute with 2 hr.

4.8. Laboratory procedure

4.9. Inoculation

The first swab was inoculate onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (Oxoid, Basingstoke, UK) and incubated at 35–37∘C for at least 48 hours aerobically.

The second was inoculate onto Blood Agar base (Oxoid, Basingstoke, Hampaire, UK) to which 5% sheep blood is incorporated, MacConkey agar, (Oxoid, Basingstoke, Hampaire, UK) and modified Thayer-martin agar (Oxoid, Basingstoke, Hampaire, UK). MacConkey agar plates incubated at 35–37∘C for 18 to 24 hours aerobically. Blood agar plates and modified Thayer-Martin agar were incubated in 5% carbon dioxide incubator. Plates with no growth after 24 hours were re incubated for a further 24 hours.

4.10. Yeast Identification

Yeasts were identified by employing conventional biochemical and assimilation test procedures [17], using CHROMagar *Candida* culture medium (Becton Dickinson) as per the instruction of the manufacture, germ-tube formation in human serum, and production of blastoconidia, pseudohyphae, and chlamydospore on cornmeal agar (Oxoid, Basingstoke, UK).

4.11. Bacterial Identification

Pure isolates of bacterial pathogen were preliminarily characterized by colony morphology, gram stain, and haemolytic reactions on blood agar plates, color change of media around the colony, odor, shape and texture on agar plate. For gram positive bacteria:- catalase, coagulase, CAMP, PYR, and Bacitracin have been used. Identification of gram negative bacteria down to genus and/or species level was done by employing an array of routine biochemical tests such As Indole, Urea, Manitol, Triple Suger Iron Agar, Lysine Decarboxylase and Lysine Deamination, Citrate Utilization, Oxidase and Motility.

4.12. Antibiotic susceptibility testing

The anti-bacterial susceptibility testing of bacterial isolates was performed by Kirby-Bauer disc diffusion method. For gram negative and *S.aureus*, muller-Hinton(Oxoid,Ltd England) was used also for fastidious bacteria such as *S.agalactae* miller-Hinton agar supplemented with 5 % sheep blood was used .

The following antimicrobial agents were employed: Ampicillin (AM–10ug), Amoxicillin/Clav. (AMC30ug), Cefoxitin(Fox-30ug), Ceftriaxone (CRO,30ug), Ciprofloxacin (CIP,5ug) Clindamycin (Da,2ug), Erythromycin (E-15ug), Gentamicin(CN,10ug), Penicillin(Pen,10ug), Tetracycline (T,30ug), Tobramycin (TOB,10), Vancomycin (Va,30ug), Amikacin (Ak ,30ug) and Trimethoprim/sulfamethoxazole (SXT, 0.05ug) . Resistance data were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2016) [64]. Reference strain of *E.coli ATCC* 25,922 and *S.aureus ATCC* 25,923 were used for quality control for antimicrobial susceptibility test. All drugs were generously provided by Ethiopian Public Health Institute.

4.13. Gram staining

The second swab was also used for smear preparation on a glass slide for Gram staining to diagnose bacterial vaginosis. For diagnosis of bacterial vaginosis, vaginal smear slides were air dried heat fixed, gram-stained and examined under oil immersion objective (1000 x magnification) and graded as per standardized, quantitative, morphological classification method developed by Nugent et al. [11]. The method involved assigning a score between 0 and 10 based on the quantitative assessment of the Gram-stain for three different bacterial morphotypes: (i) large Gram-positive rods (indicative of Lactobacillus spp), (ii) small Gram-negative or variable rods (indicative of Gardnerella, Bacteroides and other anaerobic bacteria), and (iii) curved, Gram-variable rods (indicative of Mobiluncus spp).

Each morphotype was quantitated from 1 to 4+ with regard to the number of morphotypes per oil immersion field (0, no morphotypes; 1+, less than 1 morphotypes; 2+, 1to 4 morphtypes ; 3+, 5 to 30 morphtypes; 4+,30 or more morphotypes).Then scores between 0 and 3 represented „normal vaginal flora", between 4 and 6 „intermediate vaginal flora", and scores between 7 and 10 were considered diagnostic for „BV". In this study, microbiological definition of BV was a score of 7–10 by Nugent"s method.

4.14. Data Processing and Analysis

Collected quantitative data were coded; processed, edited, and analyzed using SPSS version 20 (Statistical Package for social sciences, SPSS) statistical software for analysis. Frequency and percentage of each variable were calculated. Binary Logistic regression was used to determine presence of an association between several key variables with bactria vaginosis and vulvavaginal candidiasis. Table and Figures were used for data presentation. P-value of<0.05 will be considered statistical significant.

4.15. Data Quality Assurance

Data quality was ensured through use of standard data collection material, pre testing of the questioners, proper data collection and processing of all activities done by the principal investigator. Every activity in the laboratory was done by adherence with standard operation procedures. The specimen was kept free of contamination. All materials, equipment and procedures were adequately controlled. Preparation and performance evaluation of culture media were done as per the instruction of the manufacturer. Culture media was tested for sterility and performance tests.

The performance of equipment‟s (autoclave, incubators, refrigerator and freezer) was monitored by using standard procedures. Reference strain of *E.coli* ATCC 25-922 and *S.aureus* ATCC 25-923 was used for quality control of antimicrobial susceptibility test. The inoculums size during drug susceptibility testing was monitored by using 0.5 McFarland standards [14]. The data was checked for completeness and representativeness prior to entry.

4.16. Ethical consideration

Before starting the research work, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences and department of Medical Laboratory science and institutional review Board (IRB) of St. Paul Hospital Millennium Medical College. The respondent was given the right to refuse to take part in the study as well as to withdraw at any time during the study period. All the information obtained from the study subjects were coded to maintain confidentially. When the participants

were found to be positive for culture and /gram stain, they were informed by the hospital clinician and receive proper treatment.

4.17. Dissemination of results

The finding of the study will be submitted to Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences. In addition, a copy of this material will be given to Addis Ababa Health Bearua, st. Paul Hospital Millennium Medical College, annual conferences of professional societies and other concerned bodies. The finding of the study will also be presented to the medical scientific community and manuscript will be submitted to peer reviewed journals for publication.

5. Results

5.1. Age distribution of women complaining genital tract infections

Two hundred ten women complaining genital tract infections were examined for bacterial vaginosis and candidiasis. From these study participants, 80 of them were from St. Paul Hospital Millennium Medical College Gynecology OPD and 130 of them from Ethiopian Family guidance Model clinic. Samples were collected based on three age group brackets of 18-24, 25-44 and 45-64 years and frequency of age were 54(25.7) ,116(55.2%),40(19%) respectively with a mean age of 33 years and the patient age were 18-53.

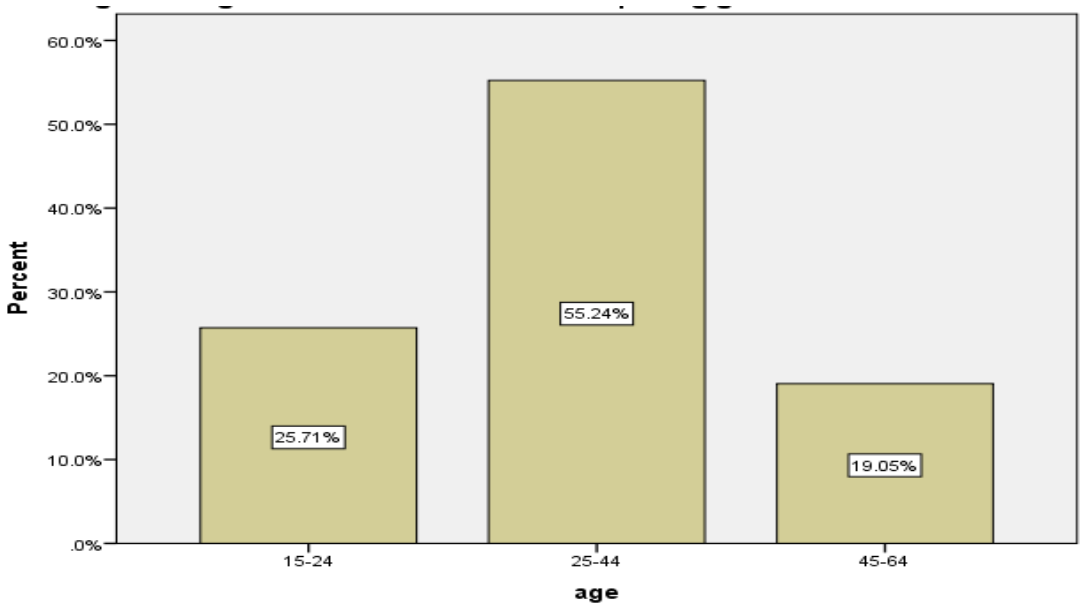

Figure 1: Age distribution of women complaining genital tract infection among women complaining genital tract infection at SPHMMC and FGAEMC from February to July, 2016.

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5.2. Total Prevalence vaginal infections among women complaining genital tract infection

Among 210 study participant the overall prevalence of vaginitis infection was 70%. Women with BV (48.6%), 13.3% were mixed infection with *candida spp*.

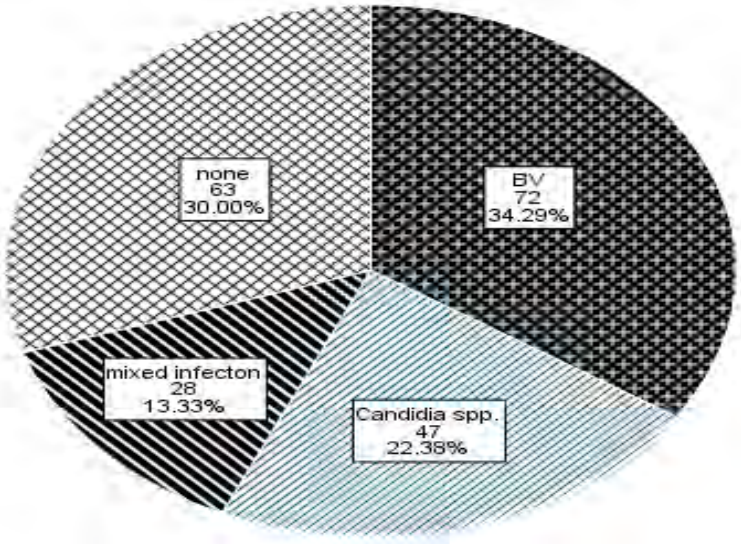

Figure 2: Prevalence of vaginal infection among women complaining genital tract infection at among women complaining genital tract infection at SPHMMC and FGAEMC from February to July, 2016.

5.3. Socio-demographic characteristics in relation with bacterial vaginosis

The overall prevalence of bacterial vaginosis was 48.6% and subgroup prevalence of bacterial vaginosis are presented in Table 1 and Table 2. Although younger women, those between the ages of 18 and 24 years had a somewhat lower prevalence 22(40.7%) of bacterial vaginosis, among the 25 years and older group the prevalence was between 55(47.4%) and 25(62.5%). Statistical analysis showed a significant correlation between BV and patients with age groups of 45-64 AOR, 3.022 (95% CL (1.201-7.606) (P. =0.019).

The prevalence of bacterial vaginosis varied with education, and marital status. Women with more than a high school education were less likely to be positive for bacterial vaginosis than those with a high school education or less (35.9% versus 44.7- 55.3%). Similarly, the prevalence

of bacterial vaginosis was higher among those unmarried study subjects (53.8%) compared with those married (44.8%) and divorced (50.0%) in this study but it is not statically significant.

Selected gynecology features were also associated with the prevalence of bacterial vaginosis. Women who reported one sex partner in the previous 12 months and 1-3 life times‟ male sex partners had a prevalence rate of 47.2%, and 43.4% respectively. Those who reported two and above sex partner in the previous 12 months and four and above life time male sex partner had a prevalence of 49% and 58%. The prevalence rate of bacterial vaginosis was higher in women with a history of abortion (53.8%) than women with no abortion history (48.8%). The prevalence of bacterial vaginois was less in patients that had previous bacterial vagnosis (46.3%) than patients with no previous bacterial vagnosis (50.4%).

Table1:-Prevalence of bacterial vaginosis (Nugent"s Gram Stain Score 7–10) by selected characteristics in relation to demographic and gynecology features (n=210) among women complaining genital tract infection at SPHMMC and FGAEMC from February to July, 2016.

Characteristics	Number	BV positive	BV Negative	COR(95%CI)	P-value(AOR)	AOR(95%CI)
Age in years						
15-24	54(25.7)	22(40.7)	32(59.3)	1		1
25-44	116(55.2)	55(47.4)	61(52.6)	1.311(0.682-2.522)	0.186	1.610(.795-3.260)
45-64	40(19)	25(62.5)	16(37.5)	2.424(1.047-5.611)	.019	3.022(1.201-7.606)
Total	210(100)	102(48.6)	108(51.4)			
Marital status						
Unmarried	65(30.9)	35(53.8)	30(46.1)	1		
Married	107(50.9)	48(44.8)	59 (55.1)	0.697(0.376-1.295)		
Divorced	38(18.1)	19(50)	19(50)	0.857(0.385-1.910)		
Total	210	102(48.6)	108(51.4)			
Education						
Illiterate	47(22.4)	21(44.7)	26(55.3)	1.442(.604-3.446)		
Primary school	59(28.1)	31(52.5)	28(47.5)	1.977(.862-4.535)		
Secondary school	65(31.0)	36(55.3)	29(44.6)	2.217(.979-5.017)		
College	39 (18.6)	14(35.9)	25(64.1)	1		
Total	210 (100)	102(48.6)				
Number of life time male sex partner						
1-3	136(64.8)	59(43.4)	77(56.6)	1		1
≥ 4	74	43(58)	31(41.8)	1.810(1.021-3.210)	.504	1.244 (0.656-2.357)
Total	210	102(48.6)	108(51.4)			
No of sex partner in the 12 months						
0	28(13.3)	15(53.5)	13(46.2)	1		
1	129(61.4)	61(47.2)	68(52.7)	0.777(0.343--1.764)		
≥2	53(25.2)	26(49)	27(50.9)	0.835(0.333--2.089)		
Total	210	102(48.6)	108(51.4)			
History of abortion						
Yes	52(24.7)	28(53.8)	24(46.1)	1.324 (0.706-2.483)		
No	158(75.2)	74(46.8)	84(53.1)	1		
Total	210	102(48.6)	108(51.4)			
Previous BV/GTI						
Yes	95(45.2)	44(46.3)	51(53.6)	0.848 (0.492-1.461)		
No	115(54.7)	58(50.4)	57(49.5)	1		
Total	210	102(48.6)	108(51.4)			

COR=Crude odd ratio, AOR=Adjusted odd ratio, P-value(AOR)= P-value by AO

5.4. **Clinical manifestations and genital hygiene in relation to bacterial vaginosis**

With regards to clinical symptoms vaginal itching, vaginal discharge, vaginal burning and vaginal odor were revealed in 127(60.4%), 210 (100%), 83(39.5%) and 135(66.4%) respectively. The prevalence of bacterial vagnoisis against clinical symptom was 51.9 %, 48.6%, 49.3% 56.2% in patients with vaginal itching, vaginal discharge, vaginal burning and odor respectively. Statistical analysis showed a significant correlation between BV and vaginal odor ARO 0.413 (95%CL (0.226-0.756) (P= 0.007).

Hygiene-related variables were divided in to two categories i.e., vaginal bathing and number of pants used per day (Table 2). The prevalence of bacterial vaginosis was less than in patients that change pants more frequently, (one to two per day) (36.9%) than that change their pants less frequently one pant for 2-4 days (57.6%). Women those not frequently change their pant was strong association between number of pants used per day with BV ARO 2.558 (95% CL (1.361-4.806) (P=0.004).Statistical analysis showed that patients that bath their vaginal frequently (≥4 times within a day) were strong correlation between BV ARO 1.832 (CL95 %((1.013-3.344) (P=0.049).

Table 2:-Prevalence of bacterial vaginosis (Nugent"s Gram Stain Score 7–10) by selected characteristics in relation to genital hygiene and clinical manifestations (n= 210) at SPHMMC and FGAEMC from February to July, 2016.

Characteristics	Number	BV positive	BV Negative	COR(95%CI)	P-value(AOR)	AOR(95%CI)
Vaginal itching						
Yes	127(60.4)	66(51.9)	61(48)	1.413(.810-2.464)		
No	83(39.5)	36(43.3)	47(56.6)	1		
Total	210	102	108			
Vaginal discharge						
Yes	210	102(48.6)	108(51.4)			
No	0	0	0			
Total	210	102	108			
Vaginal burning						
Yes	83(39.5)	41(49.3)	42(50.6)	1.056(.607-1.837)		
No	127(60.4)	61(48)	66(51.9)	1		
Total	210	102	108			
Odor						
Yes	135(64.2)	76(56.2)	59(43.7)	2.428 (1.353-4.356)	0.007	0.413(0.226-0.756)
No	75(35.7)	26(34.7)	49(65.3)	1		
Total	210	102	108			
Vaginal bathing /day				1		
1-3	128(60.9)	69(53.9)	59(46)			
≥4	82(39)	33(40.2)	49(59.7)	.576(.328 -1.010)	*0.049*	*1.832 (1.013-3.344)*
Total	120	102	108			
Number of pants used/ day						
1-2 pants/a day	92(43.8)	34(36.9)	58(63)	1	.	
1pant for 2-4 days	118(56.1	68(57.6)	50(42.3)	2.320(1.326-4.058)	*0.004*	*2.558(1.361-4.806)*
Total	210	108				

COR=Crude odd ratio, AOR=Adjusted odd ratio P-value (AOR) =P-value by AOR, CL =confidence interval

5.5. Socio-demographic characteristics in relation with vulvovaginal candidiasis

The overall prevalence of vulvocandidiasis was 41.4% and subgroup prevalence of rates of vulvocandidiasis are presented in Table 3 and Table 4. The prevalence of vulvovaginal candiasis in the study subjects was (38.8 %: 87/210). Although younger women, those between the ages of 15 and 24 years, had a somewhat lower prevalence (35.1%) of vulvovaginal candiasis than among the 25 years and older group the prevalence was between 40.0% and 44.8%. Adjusted statistically analysis showed no significant association between vulvovaginal candiasis and patients with age groups. The prevalence of vulvovaginal candiasis varied significantly with education, and marital status. Women that are uneducated were less likely to be positive for vulvovaginal candiasis (55.3%) than more educated women (35.8-38.5%). Statistical analysis showed that significant association between Illiterate women and vulvovaginal candiasis AOR 3.369(95 %(1.204-9.429).Similarly, the prevalence of vulvovaginal candidacies was higher among those unmarried and divorced study subjects (41.5%) and (41.4%) respectively compared with those married (37.4%) but it is not statically significant.

Selected gynecology features were also associated with the prevalence of vulvovaginal candiasis. Women who reported two and above sex partner in the previous 12 months and ≥4 life time male sex partner had a prevalence rates of(47.1%), and (52.7%) respectively. Those who had one sex partner in the previous 12 months and1-3 life time male sex partners had a prevalence of 45.7% and 35.2%. Statistical analysis showed a significant correlation between vulvovaginal candidiasis and ≥4 life time male sex partner AOR, 2.132 (95%CL(1.046-4.342) (P=0.037), between vulvovaginal candidiasis and one sex partner in the previous 12 months AOR, 16.784 (95%CL (4.043-69.684) (P=0.001), between vulvovaginal candidiasis and ≥ 2 sex partners in the previous 12 months, AOR, 14.988 (95%CL (3.454-65.030)) (P=0.001).

The prevalence rate of vulvovaginal candidiasis was less in women with a history of abortion (40.3%) than women with no abortion history (41.7%). The prevalence of vulvovaginal candidiasis was more in patients that had previous bacterial vagnosis or GTI (52.6%) than patients with no previous bacterial vagnosis (32.1%). Statistical analysis showed a significant associated between vulvovaginal candidiasis and previous bacterial vagnosis or GTI, AOR, 2.132 (95%CL (1.046-4.342) (P=0.037).

Table 3:- Prevalence of vulvovaginal candidiasis (culture) by selected characteristics in relation to demographic and sexual and gynecology features (210) at SPHMMC and FGAEMC from February to July, 2016.

Characteristics	Number	Candidiasis positive	Candidiasis Negative	COR(95%CI)	P-value(AOR)	AOR(95%CI)
Age in years						
15-24	54(25.7)	19(35.1)	34(62.9)	1		1
25-44	116(55.2)	52(44.8)	65(56)	2.040(1.014-4.105)	0.330	1.497(.665-3.366)
45-64	40(19)	16(40)	24(60)	2.874(1.216-6.791)	0.148	2.088(.770-5.663)
Total	210(100)	87(41.4)	123(58.5)			
Marital status						
Unmarried	65(30.9)	27(41.5)	38(58.4)	1		
Married	107(50,9)	40(37.4)	67(62.6)	0.840(.448-1.577)		
Divorced	38(18)	20(52.6)	18(47.4)	1.564(.699-3.500)		
Total	210(100)	87(41.4)	123(58.5)			
Education						
Illiterate	47(22.3)	26(55.3)	21(44.7)	2.211(.925-5.283)	0.021	3.369(1.204-9.429)
Primary school	59(28)	22(37.3)	37(62.7)	1.062(.458-2.461)	0.857	0.913(.339-2.457)
Secondary school	65(30.9)	25(38.5)	40(61.5)	1.116(.490-2.542)	0.563	0.762(.762-.303)
College	39(18.6)	14(35.8)	25(64.1)	1		1
Total	210(100)	87(41.4)	123(58.5)			
Number of life time male sex partner						
1-3	136(64.7)	48(35.2)	88(64.7)	1		1
≥ 4	74(35.2)	39(52.7)	35(47.2)	2.043 (1.148-3.635)	0.037	2.132(1.046-4.342)
Total	210(100)	87(41.4)	123(58.5)			
No of sex partner in the 12 months						
0	28(13.3)	3(10.7)	25(89.2)	1		1
1	129(61.4)	59(45.7)	70(54.2)	7.024(2.019-24.433)	0.001	16.784(4.043-69.684)
≥2	53(25.2)	25(47.1)	28(52.8)	7.440(2.001-27.669)	0.001	14.988(3.454-65.030)
Total	210(100)	87(41.4)	123(58.5)			
History of abortion						
Yes	52(24.7)	21(40.3)	31(59.6)	0.944 (.499-1.787)		
No	158(75.2)	66(41.7)	92(58.2)	1		
Total	210(100)	87(41.4)	123(58.5)			
Previous BV/GTI						2.719(1.381-5.356)
Yes	95(45.2)	50(52.6)	45(47.3)	2.342 (1.336-4.107)	0.004	
No	115(54.7)	37(32.1)	78(67.8)	1	1	
Total	210(100)	87(41.4)	123(58.5)			

COR=Crude odd ratio, AOR=Adjusted odd ratio, P-value (AOR)= P-value by AOR, CL =confidence interval

5.6. Clinical manifestations and genital hygiene in relation to vulvovaginal candidiasis

The prevalence of vulvovaginal candidiasis against clinical symptom was 55(43.3%), 87 (41.4%), 35(42.1%) and 56(41.5%) in patients with vaginal itching, vaginal burning and odor respectively. Statistical analysis showed no significant association between vulvovaginal candidiasis with clinical symptom.

Hygiene-related variables were divided in to two categories i.e., vaginal bathing and number of pants used per day (Table 4). The prevalence of vulvovaginal candidiasis was less than in patients that change pants more frequently, one to two per day 31(33.6%) than that change their pants less frequently one pant for 2-4 days 56 (47.4). Similarly, patients that bath their vaginal frequently were less affected (prevalence rate 40.2%) than that did not bath their vaginal more frequently (prevalence rate of 42.1%).In contrast, no significant associated was observed between vulvovaginal candidiasis and vaginal bathing (those bath their vaginal frequently), and vulvovaginal candidiasis with number of pants used per a day (one pant for 2-4 days).

Table 4:-Prevalence of vulovovaginal candidiasis (culture) by selected characteristics in relation to genital hygiene and clinical manifestations (n= 210) at SPHMMC and FGAEMC from February to July, 2016.

Characteristics	Number	Candidiasis positive	candidiasis Negative	COR(95%CI)	P-value	AOR(95%CI)
Vaginal itching						
Yes	127(60.4)	55(43.3)	72(56.6)	1.217(.692-2.141)		
No	83(39.5)	32(38.5)	51(61.4)	1		
Total	210(100)	87	123(58.5)			
Vaginal discharge						
Yes	210(100)	87(41.4)	123(58.5)			
No	0	0	0			
Total	210	87	123(58.5)			
Vaginal burning						
Yes	83(39.5)	35(42.1)	48(57.8)	1.052(.600-1.843)		
No	127(60.4)	52(40.9)	75(59)	1		
Total	210(100)	87	123(58.5)			
Odor						
Yes	135(64.3)	56(41.5)	79(58.5)	1.006 (0.567-1.785)		
No	75(35.7)	31(41.3)	44(58.6)	1		
Total	210(100)	87(41.4)	123(58.5)			
Vaginal bathing /day						
1-3	128(60.9)	54(42.1)	74(57.8)	1		
≥4	82(39)	33(40.2)	49(59.7)	.923(.525-1.622)		
Total	210(100)	87(41.4)	123(58.5)			
Number of pants used/ day						
1-2 pants/a day	92(43.8)	31(33.6)	61(66.3)	1		
1pant for 2-4 days	118(56)	56(47.4)	62(52.5)	1.777(1.012-3.122)	*0.054*	*0.507(0.254-1.013)*
Total	210(100)	87(41.4)	123(58.5)			

COR=Crude odd ratio, AOR=Adjusted odd ratio, P-value(AOR)= P-value by AOR ,CL =confidence interval

5.7. Bacteria isolate among women complaining genital tract infection

A total 151 bacterial isolates were recovered, of which 105(69.5%) of the isolates were gram negative and 46 (30.5%) isolates were gram positive bacteria. Of the total isolates, gam negative bacteria were the most common accounting for 69.5% of the total isolates. *E. coli* and *Klebsiella spp.* were the two predominant gram negative bacteria consisting of (20.5, and 15.2% of the total bacterial isolates respectively. Of the total bacterial isolates, gram positive bacteria accounted for S. *aureus(17.1%)* and *S. agalactaes(3.3%)* being the first and the second predominant gram positive bacteria respectively (Table 5). None of the subjects study had gonorrhea.

Table 5: -Spectrum of bacterial isolates (n =151) at SPHMMC and FGAEMC from February to July, 2016.

Species	***Number of isolates***	***%of the total***
E.coli	*43*	*20.5*
S.aureus	*36*	*17.1*
Klebsiella pneumonia	*28*	*13.3*
Enterobacter aerogens	*17*	*8.1*
Citrobacter spps.	*8*	*3.8*
S.agalacte	*7*	*3.3*
Klebsiella ozenae	*4*	*1.9*
Providencia spps.	*4*	*1.9*
S. pyogens	*3*	*1.4*
Proteus miralabis	*1*	*0.47*
Total	*151*	*71.77%*

5.8. Fungal isolate among women complaining genital tract infection

A total 120 yeast isolates were recovered, from 87 patients giving 41.4% prevalence rate of vulovaginal candidiasis. *C. albicans* and *C.krusi* were the two predominant yeasts consisting of 43.3%, and 33.3% of the total yeast isolates respectively (Table 6)

Table 6: Spectrum of fungal isolates from women complaining genital tract infection (n =87)

At SPHMMC and FGAEMC from February to July, 2016.

species	Pure culture	Mixed culture	Total	% of the total yeast Isolates
C.albican	37	15	52	43.3
C.tropical	2	8	10	8.3
C.krusi	16	24	40	33.3
Undefined yeasts	1	17	18	15.0
Total	**57**	**63**	**120**	**100**

Note: *C.albican with C.tropical(6),C.krusi(6), Undefined yeasts(3),C.krusi with C.tropical(2),Unedified yeasts(14)*

5.9. Antibiotic susceptibility pattern of gram positive bacteria

Table 7 summarizes the overall drug susceptibility profile of gram positive bacteria against eleven antibacterial drugs tested. The highest overall resistance rate of gram positive bacteria was observed against Penicillin (67.4%), followed by Tetracycline (58.7%) and Erytromcine Clindamycin (45.6%) while all isolates were 100 % sensitive Vancomycine) followed by Tobramycin (88.8%), Clindamycin (86.9%) and Gentamicin (80.6%). *S. aureus* the most frequently isolated gram positive bacterium was 88.8% sensitive to Tobramycin and 86.1% to Clindamycin. As depicted in table 5, *S. agalactaae,* the 2nd most frequently isolated gram positive bacterium was 100% susceptible to Pen, FOX and Clindamycin.

Table 7:- Percentage in vitro antibacterial susceptibility pattern of all gram positive bacteria isolates (n=46) at SPHMMC and FGAEMC from February to July, 2016.

Species	Antibacterial drugs										
	Pen	FOX	E	DA	SXT	TE	CN	VA	CRO	CPR	TOB
s.aureus(36)	13.9	97.2	41.7	86.1	58.3	11.1	80.6	-	-	77.7	*88.8*
s.agalacte(7)	100	100	85.7	100	57.1	42.8	ND	100	100	-	-
S.pyogen (3)	100	100	66.67	66.67	33.3	33.3	-	100	100	-	-
All gram positive isolate (46)	32.6	97.2	50	86.9	56.5	17.3	80.6	100	100	77.7	88.8

Pen =Penicillin, Fox= Cefoxitin, SXT= Trimethoprim/sulfamethoxazole, CRO= Ceftriaxone, CIP=Ciprofloxacin, Da =Clindamycin,E=Erythromycin ,CN=Gentamicin,TE=,Tetracycline,TOB=Tobramycin , VA= vancomycin , __=Not tested

5.10. Antibiotic susceptibility pattern of gram negative bacteria

The overall antibiotic susceptibility profile of gram negative bacteria against the nine antibacterial drugs tested is summarized in Table 8. Tetracycline had the highest overall resistance rate (77.3%) against gram negative bacteria followed by Ampicillin (77.1%) and amoxicillin (70.6%). Gram negative bacteria showed sensitivity towards Amikacin (85.7%) and Tobramycin (82.8%).

As far as species specific antimicrobial resistance rates are concerned, *E. coli*, the most frequently isolated bacterium, showed 76.7 to both Ampicillin and Tetracycline and, 60.5% to Amoxicillin/Clav. The least resistance rate (13.9%) of the bacterium was observed against Amikacin, Tobramycin and Gentamicin. *K. pneumonia*, the second most commonly isolated

gram negative bacterium exhibited a resistance rate of 85.7% against Trimethoprim/sulfamethoxazole and 82.1% to tetracycline. The least resistance rate (17.8%) of the bacterium was observed against Amikacin. *Entrobacter aerogens* the 3rd most frequently isolated gram negative bacteria was 82.4% resistant to Ampicillin.

Table 8: Percentage of antibacterial susceptibility pattern of all gram-negative bacteria isolates (105) at SPHMMC and FGAEMC from February to July, 2016.

Species	Antibacterial drugs								
	AM	AMC	SXT	TE	CN	CRO	CPR	TOB	AK
E.coli(43)	23.3	32.6	60.5	18.6	83.7	76.7	79.1	86.04	86.04
Klebsiella pneumonia(28)	14.3	14.3	14.3	17.8	78.5	60.7	57.1	78.5	82.1
Klebsiella ozenae(4)	0	0	0	0	50	50	50	75	100
Enterobacter aerogens (17)`	0	17.6	28.1	5.9	70.6	70.6	58.8	76.7	82.35
Citrobacter spp.(8)	25	0	37.5	25	75	62.5	75	87.5	87.5
Proteus miralabis(1)	100	100	100	100	100	100	100	100	100
Providencia spp(4)	100	50	50	50	100	75	100	100	100
All gram negative isolate	18	22.8	39	18	79	68.5	69.5	82.8	85.7

AM =Ampicillin, AMC=Amoxicillin/Clav, SXT= Trimethoprim/sulfamethoxazole, CRO= Ceftriaxone, CIP=Ciproflox, CN=Gentamicin ,TE=Tetracycline, TOB=Tobramycin, AK=Amikacin, S= sensitive, R= resistance. I=interpretation

6. Discussion

From 210 women complaining genital tract infection, prevalence of vaginal infections overall, was 147 (70 %). It was lower compared to reports in Lybia by Khamees et al. (86%) [5],and West-Cameroon by Kouamouo *et al* (71%) [41] And higher than previous study in Ethiopia, Bahrdar by Mulu M.,et al. 15.4% [28].This may happen because of method difference in study population. For instance, in this study only symptomatic women were included.

The overall prevalence of bacterial vaginosis in the present study as determined by Gram stain Nugent scoring criteria was 48.6%. Though the prevalence rate of bacterial vaginosis in the present study was well within the reported range, it was relatively higher than the prevalence rates of BV obtained in similar local studies by Ayenalem ,et al., Mengistie et al., Mulu et al. [26, 27, 28] and the same as the prevalence rate of 50% that has been reported in a study conducted in Veitnam by Go et al. [51]. Local studies reported prevalence rates of bacterial vaginosis in the range of 2.8% [28] to 32% [26]. Lower prevalence of bacterial vaginosis than the present study were also reports from different sub-Saharan countries like Kenya (37%), Botswana , (38%), and Zimbabw (32.5%) [52-54] and higher result reported in India by Thulkar et al.,(53.8%) [37]. Disparity in the prevalence rates of BVs in different studies could result from difference in the definition of BV, methodology, and size and type of study population.

Although the cause of BV remains unclear, BV has been associated with demographic, sexual, Reproductive health, and behavioral Characteristics. In line with this, our work studied the association of bacterial vaginosis with many of these parameters. The results of the present study showed that patients with age groups of 45-64 (60%), married (50.9%) and with education level of secondary school (55.3%) were more affected. Bacterial vaginosis however was not significantly associated with these socio-demographic characteristics except in age.

Women aged 45-64 3.022 times high likely to be risk for BV than women ≤44 age AOR, 3.022 (95% CL (1.201-7.606) (P. =0.019). A highest proportion of BV in age groups greater than 45 years in the present study was in good agreement with studies conducted in Shandong by Xueqiang F et al. [55], Indonesia by Ocviyanti et al. [56] and Bangladesh by Yusuf et al. [57]. Similarly, a previous study has found that bacterial vaginosis prevalence increased with age. In a population of individuals seeking STD treatment, 23% of women aged 14–24 years had bacterial

vaginosis compared with 33% of women aged 25 years and older [58]. An elevation of pH in women at the age of greater than 45 years as the result of a decline in, the level of estrogen is not optimal for the growth of lactobacilli but conducive for the growth of other microorganisms causing bacterial vaginosis.

Lack of education has been found to be significantly associated with bacterial vaginosis by Bhalla et al. [38]. However, our finding in this regard like other studies in Shandong by Xueqiang et al., [55, 59] contradicted with the association of lack of education with BV since the adjusted analyses in the present study confirmed associations of bacterial vaginosis with higher level of education and since this study conducted in Shandong rural area so variation can be due to the socio-demographic factor.

Of all risk factors explored thus far, sexual behavior related characteristics: no significant correlation was observed between life time number of sex partners and BV. Our result in this regard was supported by previous studies in Ethiopia [27], [28] and study in Brazil [10] and disagree with study conducted by Allsworth [59], that reported multiple or new sex partners increased the risk of acquiring BV by a factor of 1.6 –2.5.

The number of sex partner in the 12 months was not statistically significant in our study contrast to study in Bahrdar, Ethiopia [28] women who have no sex partner in the 12 months was statistically significant association BV . In contrast to other findings, no significant correlation was observed between BV and number of abortion in this study [7]. As far as personal hygiene is concerned, 1.832 times high likely to be risk for bacterial vaginosis compared ≥4 times of vaginal bathing per day with one to three times of vaginal bathing per day AOR, 1.832 (95%CL (1.013-3.344) (p=0.049).

Regarding the number of pants used per day, 2.558 times a high likely to be risk for bacterial vaginosis compared women those used one pant for two to four day with women those used one to two pant for one day ARO 2.558 (95% CL (1.361-4.806) (P=0.004).

In this work the association of bacterial vagionisis with clinical symptoms such as vaginal itching, vaginal burning, vaginal discharge and odor was studied. Of these, clinical symptoms, bacterial vaginosis was statistically associated with odor ARO 0.413 (95%CL (0.226-0.756) (P= 0.007) which agree with study conducted in Iran P<0.001 [8].

A prevalence rate of vulvovaginal candidiasis in the present study was 41.4%. This is higher than the prevalence of vulovaginal candidiasis reported in local study 8.3 % [28], Iran 35.76%)[13], Ramadi 18.5%[39] Iraqi 38% [40] in Nigeria 33.33%[42] and low than study conducted in Kenya 60%[44], in Nigeria 47.7%[45] . Disparity in the prevalence rates of vulvovaginal candidiasis in different studies could result from difference in methodology, and size and type of study population.

Of the socio-demographic and behavioral characteristics we examined, Women that are uneducated were 3.369 times high likely risk to be positive for vulvovaginal candiasis than more educated women 3.369(1.204-9.429) (p=0.021).

Women who have ≥4 life time male sex partners were 2.132 times high likely risk for vulvovaginal candiasis than one up to three life time male sex partners AOR, 2.132 (95%CL (1.046-4.342) (P=0.037). Women who have one sex partners in the last 12 months 16.784 times high likely risk for vulvovaginal candiasis than who had no sex partners in the last 12 months AOR, 16.784 (95%CL (4.043-69.684).Similarly, Women who have two and above sex partners in the last 12 months 14.988 times high likely risk for vulvovaginal candiasis than who had no sex partners in the last 12 months AOR, 14.988 (95%CL (3.454-65.030) (P=0.001).

In contrast to study in Bahrdar, Ethiopia [28] one sex partners in the last 12 months and Women who have two and above sex partners in the last 12 months had strong significant correlation with vulvovaginal candidiasis. In Bahrdar study women who had no sex partners in the last 12 months the difference could be, since the adjusted analyses in the present study confirmed associations of vulvovaginal candidiasis with women who had no sex partners in the last 12 months.

Women who have previous bacterial vagnosis 2.719 times high likely risk for vulvovaginal candiasis than patients with no previous bacterial vagnosis AOR, 2.719 (95%CL (1.381-5.356) (P=0.001).

In the present study a total of 120 yeast isolates were recovered from 87 patients. Of yeast isolates *C. albicans* was the major yeast accounting for 43.3%. Our result in this regard was in agreement with a study conducted in the United States and Neptune [60]. However, the proportion of yeast isolates accounted by non-albicans yeast species (56.7%) in the present study

was not in line with similar study conducted in the United States [60]. The significance of this finding is that non-albicans yeast species may replace *C. albicans* under selective pressure of fluconazole, resulting in infections refractory to the current fluconazole based treatment in Ethiopia. Like other African countries, the present guideline of the Ethiopian Ministry of Health (MOH) for the management of candidiasis includes fluconazole as a first choice drug and ketoconazole, miconazole ointment as alternative antifungal agents [61, 62]. Although clinical and in vitro resistance to C albicans fortunately is rare, non-albicans Candida species are less likely to respond to azole antifungal therapy [63]

In the present study a total 151 bacterial isolates were recovered, of which 69.5% of the isolates were gram negative and 30.5% isolates were gram positive bacteria. *E. coli* and *Klebsiella spp.* were the two predominant gram negative bacteria while *S. aureus* and *S. agalactaes* were the two predominant gram positive bacteria. Among the overall bacteria isolate, *Escherichia coli(20.5%*) was the highest prevalence isolate followed by Staphylococcus aureus (17.1%) which is comparable with other studies like study conducted in West-Cameroon by Kouamouo et al ,in Nigeria by Lawrence et al., in India by Lakshmi,et al. [41, 47, 36]. All bacterial isolates were tested against an array of antibacterial antibiotics. None of the subjects study had gonorrhea which contradicts with study in Bahrdar, Ethiopia [28] since the sample was high vaginal swab; the probability of getting Gonorrhea was less.

The overall drug resistance rates of gram negative bacterial isolates ranged from 14.3% to Amikacin 77.3% to Tetracycline. *E. coli,* the most frequently isolated gram negative bacterium showed a high level of was resistance (76.7%) to tetracycline and ampicillin while almost 86 % of *E. coli* isolates were susceptible to Amikacin.

The overall drug resistance rates of gram positive bacterial isolates ranged from 0% to vancomycin and 67.4% to penicillin. *S. aureus* , the most frequently isolated gram positive bacterium revealed a high level of resistance to pencicillin (86.1%) and tetracycline 63.8%.Availability of antimicrobials without prescription and inappropriate dosing schedule may explain the isolation of high level of drug resistance in the present study.

7. Limitations of the Study

- Some yeast are not identify up to species level.

8. Conclusion

In summary, the results obtained demonstrated a high prevalence of bacterial vaginosis and genital candidiasis. Among bacteria isolate 105(69.5%) were gram negative and 46 (30.5%) isolates were gram positive bacteria. The overall drug resistance rates of gram negative bacterial isolates ranged from 14.3% to Amikacin 77.3% to Tetracycline. *S. aureus* , the most frequently isolated gram positive bacterium revealed a high level of resistance to pencicillin (86.1%) and tetracycline 63.8%.

9. Recommendations

- Many such types of studies are recommended to be conducted across the country
- We recommended concerned to formulate laboratory based guidelines for choosing effective antibiotic therapy.

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Annex I: English Versions of Participant Information Sheet

My name is Yeshiwok Abebaw. I am a laboratory technologist postgraduate student at Addis Ababa University. Now I am conducting a study entitled **"Bacterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection at St. Paul's Hospital Millennium Medical College Gynecology and Ethiopia Family Guidance Model Clinic, Addis Ababa, Ethiopia, 2016."**

You are invited to participate in this study. Please read or listen the following statements and ask any unclear points before you agree to participate. If you agree to be included in this study, I would like to ask you to sign on a document to show your agreement; participate accordingly, and give clinical specimen.

Introduction

The topic of this study is "**bacterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection at St. Paul's Hospital Millennium Medical College Gynecology and Ethiopia Family Guidance Model Clinic ,Addis Ababa, Ethiopia, 2016**". Vaginitis is a very common disease of women all over the world including Ethiopia so this study may determine Prevalence of bacterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection and their drug sensitivity profile and the result of the study can be helpful in patient management. Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw yourself at any time, there will be no consequences and you will get all the services provided in the hospital with no problem. If you decide to participate, you have to sign on the assent/ permission template form and you may obtain a copy of this information sheet.

Expected from participants

What we expect from you is your willingness to give us high vaginal swap. Sample will be collected using sterile cotton swap and test tubes by experienced nurse. Being asked to give sample does not necessarily mean that you have the disease. When you are found to be positive for the micro-organism, you will be informed by the health worker and receive proper treatment. You need to know that your results might be discussed with other appropriate individual out of

this hospital. But your name, address will not be disclosed rather an identification code will be used in such conditions.

Time required participating

You will spend 10-15 minutes until the specimen is collected and permission form is signed.

Risks of participant

Specimen collection will have no effect and you will not get any risk as the sample will be collected by well trained professionals. The sampling procedure may cause some discomfort and minor bleeding. If there comes any discomfort; we shall offer you necessary medical treatment freely.

Confidentiality

The information in your records is strictly confidential. All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional will have access the information. The information will be encoded in a computer and saved with password protection.

Benefits of participation

By participating, you will get no financial benefits. Even though there is no direct benefit due to participation in this study, the findings of the study is useful for better understanding of the problems of vaginitis. You will also obtain all the results of the analysis for free and communicated to your physician for the appropriate management.

Rights of participants

Your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

Communication

In case if you have any questions, unclear ideas and doubt about the project, contact addresses ar

Investigator: Yeshiwork Abebaw (BSc), DMLS; AMU, +251913447415

Email- Yeshi885@gmail.com

Advisor: Adane Bitew (PhD), DMLT, AAU +251911039162

For additional information, please contact Addis Ababa University, College of Health Sciences,

Department of Medical Laboratory Sciences at: Telephone +251112755170

Annex II English Versions of Consent form

This page contains an agreement signature to participate in the study entitled with "Bacterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection at St. Paul's Hospital Millennium Medical College Gynecology and Ethiopia Family Guidance Model Clinic ,Addis Ababa, Ethiopia, 2016". So please read the following points and sign your signature at the end in the space provided.

1. I understand the objective of the study "Bacterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection at St. Paul's Hospital Millennium Medical College Gynecology and Ethiopia Family Guidance Model Clinic, Addis Ababa, Ethiopia"

2. I know that the information/ specimen that I will give used for this study only.

3. I understand that, all the information given for the study and the results are confidential.

4. I understand that I will not get any money for my participation.

5. I understand that I have a right to stop from participation any time in the study.

6. I understand all the information which is explained by specimen collector/Nurse.

Participant Code:_____

Signature of the participant: _____Date: _____

Annex III: Amharic Versions of Participant Information Sheet

እኔ የሺወርቅ አበባው እባላለሁ፡፡ በአዲስ አበባ ዩኒቨርሲቲ ፣ ጤና ሳይንስ ኮሌጅ ፣ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የሁለተኛ ዲግሪ ተማሪ ስሆን የምርምር ስራየን በመስራት ላይ እገኛለሁ፡፡ በመሆኑም እርስዎም በዚህ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡ በጥናት ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈርሙ እጠይቃለሁ፡፡

መግቢያ

የጥናቱ ርዕስ "በመራቢያ አካላቸዉ ላይ ሕመም ይሰማኛ ልብለዉ ከሚመጡ ሴቶች ላይ ያለዉን የባክቴርያ እና ፈንገስ ስርጭት ቅዱስ ጳዉሎስ ሚሊኒየም ሜዲካል ኮሌጅ የማህፀን እና ፅንስ ክፍል እና በኢትዮጵያ ቤተሰብ *መምሪያ ሞዴል ክሊኒክ ለታደሙ ህመምተኞች ፤ አዲስ አበባ ፤ ኢትዮጵያ* " በሚል ርዕስ እያጠናሁ እገኛለሁ፡፡ ይህ ጥናትም በተሳታፊ ሙሉ ፈቃደኝነት ላይ ተመስርቶ ባክቴርያ እና ፈንገስ በሴቶች መራቢያ አካል ላይ ያላቸዉን ስርጭት እና ለመድሀኒቶች ያላቸዉን ተላምዶ ለማወቅ እና አማራጭ መንገዶችን ለመጠቆም ያስችላል፡፡

ከጥናቱ ተሳታፊ የሚጠበቁ

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ከብለተዎ ናሙና እንዲወሰድ እና ለጥናቱ እንዲዉል መስማማት ይጠበቅቦታል፡፡የጤና ባለሙያ ከእርሰዎ ናሙናዉን ይሰበስባል ፡፡ከተወሰደዉም ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙ ለሥራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅቦታል፡፡ይሁን እንጂ ይህ ዓይነቱ መረጃ የእርሶን ማንነት የማገልጹ ማስረጃዎን ማለትም ስም ፣አድራሻ ና የመሳሰሉት መረጃዎች አይጨምርም፡፡ይልቁንም ለዚህ ጥናት አገልግሎት ብቻ የሚዉል መለያቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ናሙና ሰጡ ማለት በሽታው ይገኝብዎታል ማለት አይደለም፡፡ በእርሰዎ ናሙና ውስጥ የበሽታ አምጭ ተህዋስያን ቢገኝ ከጤና ባለሙያዉ አስፈላጊውን ህክምና ያገኛሉ፡፡

ተሳታፊዉ የሚያጠፋዉ ጊዜ

የተዘጋጀዉን የስምምነት ቅጽ ለመፈረምና ናሙና ለመስጠት 10-15 ደቂቃ ያስፈልጋል፡፡

በጥናቱ በመሳተፍ የሚስከትላቸዉ ችግሮች

ናሙና በሚሰበሰብበት ወቅት ምነም አይነት ችግር አያስከትልቦትም፡፡ሆኖም ናሙናዉ በሚወሰድበት ጊዜ ትንሽ የህመም ስሜት ሊኖር ይችላል፡፡የተለየ የሕመም ስሜት ከተሰማዎት አስፈላጊዉን እርዳታ በነፃ እናደርጋለን::

የመረጃዉሚስጥራዊነት

ማንኛዉም የሰጡት መረጃ እና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦረቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ይህን ማህደር ሊያገኙ የሚችሉ የተወሰኑ የጥናቱ ተባባሪ ሠራተኞች ብቻ ናቸዉ፡፡ከዚህም በላይ ስለ እርስዎ ያለዉን ማንኛዉንም መረጃ በመረጃ ማህደር ዉስጥ በኮድ እንዲቀመጥይደረጋል፡፡

በጥናቱ በመሳተፍ የሚያስከትላቸዉ ጥቅሞች

ይህ ጥናት የማስተርስ ዲግር መመረቂያ እንደ መሆኑ መጠን በመሳተፍዎ የሚያገኙት የገንዘብ ጥቅማጥቅም የለም ለወደፊት በተመሳሳ ይሁኔታ ዉስጥ ላሉ በሽተኞች በመረጃ ላይ የተመረተ ህክምና ለመስጠት ያግዛል ከፈለጉ የላቦራቶሪ ዉጤቶችን በነፃ ያገኛሉ እንዲሁም ስለ አስፈላጊዉ ህክምና ከሀኪምዎ ጋር ይነጋገራሉ፡፡

የጥናቱተሳታፊዎችመብት

ትብብርዎ ሙሉ በሙሉ በፍቃደኝነት ላይ የተመሠረተ ና ተሳትፎዎን መተዉ ና በማንኛውም ሰዓት ጥናቱን ማቆምይችላሉ፡በጥናቱ ዉስጥ ያሎትን ተሳትፎ በማንኛዉም ጊዜ የማጀረጥ ሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራሶን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም ዓይነት የሆስፒታል አገልግሎት አይኖርም፡፡ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛዉንም ዓይነት ጥያቄ የመጠቅ ና ገለፃ የማግነት መብት አለዎት፡፡የላቦራቶሪ ምርመራ ዉጤቱንም በነፃ ማግኘት ይቻላል፡፡

ግንኙነት ና ጥያቄ

ይህን ጥናት በተመለከተ ወይም ከዚህጋራ በተዛመደ መልኩ ስለ ሚያጋጥዉ ድንገተኛ ችግር ወይም ጥያቄ ካሎት በሚከተለዉ አድራሻ ይጠቀሙ፡፡

ተመራማሪ፣የሺወርቅ አበባው (ቢ.ኤስ.ሲ)

ሞባይል +251913447415

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል! የጤናሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ኢ-ሜይል፤ Yeshi885@gmail.com

አማካሪ፣አዳነቢተዉ(ፒ.ኤች.ዲ)
ሞባይል +251911039162

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል! የጤናሳይን ስኮሌጅ

አዲስ አበባዩኒቨርሲቲ

ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ፤የሕክም ና ላብራቶሪ ሳይንስ ትምህርት ክፍል ይጠይቁ;

ስልክ-+251112755170

Annex IV: Amharic Versions of Consent form

የተሳታፊ ስምምነት ቅጽ

ይህገጽ “Bacterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection at St. Paul’s Hospital Millennium Medical College Gynecology and Ethiopia Family Guidance Model Clinic ,AddisAbaba,Ethiopia,2016.” **“በመራቢያ አካላቸዉ ላይ ሕመም ይሰማኛ ልብለዉ ከሚመጡ ሴቶች ላይ ያለዉን የባክቴርያ እና ፈንገስ ስርጭት ቅዱስ ጳዉሎስ ሚሊኒየም ሜዲካል ኮሌጅ የማህፀ እና ፅንስ ክፍል እና በኢትዮድያ ቤተሰብ መምሪያ ሞዴል ክሊኒክ ለታደሙ ህመምተኞች ፤ አዲስ አበባ ፤ ኢትዮጵያ”**በሚል ርዕስ የተሳታፊ ስምምነት ቅጽ ነዉ።በመሆኑም እባክዎን በዚህ በታች የተዘረዘሩትን ነጥቦች ይረዱና፤ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈርሙ እጠይቃለሁ።

1.እኔ“ “በመራቢያ አካላቸዉ ላይ ሕመም ይሰማኛ ልብለዉ ከሚመጡ ሴቶች ላይ ያለዉን የባክቴርያ እና ፈንገስ ስርጭት ቅዱስ ጳዉሎስ ሚሊኒየም ሜዲካል ኮሌጅ የማህፀ እና ፅንስ ክፍል እና በኢትዮድያ ቤተሰብ መምሪያ ሞዴል ክሊኒክ ለታደሙ ህመምተኞች ፤ አዲስ አበባ ፤ ኢትዮጵያ” የሚለዉጥናትአላማበደንብተገንዝቤአለሁ።

2.ከእኔየሚወሰደዉናሙናለጥናቱአላማብቻእንደሚዉልተረድቻለሁ።

3.ሁሉምመረጃዎችእናየናሙናዉጤቱምስጢራዊመሆኑንተገንዝቤአለሁ።

4.በጥናቱላይበመሳተፌምንምየገንዘብክፍያእንደማላገኝተረድቻለሁ።

5.በጥናቱያለመሳተፍእንዲሁምበማንኛዉምጊዜየማቃረጥመብትእንዳለኝአዉቄአለሁ።

6.ሁሉምመረጃዎችበአስተባባሪዉ/ዎችተገልጾልኝበደንብተረድቻለሁ።

የተሳታፊ ሚጥር ቁጥር _____

የተሳታፊ ፊርማ፡ ----------

ቀን፡----------

Annex V: Data Collection Form

For The Study "**prevalence and species distribution of bacterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection at St. Paul's Hospital Millennium Medical College Gynecology and Ethiopia Family Guidance Model Clinic ,AddisAbaba,Ethiopia,2016**"

Date_____ Study Serial Number_____

Socio-demographic and reproductive health question		Choices
No	Question	
100	Age _____	
101	Marital Status	1.Married 2. Single 3. Divorced
102	Highest level of Education	1.Illiterate 2.Primary 3.Secondary 4.College and above
103	Life time number of sexual partners	1.one 2.two 3.three 4.four and above
104	Number of sexual partners for the last 12 months	1.one 2.two and above
105	History of abortion	1.Yes 2.No
106	past history of BV/UTI	1.Yes 2.No
genital hygiene and clinical manifestations		
200	Vaginal discharge	1.Yes 2.No
201	Vaginal itching	1.Yes 2.No
202	Vaginal burning	1.Yes 2.No
204	Vaginal discharge	1.Yes 2.No
204	Vaginal bathing /a day	1.1 time 2.2 time 3.3 time 4.≥4 time
205	Number of pants used/ day	1-2 pants/a day 1pant for 2-4 days

Annex VI:Amharic Versions translated questioner

“በመራቢያ አካላቸዉ ላይ ሕመም ይሰማኛ ልብለዉ ከሚመጡ ሴቶች ላይ ያለዉን የባክቴርያ እና ፈንገስ ስርጭት ቅዱስ ጳዉሎስ ሚሊኒየም ሜዲካል ኮሌጅ የማህፀ እና ፅንስ ክፍል እና በኢትዮድያ ቤተሰብ መምሪያ ሞዴል ክሊኒክ ለታደሙ ህመምተኞች ፤ አዲስ አበባ ፤ ኢትዮጵያ”ለሚካሄደው ጥንት የተዘጋጀ ጥያቄ

ቀን፡-_____ የተሳታፊ ሚጥር ቁጥር _____

1. የተሳታፊዋች የአኗኗር ሁኔታ		
ተ.ቁ	ጥያቄ	
100	እድሜሽ በአመት_____	
101	የጋብቻ ሁኔታሽ	1.ያላገባ 2. ለ.ያገባ 3.አግብቶ የፈታ
102	የተማርሽዉ/ሩት ከፍተኛ ትምርት	1.ትምህርት አልተከታተሉም 2.የመጀመርያ ደረጃ 3. ሁለተኛ ደረጃ ሠ 4.ኮሌጅ ዩንቨርስቲ
103	በሂወት ዘመንሽ/ዎት ከምን ያህል ሰው ጋር ግብረስጋ ግኑነት ፈፅመሻል/ ዋል?	1.አንድ ሰው 2.ሁለት ሰው 3.ሦሥት ሰው 4.አራት ሰው እና ከዛ በላይ
104	ባለፉት 12 ወራትስ ከምን ያህል ሰው ጋር ግብረስጋ ግኑነት ፈፅመሻል	1. ከአንድ 2.ከሁለት
105	አስወርዶሽ /ዎት ያውቃል?	1.አዎ 2. ለ.የለም
106	ከዚህ በፊት አሁን ያመመሽን አይነት ህመም አሞሽ/ዎት ያዉቃል	1.አዎ 2. ለ.የለም
ከህመሙን ጋር ተያያዥ ጉዳዮች		
200	ከብልተሽ/ዎት ከፍተኛ ፈሳሽ ይወፈጣል	1.አዎ 2. ለ.የለም
201	ብልትሽን/ዎትን ያሳክክሻል /ዎታል?	1.አዎ 2. ለ.የለም
202	ብልትሽን/ዎትን ያቃጥልሻል?	1.አዎ 2. ለ.የለም
204	ከብልተሽ/ዎት የሚወጣው ከፍተኛ ፈሳሽ ሽታ አለው	1.አዎ 2. ለ.የለም
204	ብልትሽን በቀን ምን ያህል ጊዜ ተታጠቢየለሽ	1.አንድ ጊዜ 2.ሁለት ጊዜ 3.ሦሥት ጊዜ 4.አራት ጊዜ
205	የዉስጥ ልብስሽን(ፓንት) በአብዛኛዉ በምን ያህልጊዜ ትቀይሪለሽ	1.በቀን 1-2 2. ፓንት 2-4 ቀን

Annex V: Laboratory activity

Laboratory protocol

- Provide written consent form for sexuality active women willing to participate in the study prior to sample collection
- Collection clinical data from physician
- Socio-demographic data was collected with well standardized questioner

Specimen collection

1. The patient is positioned in lithotomy position on the exam table (as for a pelvic examination).

2. Using two sterile rayon tipped applicator stick swabs to obtain a sample of vaginal discharge. The fluid specimen can be obtained directly from the mucosa of the posterior vagina

Laboratory procedure

1. **Gram Stain for Bacterial Vaginosis(Nugent's method)**

Scan the slide using a low power objective to locate any clusters of epithelial cells. The flora in these areas should be noted. Switch to the oil immersion lens (x1000) and examine between 10 and 20 representative fields to observe cell morphology and Gram reaction. The BV score for Gram staining will be calculated by Nugent"s method (1991).

1.1. Materials

Gram stain reagents: Crystal violet, Gram"s iodine, Decolorizer, Safranin **,**Slides, inoculating loops,Bunsen burner/slide warmer for heat-fixing slides

1.2. Staining

Do not apply stains, water, or decolorizer directly to specimen area. Apply drops near the frosted end of the slide, allowing the reagent to flow over the remaining surface.

- Flood the fixed smear with crystal violet for 30 seconds.
- Decant crystal violet and rinse slide gently with running tap water.

- Rinse off excess water with Gram's iodine, and then flood the slide with Gram's iodine for 30 seconds.
- Rinse off iodine gently with flowing tap water.
- Decolorize by letting the reagent flow over the smear while the slide is held at an angle or tilt slide. Stop when the runoff becomes clear (1-5 seconds).
- Adjust decolorization time to thickness of smear.
- Remove excess decolorizer with gentle flow of tap water.
- Flood with safranin and allow remaining for 30 seconds.
- Remove excess safranin with a gentle flow of tap water.
- Drain slide and air dry in an upright position.

1.3. Examination of stained smears

Reading Stained Slides

- Scan the slide using a low power objective to locate any clusters of epithelial cells (clue cell).
- Switch to the oil immersion lens (x1000) and examine between 10 and 20 representative fields to observe cell morphology and Gram reaction.
    - Lactobacillus spp-large Gram-positive bacilli
    - Gardnerella spp-small Gram-variable bacilli
    - Mobiluncus spp-curved Gram-variable bacilli
- The BV score for Gram staining will be calculated by Nugent‟s method (1991. Calculate a total numerical score by adding the scores for the three morph types

Interpretation and Reporting

QUANTITATIVE VAGINAL GRAM STAIN REPORT FORM

Pt. name:	**Micro #:**	**Date:**
Pt. ID #:		**Tech:**

Morphotype	**Number seen/OPF**					**Score**
	None	**≤1**	**1-5**	**5-30**	**>30**	
Gardnerella/Prevotella (Gram var.coccobac.)	**0**	**1**	**2**	**3**	**4**	
Mobiluncus	**0**	**1**	**2**	**3**	**4**	
Lactobacillus	**4**	**3**	**2**	**1**	**0**	
				Total Score		_____

BV Interpretation for score:

0-3 = Normal	**4-6 = Intermediate**	**7-10 = Bacterial Vaginosis**

Other observations	**Rare**	**Few**	**Numerous**
Yeast			
PMNs			
Predominant bacterium assoc. with PMNs: _____			

1.4. Preparation of Gram Stain Reagents (Hucker's Modification)

- **Crystal violet contains two solutions (solution a and b)**

Solution a

Solution a	Solution b
Crystal violet (certified) 20 g	Ammonium oxalate 8.0 g
Dissolve in 95% ethyl alcohol 200 ml	Dissolve in distilled wate 800 ml

Mix solutions a and b. Let stand overnight. Filter through coarse filter paper. Store in a brown screw cap bottle. Stain is stable for up to one year at room temperature.

- **Gram's iodine**

Dissolve iodine (3.3 g) and potassium iodide (6.6 g) in small amount of water (about 20 – 50 ml). Add remaining water (990 ml) and mix well. Store in a dark brown screw cap bottle, protected from light to avoid degradation. Solution is stable for up to one year at room temperature

- **Decolorizer**

95% ethyl alcohol(1200 ml) and Acetone(400 ml)Mix and store in a screw cap bottle. Solution is stable for up to one year at room temperature.

- **Safranin Counterstain**

Safranin O (certified) (2.5 g) Dissolve in 95% ethyl alcohol(100 ml) and Add distilled water (900 ml) then Mix well and store in a brown screw-cap bottle. Stain is stable for up to one year at room temperature.

1.5. Quality Control

Prepare Gram stain QC Slides: Inoculate Tryptic Soy Broth (TSB) with colonies of S. aureus ATCC 25923 and E. coli ATCC 25922. Incubate at 35oC for a few hours to obtain a faintly turbid broth. Label several slides (Gram stain QC, preparation date). Make smears using 2 drops per slide spread to about 2 cm size. Air dry then heat-fix or methanol-fix; store in a box at room temperature.

2. Identification of bacteria and fungus

Suspected growth from vaginal swab on blood agar,macConkey, modified Thayer martin agar and sabroude dextrose agar

A. Significant growth/present B. Significant growth/absent

Identification for suspected colonies

2.1. For gram positive bacteria

a. Colony morphology (size, shape, hemolysis and color

b. Gram stain

c. Perform appropriate biochemical identification test(catalase, coagulase, CAMP test bacitracin)

2.2. For gram negative bacteria

a. Lactose fermenter from macConke agar (lactose fermenter and non-lactose fermenter)

b. Biochemical identification test (indole ,urea ,manitol,triple suger iron agar citrate ,motility,lysinedecarboxylase,oxidase)

2.3. For gonoria isolation identification done by

a.Gram stain

b. biochemical test (30%H_2O and oxidase)

2.4. For fungal isolation identification done by

a. Perform wet mount

b. Color identification on chromo agar

2.5. Drug susceptibility for bacteria

Antibiotic	Abbrev. & conc. (µg/ml)	Sensitive	Intermediate	Resistance
Ampicillin	AM- 10			
Amoxicillin/Clav.	AMP – 30			
Cefoxitin	FOX-30			
Ceftriaxone	CR0 – 30			
Ciprofloxacin	CIP – 5			
Clindamycin	Da – 2			
Erythromycin	E-15			
Gentamicin	CN – 10			
Penicillin	PEN– 10			
Tetracycline	Tet – 30			
Tobramycin	TBO- 10			
Vancomycin	Va – 30			
Ceftriaxone	CRO-30			
Trimethoprim/sulfamethoxazole	SXT, 0.05ug			
Amikacin	Ak -30			

2.6. Gram Stain Procedure for culture identification

The Gram stain is used to classify bacteria on the basis of their forms, sizes and cellular morphologies and Gram reactions. Bacteria stain either Gram-positive or Gram-negative on the basis of differences in their cell wall compositions and architectures. Gram-positive species have a thick peptidoglycan layer and large amounts of teichoic acids; they are unaffected by alcohol decolorization and retain the initial stain, appearing deep violet. Gram-negative species have a single peptidoglycan layer on their cell wall attached to an asymmetric lipopolysaccharide-phospholipid bilayer outer membrane interspersed with protein; the outer membrane is damaged by the alcohol decolorizer, allowing the crystal violet-iodine complex to leak out and be replaced by the counterstain.

Interpretation of Gram-stained smears involves consideration of staining characteristics, cell size, shape and arrangement. These characteristics may be influenced by many factors, including culture age, medium, incubation, atmosphere, staining methods and presence of inhibitory substances.

2.6.1. Materials

Gram stain reagents: Crystal violet, Gram‟s iodine, Decolorizer, Safranin

Slides, inoculating loops, sterile applicator sticks, Normal Saline Solution (NSS), transfer pipettes

Bunsen burner/slide warmer for heat-fixing slides

Specimen: Colonies growing on solid medium, <24 hours old from non-inhibitory media.

2.6.2. Procedure

2.6.2.1. **Smear Preparation**:

- Use clean slides frosted on one end. Label slides appropriately using pencil.
- Place a drop of sterile NSS on slide.
- Transfer a small portion of colony with a sterile applicator stick or inoculating loop. Mix gently to emulsify.
- Staining procedure is similar with bacteria vaginosis
- Examination of stained smears
    - Gram-positive: deep violet
    - Gram-negative: pink or red

2.7. Biochemical test

2.7.1. Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase such as Staphylococci from non-catalase producing bacteria such as Streptococci.

2.7.1.1. **Principle**:

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it in to contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more that 24 hour old

2.7.1.2. Material Required

Hydrogen peroxide (3% H_2O_2) ,Test tubes Swab

2.7.1.3. Method

- Pour 2-3 ml of the hydrogen peroxide solution into a test tube.
- Using a sterile wooden stick or a glass rod, remove several colonies of the test organism and 3.immerse in the hydrogen peroxide solution.
- Look for immediate bubbling.

2.7.1.4. Results

Active bubbling ----- Positive test- Catalase produced

No release of bubbles ----- Negative test - No catalase produced

2.7.2. Coagulase Test

This test is used to differentiate Staphylococcus aureus which produces the enzyme coagulase, from S.epidermidis and S.saprophyticus which do not produce coagulase.

2.7.2.1. Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.Two types of coagulase are produced by most strains of S.aureus. Free Coagulase which converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by the appearance of a fibrin clot in the tube test.

2.7.2.2. Bound coagulase (clumping factor)

It is found on bacterial cell surface which converts fibrinogen directly to fibrin without requiring a coagulase reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test. It is usually recommended that a tube test should be performed on all negative slide tests. A tube test must always be performed if the result of the slide test is not clear, or when the slide test is negative.Before performing a coagulase test, examine a Gram stained smear to confirm that the organism is a Gram positive coccus.

2.7.2.3. Material Required

EDTA anticogulated human plasma

Slide test method (detects bound coagulase)

Place a drop of distilled water on each end of a slide or on two separate slides Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.Add a loopful (not more) of plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds.No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping

2.7.2.4. Results

Clumping within 10 seconds…...S. aureus

No clumping within 10 seconds :No bound coagulase

2.7.2.5. Test tube method (detects free coagulase)

- Take three small test tubes and label as:
    - T = test organism [18 – 24 hr broth culture]
    - Pos = positive control. (18 – 24 hr S. aureus broth culture)
    - Neg = Negative control (sterile broth)
- Pipette 0.2ml of plasma in to each tube
- Add 0.8ml of the test broth culture to tube T.
- Add 0.8ml of the S.aureus culture to the tube labeled as „pos"
- Add 0.8ml of sterile broth to the tube labeled as „Neg
- After mixing gently, incubate the three tubes at 35 – 37oC.
- Examine for clotting after 1 hour.

If no clotting has occurred, examine after 3 hours. If the test is still negative, leave the tube at room temperature overnight and examine again by tilting the tube gently.

2.7.3. CAMP Factor Test

2.7.3.1. Principle

Group B streptococci (*Streptococcus agalactiae*) produce a thermostable, extracellular, diffusible protein that acts synergistically with the beta-lysin produced by *Staphylococcus aureus* to produce a zone of enhanced lysis of sheep erythrocytes. The protein was named CAMP factor for the initials of the authors of the manuscript that first described the phenomenon.

2.7.3.2. Materials

- Sheep blood agar (SBA) plates
- Fresh culture (18-24 hours) of *S. aureus* ATCC 25923
- Inoculating wire, Bunsen burner, or disposable sterile inoculating loop

2.7.3.3. Specimen

Overnight growth of test isolate on SBA or any non-selective medium

2.7.3.4. Procedure

- Label a blood agar plate
- Using a loop, streak *S. aureus* in a straight line down the center of the blood agar plate
- Streak colonies of the test organism at a 90-degree angle (right angle) to the *Staphylococcus* streak. Do not allow the two lines to touch each other.
  Note: Multiple isolates can be tested on a single plate if they are 3 to 4 mm apart.
- Incubate plate at 35°C in 5 – 10% CO_2 for 18-24 hours.
- The next day, observe for characteristic arrowhead hemolysis.
- Record results.

2.7.3.5. Interpretation

a. Positive: Formation of a distinct arrowhead hemolysis at the intersection of *S. aureus* and test organism streaks.
b. Negative: Absence of arrowhead hemolysis

2.7.4. Bacitracin Disk Test

2.7.4.1. Principle

The bacitracin disk susceptibility test is commonly used to presumptively identify group A beta-hemolytic streptococci. Group A *Streptococcus* is differentiated from other groups of beta-hemolytic streptococci by the formation of a zone of inhibition around a disk impregnated with 0.04 unit of bacitracin.

2.7.4.2. Materials

a. Bacitracin disks, 0.04 unit
b. Sheep blood agar (SBA) plates
c. Sterile inoculating loop, forceps

2.7.4.3. Specimen

Overnight growth of test isolate on SBA. Test may be performed on isolates from primary culture plates. Organisms used for testing must be isolated and not mixed with normal flora.

2.7.4.4. Procedure

a. Divide a SBA plate into two halves and label appropriately.
b. Using a loop, touch a colony of the test organism. Inoculate half the surface of SBA.
   Note: Two organisms can be tested on a single plate if the plate is divided into two halves.
c. Streak the plate in at least two directions to obtain confluent growth.
d. Using sterile forceps, place a bacitracin disk in the center of each inoculated area.
e. Press disk gently with the sterile forceps so that the disk adheres firmly to the agar surface.
f. Incubate plate at 35°C in 5 – 10% CO_2 for 18-24 hours.
g. The next day, observe for zone of inhibition around the disk.
h. Record results.

2.7.4.5. Interpretation

a. Susceptible: Any zone of inhibition
b. Negative: No zone of inhibition

2.7.5. Triple sugar Iron (TSI) & Hydrogen sulfide production (H2S):

Looks at fermentation of glucose, lactose, and sucrose and checks if hydrogen sulfide and gas is produced in the process. Basically a pH indicator will change the color of the media in response to fermentation. The color change that occurs in the tube will indicate what sugar or sugars were fermented. The presence of a black color indicates that H2S was produced. In this media, H2S reacts with the ferrous sulfate in the media to make ferrous sulfide, which is black in colour.To inoculate, use a needle to stab agar and then use a loop to streak the top slanted region.

SLANT COLOR:	Interpretation
RED	does not ferment either lactose or sucrose
YELLOW	Ferments lactose and/or sucrose
BUTT COLOR/CONDITION	**Interpretation**
RED	no fermentation of glucose
YELLOW	some fermentation of glucose has occurred, acid has been produced
GAS FORMED	Seen as cracks in the agar, bubbles, or the entire slant may be pushed out of the tube.
BLACK	H_2S has been produced

2.7.6. Motility test

To perform this test, the bacterial sample is inoculated into motility media using inoculating straight wire. Simply stab the media in as straight a line as possible and withdraw the needle very carefully to avoid destroying the straight line.After incubating the sample for 24-48 hours, observations can be made. Check to see if the bacteria have migrated away from the original line of inoculation. If migration away from the line of inoculation is evident then you can conclude that the test organism is motile (positive test). Lack of migration away from the line of inoculation indicates a lack of motility (negative test result).

2.7.7. Oxidase test/Cytochrome oxidase test

The oxidase test is used to detect bacteria that produce the enzyme cytochrome oxidase which catalyze oxidation of reduced cytochrome by oxygen molecule .It assist in the identification of Pseudomonas and Neisseria which are oxidase positive.

2.7.7.1. Principle

A piece of filer paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. When the organism is oxidase-producing, the Phenylenediamine in the reagent will be oxidized to a deep purple colour. Occasionally the test is performed by flooding the culture plate with oxidase reagent .It can be useful when attempting to

isolate N.gonorrhoeae colonies from mixed cultures in the absence of a selective medium. The oxidase positive colonies must be removed and subcultured within 30 seconds of flooding the plate

2.7.7.2. Method using an oxidase regent strip

Moisten the strip with a drop of sterile water.

Using a piece of stick or glass rod (not an oxidized wire loop) remove a colony of the test organism and rub it on the strip.

Look for a red-purple colour within 20 seconds.

NB: Red-purple colour…….positive oxidase test.

Controls

Positive oxidase control: Pseudomonas aeruginosa

Negative oxidase control: Escherichia coli.

2.7.8. Urease test using Christensen's (modified) urea broth

This test is used to detect the enzyme urease, which breaks down urea into ammonia.Testing for urease enzyme activity is important in differentiating enterobacteria. Proteus strains are strong urease producers.

2.7.8.1. Principle

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease-producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.

2.7.8.2. Method

Inoculate heavily the test organism in a bijou bottle containing 3 ml sterile Christensen‟s modified urea broth.Incubate at 35-370C for 3-12 h (preferably in a water bath for a quicker result).Look for a pink colour in the medium.

Results

Pink colour……..Positive urease test

No pink colour…… Negative urease test

2.7.8.3. Indole test

The test detects the ability of an organism to produce indole from Tryptophan. Testing for indole production is important in the identification of enterobacteria.

2.7.8.4. Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac‟s or Ehrlich‟s reagent which contains 4(p)-dimethylamino-benzaldehyde. This reacts with the indole to produce a red coloured compound.

2.7.8.5. Material required

- Kovac‟s or Ehrlich‟s reagent
- Bijou bottle/test tube

2.7.8.6. Method

- Indole test using tryptone water and kovac‟s reagent.
- Inoculate the test organism in a bijou bottle containing 3 ml of sterile tryptone water.
- Incubate at 35 – 37oC for up to 48 hr
- Test for indole by adding 0.5ml of Kovac‟s reagent and shake gently.
- Examine for a red color in the surface layer with in 10 minutes.

Results

Red surface layer……Positive indole test

No red surface layer……. Negative indole test

2.7.9. Citrate utilization using Simmon's citrate agar

The test detects the ability of an organism to use citrate as its only source of carbon. This test is one of several techniques used occasionally to assist in the identification of enteric bacteria.

2.7.9.1. Principle

Some bacteria can obtain energy in a manner other than by the fermentation of carbohydrate by using citrate as source of carbon. The utilization of citrate by a test bacterium is detected in citrate medium by the production of alkaline by-products. The medium includes sodium citrate as the sole source of carbon and ammonium phosphate as the sole source of nitrogen.

Bacteria that can use citrate can also extract nitrogen from the ammonium salt, with the production of ammonia (NH+), leading to alkalinization of the medium.

In the presence of the indicator Bromothymol blue the medium will be converted from green (at pH 6.0) to blue (at a pH above 7.6).

2.7.9.2. Material required

- Simmon"s citrate medium/agar
- Inoculating loop

2.7.9.3. Method

- Prepare slopes of the medium in bijou bottles as recommended by the manufacturer (store at 2-8 C)
- Using a sterile straight wire, first streak the slope with a saline suspension of the test organism and then stab the butt.
- Incubate at 35 0C for 48 hours
- Look for a bright blue colour in the medium

2.7.9.4. Results

Bright blue----------Positive citrate test

No change in colour of medium ----------Negative citrate test

2.7.10. Antimicrobial Susceptibility Test:(Kirby Bauer Method)

2.7.10.1. Principle

Kirby Bauer (KB) is a standardized procedure for performing AST by disk diffusion. A standardized inoculum of the bacteria is swabbed onto the surface of a Mueller Hinton agar (MHA) plate. Filter paper disks impregnated with antimicrobial agents are placed on the agar. After overnight incubation, the diameter of the zone of inhibition around each disk is measured. By referring to the standardized tables compiled by CLSI, a qualitative report of susceptible, intermediate or resistant can be obtained.

2.7.20.2. **Materials**

- MHA, Tryptic Soy Broth (TSB), Normal Saline Solution (NSS)
- Antimicrobial disks
- 0.5 McFarland Standard
- Sterile cotton swabs
- .Ruler or caliper

2.7.10.3. Specimen

Pure culture of the organisms from an 18-24 hour agar plate, preferably a nonselective medium like sheep blood agar.

2.7.10.4. Procedure

- Bring agar plates and antibiotic disks to room temperature before use.

Prepare bacterial suspension.

- Select 3 – 5 well-isolated colonies of the same morphologic type from an agar plate culture.
- Touch the top of each colony with a loop and transfer the growth into a tube containing 4 – 5 ml of TSB or NSS.
- Mix well and adjust turbidity with broth or NSS to match 0.5 McFarland standard.

- Within 15 minutes of adjusting turbidity, dip a sterile cotton tipped applicator swab into the inoculum and rotate against the wall of the tube to remove excess inoculum.
- Swab entire surface of the agar plate three times, rotating plate approximately 60o between streaking to ensure even distribution. As a final step, swab the rim of the agar.
- Allow inoculated plate to stand 3 -15 minutes (no longer than 15 minutes) before applying disks.
- Apply antibiotic disks to agar surface using sterile forceps or dispenser. Apply gentle pressure to ensure complete contact of disk with agar.
- Do not relocate a disk once it has made contact with agar surface. Instead, place a new disk in another location on the agar.
- Place no more than 12 disks on 150 mm plate and no more than 5 disks on 100 mm plate.
- plate and incubate within 15 minutes of disk application. Incubate for 16 – 18 hours at 35 ± 2oC in an ambient air incubator
- Reading and Interpretation

Read plates only if lawn of growth is confluent. If individual colonies are apparent, the inoculum was too light and the test must be repeated.

- Hold inverted plate a few inches above a black nonreflecting surface. Illuminate plate with reflected light.
- Use ruler held on the back of the plate to measure the diameter of zone of inhibition.
- Measure the diameters of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the inhibited growth.
- Measure the zones to the nearest millimeter (mm).
- Refer to CLSI M100 tables for interpretation of zone sizes.
- Reporting

Report the organisms as either Susceptible (S), Intermediate (I), or Resistant (R) to the antimicobial agents that have been tested.

2.3. Preparation of the chromagar media

Disperse slowly 47.7 g of powder base in 1L of purified water. Stir until agar is well thickened. Heat and bring to boiling (100°C) while swirling or stirring regularly. Do not heat to more than 100°c. Do not autoclave at 121°C. Cool in a water bath to 45-50°C, swirling or stirring gently. Pour medium into sterile Petri dishes and Let it solidify and dry. Store in the dark before use. Prepared media plates can be kept for one day at room temperature so we store the sample in refrigerator by sub culture on SDA to process sample in mass.

Inoculation

Related samples can be processed by direct streaking on the plate. Since our sample plate is stored in the refrigerator, we were allowed to warm to room temperature before inoculation. Then streak sample onto chromagar plate. Incubate in aerobic conditions at 30-37°C for 48 hours

Interpretation

Microorganism Typical colony appearance C.albicans → green, C.tropicalis → metallic blue ,C.krusei → pink

Annex VII: Declaration

Title of project:B**acterial vaginosis and vulovvaginal candidiasis among women complaining genital tract infection at St. Paul's Hospital Millennium Medical College Gynecology and Family Guidance Association Ethiopia Model Clinic, Addis Ababa, Ethiopia,2016**.

I, the undersigned, declare that this MSc research project is my original work. It has not been presented for a degree in any other University. False statements could be cause for invalidating this research project and may lead to other administrative or legal action.

Principal investigator:

Name: Yeshiwork Abebaw (BSc) Signature: _____ Date: _____

Advisor (s):

Name: Adane Bitew (MSc, PhD) Signature: _____ Date_____

Co-advisor

Ms. Amete Mihiret (Bsc,MSc) Signature: _____ Date: _____

Mr.Anteneh Tessema (Bsc,MSc) Signature: _____ Date: _____

Dr.Dilayehu Bekel(gynecologists) Signature: _____ Date: _____

Curriculum Vitae

Amete Mihret Teshale, MSc Microbiologist

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EDUCATION 2007/2010 Hawassa University,Hawassa,Ethiopia
BSc/Medical Laboratory Technology

POST GRADUATE TRAINING 2012/2014 Addis Ababa University, Addis Ababa, Ethiopia MSc/Diagnostic and Public Health Microbiology

LICENSES

10/03/2009 Federal Democratic Republic of Ethiopia Junior Medical Laboratory Technologist

CERTIFICATIONS

08/15-16/2016 Ethiopian Public Health Institute &
Ethiopian Public Health Laboratory Association
Laboratory bio-safety and bio-security

02/1-5/2016 Ethiopian Public Health Institute,
Center for Disease Control and prevention and
American Society for Microbiology
Microbiology Training of Trainers Workshop-Part I

05/23-31/2016 Ethiopian Public Health Institute,
Center for Disease Control and prevention
and American Society for Microbiology
Microbiology Training of Trainers Workshop-Part II

02/2015 Ethiopian Public Health Association Presentation of scientific paper at the 26th annual conference of EPHA

10/12-14/2015 Johns Hopkins University Center for Communication Programs (JHU.CCP) Ethiopia Qualitative Research Methods and Analysis

09/18-22/2014 Ethiopian Public Health Institute & Ethiopian Medical Laboratory Association Laboratory Quality Management System Training

11/15-20/2014 Ethiopian Public Health Laboratory Association Basic Diagnostic Bacteriology training

09/24-26/2014 Ethiopian Public Health Institute, Cornell University"s Mann Library & Research4life Programs Electronic Library Resources Training Workshop on TEEAL and AGORA for Ethiopia

11/17-20/2014 Norwegian Institute of Public Health (NIPH), Oslo, Norway & Center for Disease Control and Prevention (CDC), Atlanta, Georgia, U.S.A Laboratory diagnosis of bacterial meningitis by use of real-time PCR

06/24-07/03/2014 Ethiopian Health and Nutrition Research Institute & Center for Disease Control and prevention CD4, Chemistry and Hematology laboratory diagnosis

07/04-13/2014 Ethiopian Health and Nutrition Research Institute & Center for Disease Control and prevention Rapid HIV Testing and AFB Smear Microscopy

PROFESSIONAL APPOINTMENTS

01/2011-09/2014 Dilla University Referral Hospital Medical Laboratory Technologist
04/2014-08/2014 Girum General and Specialty Hospital Medical Laboratory Technologist
07/2014 - Ethiopian Public Health Institute Associate Researcher, Microbiology

MEDICAL AND SCIENTIFIC SOCIETIES

04/2013 Ethiopian Public Health Association

07/2014 Ethiopian Medical Laboratory Association

09/2014 American Society for Microbiology

08/2016 Ethiopian Public Health Laboratory Association

PUBLICATIONS

1. Walelgn Dessie, Gebru Mulugeta, Surafel Fentaw, Amete Mihret, Mulu Hassen, Engida Abebe. Patterns of Bacterial Pathogens and Their Susceptibility Isolated from Surgical Site Infections at Selected Referral Hospitals, Addis Ababa, Ethiopia. Int J Microbiol: 2016.

2. amete Mihret Teshale, 2kassu Desta, 2gebru Mulugeta, 1nega Asamene, 3malede Birara, 1 surafel Fentaw, 1 rajiha Abubeker, 1meseret Asefa, 1degefu Beyene, 2mulu Hassen, 2walelign Dessie, 1elias Seyoum, Prevalence And Antibiotics Susceptibility Pattern Of Common Bacterial Uropathogens Isolated From Pregnant Women Attending Antenatal Care Clinic At St. Paul Hospital Millennium Medical College And Selam Health Center, Addis Ababa, Ethiopia. International Journal OF Medical Science and Clinical Invention.Volume 2, September 2015 3. 3.Surafel Fentaw,Tamirat Tadesse,Teklil Beza,Rajiha Abubeker, Negga Asamene,Meseret Assefa, Amete Mihret, Degefu Beyene.Ciprofloxacin resistance among Neisseria gonorrhea isolates obtained from genital samples referred to Ethiopian Public Health Institute. Ethiopian Journal of Laboratory Medicine,Volume 2,December 2015.

4. Dinkineh Abebe,Amha Kebede,Sissay Menkir,Rajiha Abubeker,Amete Mihret,Surafel Fentaw,Degefu Beyene,Meseret Assefa,Nega Asamene. Prevalence of Antibiotic Resistant

Salmonella Species and selected Intestinal Protozoan Parasites in Harar Hiwot Fana Hospital, Ethiopia. American Journal of Biochemistry and Molecular Biology, 2014

PERSONAL DATE OF BIRTH:

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MARITAL STATUS Single

Amete Mihret Teshale

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EDUCATIONAL BACKGROUND

Elementary school Amanuel NO.2 Primary and Secondary School (1997-2003)

Secondary school Amanuel Senior Secondary School (2004-2005) Preparatory school Dembecha Senior Secondary School (Grade 11), (2006) Gojjam Ber Senior Secondary School (Grade 12), (2007)

Higher education

Hawassa University (2008-2010)

Addis Ababa University (2012-2014)

Amete Mihret Teshale Associate Researcher I Ethiopian Public Health Institute Addis Ababa Mobile:+251913773888 Email:amete2004@gmail.com

COMPUTER SKILLS

 Operating systems Microsoft windows Programming language Data base servers Application software

COMMUNITY SERVICES

 Community based training program (CBTP) and Team Training Programs (TTP) at different rural areas during three year education of my medical laboratory technology department with collaboration with other departments in the college of health science, Hawassa University.

 Mapping of lymphatic Filariasis and Podoconiosis program in Ethiopia prepared by Federal Ministry of Health and Ethiopian Health and Nutrition Research institute I have participated in different rural part of the country.

HOBBIES

 Attending religious programmes

 Listening spiritual song and preaches

 Sharing of different ideas and information with my friends

 Reading different books and magazines

 Appreciating nature

REFERENCES

1. Aster Tsegaye (Assistant professor of Immunology,Ph.D,), tsegayeaster@yahoo.com, mobile,0911696085.

2. Adane Bitew (Professor of Mycology, Assiastantprofessor of Microbiology,Ph.D,); adane-bitew@yahoo.com , mobile;0911039162.

3. Kassu Desta (Assistance professor,Medical microbiologist,Ph.D. candidate,Msc,Bsc); kassudesta2020@gmail.com. 4. TedilaMindaye (Ph.D candidate, Msc, CLS);Chair, Department of Medical Laboratory Science: tedlamin@yahoo.com,mobile;0911634324. 5. Surafel Fentaw (Researcher,BSc,MSc,M.Phil):sura4f@gmail.com

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1. **EDUCATINAL BAKGROUND**

Year University/School Awards Received Sept 2007-July 2009

Addis Ababa University M.Sc. in Statistics Sept 2002- Aug 2006

Addis Ababa University B.Sc. in Statistics

2. **Trainings and Computer Skills**

 Longitudinal Data Managment and Analysis, using STATA Software (topic including GEE and Mixed Models), Organized by EPHA and CDC, 2011, Addis Ababa, Ethiopia. Statistical Software Training in SPSS, SAS, STATA (2), GIS/GPS, R, CSPRO, MINITAB, HRS, Inter VA, Epi Info, Epi Data, S-PLUS

 Higher Diploma Programme (HDP), Basic Instructors Teaching Skill

 Research Proposal Development, Arba Minch University, 2009 & 2010

 Design of Experiments using R Statistical Software for Practitioners and Statisticians, Jimma University (Ethiopia), University of Gent and University of Leuven (Beligium), 2013

 Microsoft office (word, Excel, Power point, Access, …), DBMS, Internet Basic & Trouble Shooting and Others

3. **EXPERIENCE**

 Data Analyst, Ethiopian Public Health Institute, 09 April 2016 to present, temporarily for one year.

 Lecturer, Addis Ababa University, Department of Statistics 2012 to present (leave without pay).

 Project Manager, Key Indicator (Labor Market) Formulation and Development (KIFaD) project, Ethiopian Statistical Association (ESA) collaboration with Central Statistical Agency (CSA) and Addis Ababa Bureau of Labor and Social Affairs (BoLSA) project, from 01 March to 30 August, 2016.

 Lecturer (2009-12), Assistant Graduate I & II (2006-09,) Arba Minch University, Department of Statistics

 Data Manager, 01 Sept 2009 to 30 Nov 2010 and Data Analyst (Statistician), Researcher, Member and Secretary of Principal Investigators, 01 Dec 2010 to 17 July 2012, Demographic and Health Development Program: Demographic Surveillance System and HIV/ADIS Mortality projects, Arba Minch University and EPHA and CDC (2009 - 2012)

 Supervisor, pilot survey project in Tigray region on Mobile Technology data collection, Ethiopian Statistical Association collaboration with CSA and ECA, 01 March -30 April 2015 Monitoring and Evaluation Member, pilot survey project in Tigray region on Mobile Technology data collection, Ethiopian Statistical Association collaboration with CSA and ECA, 15 Sept 2014 up to 30 April 2016.

 Data Analysis (Statistician): for different consultant and research firms/plc

 Research Coordinator, Department of Statistics, Arba Minch University, 2009-2012

 Trainer: SPSS (5 times), STATA (8), SAS (4), R(4), MINITAB(3), Epi Info (3); to staff members of Arba Minch University (sept 2009 - 2012), Ethiopian Statistical Association from Sept 2012 to present, Bahir Dar University, Ethiopian Civil Service University, Unity University, for different governmental and nongovernmental organizations (a lot of certificates if you need I can list and bring all upon request) to list some.

 Thought different applied and theoretical statistical courses in Addis ababa University (AAU) and Arba Minch University (AMU): Some of them:

 different Computing Courses [Statistical Computing I (SPSS & MINITAB) and Statistical Computing II (R & SAS)]

 Statistical Consultancy and Research Methods, Research Methods in Statistics, Senior Research Project I & II

 Design and Analysis of Experiments Regression Analysis Fundamentals of Multivariate Analysis

 Epidimology and Biostatistics

 Statistical Theory II (Statistical Inference),

 Social and Economic Statistics, Social Statistics (Health, Education, Labour Statistics) Demography

 Introduction to Statistical Methods I & II

 Introduction to Statistics for various departments

 Statistics for Biologists (Fundamentals of Biostatistics) Introduction to Statistics and Probability for different engineering departments

 Biological Data Analysis (AMU, for postgraduate students in Biology) Categorical Data Analysis

 Biostatistics and Epidemiology

 Biostatistics (for postgraduate students in St. Paul's Hospital Millennium Medical College, Department of Public Health (Part time Instructor))

4. RESEARCH (PUPLICATIONS)

 Anteneh Tessema Yalew and M. K. Sharma (2010). Construction and Analyses of Complete Diallel Cross in PBIB Designs, Germany. (Monograph, ISBN: 978-3-639-26483-8)

 pilot survey project on Mobile Technology based data collection, July 2016

 Thematic Research: Factors Affecting Access and Utilization of Preventive Reproductive Health Services of Youth University Female Students in Ethiopia: A Multilevel Regression Analysis. (PI: Dejen Tesfaw, I am member of the project and other 5 members, we win 2.4 million birr for four years. we already accomplished the first year.) (on going)

 Key Indicator (Labor Market) Formulation and Development (KIFaD) research project (ongoing)

5. **RESEARCH INTEREST**

 Model development and application of Statistical modelling in Medical and Health Sciences, Design and Analysis of Health and Agricultural Experiments (Statistical Genetics, Biological Sciences)

6. Professional Memberships

 Member (Sept 2006) of Executive Committe (from June 2012 up to now), Ethiopian Statistical Association (ESA)

 Board member (August 2012 up to now), Ethiopian Biometrics Society (EBS), Member International Biometrics Society and Secretary of Ethiopian Group (Sept 2015 till now). Regular Member, International Statistical Institute

 Member, Ethiopian Public Health Association (EPHA) , Sept. 2012 - Present

 Member, Arba Minch University Instractors Association under Ethiopian Teachers Association (ETA), December 2009 - July 2012. 7. Attended Conferences, Symposiums, Seminars and Workshop/Panel Discussion (if you need I will list all upon request) 8. Research Paper Presentations

 Application of Design and Analysis Experiments in Agricultural Sciences, Haramaya University, International Conference, 25-27 March, 2016

 Application of Spatiotemporal analysis in public health, 27 June, 2015

 Application of PBIB Designs in Agriculture, Ethiopian Biometrics Society : Oct. 2013

 Factors Affecting the Use of Contraceptives and its Linkage with Population Growth: A Comparative Study between Arba Minch Town and Uba Debre Tsehay District, AMU, 2012

 Longitudinal data analysis on VA in Arba Minch Zuria District, AMU, 2011

 Construction And Analyses of Complete Diallel Cross in PBIB Designs, AMU, 2010 9. Language Proficiency

 Amharic: Native language.

 English: Excellent in listening, spoken, reading and written.

 Ge'ez: Very good in reading and listing, good in writing and speaking (Diploma in Ge'ez) 10. References Alemayehu Worku (Ph. D), Professor in Addis Ababa University, School of Public Health, Department of Biostatistics and Epidemiology, Addis Ababa, Ethiopia. alemayehuwy@yahoo.com

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Birhanu Teshome (PhD), Assistant Professor in Addis Ababa University, Department of Statistics and Graduate Program Coordinator of the Department, Addis Ababa, Ethiopia.

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