ADDIS ABABA UNIVERSTY

COLLEGE OF HEALTH SCIENCE

SCOOHL OF ALLIED HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCENCES

BACTERIAL BLOODSTREAM INFECTIONS AND THEIR ANTIMICROBIAL SUSCEPTIBLITY PATTERN AMONG HIV/AIDS PATIENTS AT FELEGE HIWOT REFERRAL HOSPITAL, BAHIR DAR, AMAHARA REGIONAL STATE, NORTH WEST ETHIOPIA.

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Bacterial blood stream infections and their antimicrobial susceptibility pattern among HIV/AIDS patients at Felege Hiwot referral hospital, Bahir Dar, Amahara Regional state, Northwest Ethiopia.

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A thesis submitted to Addis Ababa University, School of Allied Health Sciences, College of Health Sciences, Department of Medical Laboratory Science for partial fulfillment of Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty).

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Chairman of Department or Graduate program Committee

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Abbreviations

AAU	Addis Ababa University
AIDS	Acquired Immune Deficiency Syndrome
AOR	Adjusted Odds Ratio
ART	Anti-retroviral Therapy
AST	Antimicrobial Susceptible Test
ATCC	American Type Culture Collection
BAP	Blood Agar Plate
BHI	Brain Heart Infusion
BSD	Broad Spectrum Drug
BSI	Blood Stream Infection
CA	Community Associated
CAP	Chocolate Agar Plate
CD	Cluster of Differentiation
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standard Institute
CONS	Coagulase Negative Staphylococcus
CVC	Central Venous Catheter
DRERC	Departmental Research and Ethics Review Committee
ECDC	European Centre for Disease Prevention and Control
FHRH	Felege Hiwot Referral Hospital
HAART	Highly Active Anti-Retroviral Therapy
HCA	Health Care Associated
HIV	Human Immune Deficiency Virus

HTM	Haemophilus Test Medium
LDC	Lysine Decarboxylase
MAC	Mac Conkey Agar plate
MDR	Multidrug Resistance
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MRCONS	Methicillin Resistant Coagulase Negative *Staphylococcus*
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSA	Mannitol Salt Agar
SOPs	Standard Operational Procedures
SPSS	Statistical Packages for Social Sciences.
WHO	World Health Organization

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Abstract

Background: Bacterial bloodstream infections are important opportunistic infections which cause high morbidity and mortality among HIV infected individuals. Bacterial bloodstream infections among HIV/AIDS Patients in Ethiopia is poorly known. Therefore, determining the etiological agents of bacterial bloodstream infections and their *in vitro* antimicrobial susceptibility pattern among HIV infected patients in Ethiopia is one of the highest priorities.

Objective: The aimed of this study was to determine the extent of bacterial bloodstream infections and their *in vitro* antimicrobial susceptibility pattern among HIV/AIDS patients attending ART-clinic from July 15, 2016 to December 15, 2016 G.C at Felege Hiwot Referral Hospital, Bahir Dar, Amhara National Regional State North West Ethiopia.

Methods and Materials: A hospital based cross sectional study was conduct among 384 HIV/AIDS patients attending ART- clinic at Felege Hiwot Referral Hospital during the study period. Venous blood samples were collected under strict aseptic condition. Collected blood samples were inoculated on blood culture bottles and incubated aerobically at 37^o c for seven days for the presence of visible sign of microbial growth. Sub cultures were done on the BAP, CAP and MAC (Oxoid). Microorganisms were isolated and identified using standard biochemical techniques. Antimicrobial susceptibility tests for each isolated bacteria were determined by disc diffusion method. Quality of the data was assured through standard operational procedures (SOPs). Data was entered, checked for completeness and analyzed using SPSS version 20.

Result: This study confirmed that among HIV/AIDS patients, 123 (32%) were infected with bacteremia. Prevalence of blood stream infection was higher in 1-14 years of age group (53.8%) than other age group. Higher percentage of positive blood culture isolates among HIV/AIDS patients having CD4 count of <200 cells/μL (76.5%) and used of intravenous catheter (68.6%).The major isolates were *Staphylococcus aureus* 49.3% (38/77), *Coagulase Negative Staphylococcus* 35% (n=27/77) and *Klebsiella pneumoniae* 41.3% (19/46). Majority of isolates 50(40.7%) were resistant to five and above antimicrobial agents.

Conclusion: Prevalence of bacterial blood stream infections (32%) among HIV/AIDS patients was higher in this study. Positive blood culture isolates was higher among hospitalized HIV/AIDS patients with intravenous catheter and low $CD4^+$ lymphocyte count. The most leading pathogens were *S. aureus, CONS and K. pneumonia* and majority of isolates were multidrug resistant bacteria.

***Key words**: Bacteremia, HIV, Antimicrobial Susceptibility patter*.

1. Introduction

1.1.Background

HIV/AIDS has been identified as one of the major risk factor for bloodstream infection. According to previous data, bacteremia is 10^{th} leading cause of death in individuals aged 45 years older among HIV/AIDS infected patients in USA [1].

There is worldwide increase in the incidence of bacterial bloodstream infection [2]. Several factors exposed patients with HIV infection to bacterial infection such as phagocytic cell dysfunction, abnormality in humoral and skin and cell mediated immunity and skin and mucous membrane defects [3]. Use of intravenous catheter, low $CD4^+$ lymphocyte count and intravenous drug use [4, 5] are the most frequently indicated risk factors for bacterial bloodstream infections among a patients with HIV infection [3].

Bacterial bloodstream infections constitute a significant public-health problem and present an important cause of morbidity and mortality in HIV/AIDS infected patients. Study which was conducted in Genoa, Italy, showed that *non typhoid salmonellae*, *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *coagulase negative staphylococci* are the most important pathogens which caused blood stream infection and Intravenous catheter associated infection, skin-soft tissue infection and endocarditis are associated with Gram-positive bacteremia. In other hand, among the Gram-negative, *non-typhoid Salmonella* have been previously correlated to sepsis among hospitalized HIV/AIDS patient [6].

According to a review of population based studies on the incidence of bloodstream infection, the most common etiological agents of bloodstream infection are *Escherichia coli, Staphylococcus aureus and Streptococcus Pneumonia* which occur at approximated rates of 35, 25 and 10 per 100,000 population, respectively [7].

The emergence bacterial drug resistance are risk of worse clinical out come and consume more health care resources [8]. HIV/AIDS infected patients obviously co-infected with high level of antibiotic resistant pathogenic bacteria including Methicillin Resistant *Staphylococcus aureus* (MRSA), B-lactamase producing *Entrobacteriacae* and the MDR *Psedomonas aeruginosa* [9].

Several researches from different corners of the world showed increased prevalence of bacterial bloodstream infections [2, 3 & 7]. However, the incidence of bacterial bloodstream infections and their antimicrobial susceptibility profile and predisposing risk factors among HIV/AIDS patients in Ethiopia are poorly known. Therefore, determining the magnitude and distribution of bacterial origin of bloodstream infections among Ethiopian HIV/AIDS patients will help in the development of strategies for early diagnosis and proper treatment of suspected patients. Expected outcomes of this project will be incorporated into regional or national guidelines.

1.2. Statement of the Problem

Bloodstream infections constitute a significant public-health problem and present an important cause of morbidity and mortality in HIV/AIDS infected patients. A survey among HIV 1 patients in Malawi showed that 30% had bloodstream infection and mortality was higher among HIV infected patients with BSIs than without patients without BSI. [10].

Previous studies showed as *CONS* have been implicated as important causes of nosocomial bloodstream infections in patients that are immunocompromised and *S. aureus* is the second most common pathogen that is responsible for causing bloodstream infection among HIV infected patients [11]. Another study in Thailand revealed that Gram negative bacteria (39.6%) are among the common causative pathogen in bloodstream infections among HIV/AIDS infected patients [12].

Bacterial bloodstream infections among HIV/AIDs infected patient mostly caused by multidrug resistant bacteria (64.7%) including *Methicillin Resistant Staphylococcus aureus* (MRSA) and β-lactamase producing *Gram negative bacilli* [9].

The study which was conducted in Gondar, Ethiopia, showed that 31% bacterial blood stream infections and among bacterial isolates, (80.6%) was multidrug resistant to five and above antimicrobial agent. *Staphylococcus aureus* and *Coagulase negative staphylococcus* (CONS) were the most leading multidrug resistant pathogen among HIV/AIDS patients [13].

Another study which was conducted in Addis Ababa, Ethiopia, among forty HIV positive patients, 10% (4/40) were blood culture positive [14]

Since limited information is available in Ethiopia regarding prevalence of Bacteremia, its species distribution, predisposal factors and their antimicrobial susceptibility pattern, this study is aimed at investigating various parameters of bloodstream infections.

1.3.Significance of the study

Bacterial are the major bloodstream infections which are the most important causes of morbidity and mortality in HIV/AIDS infected patients. Due to frequent administration of antibacterial agents in the whole course of HIV/AIDS patient management, the emergence of antibiotic resistance strain is now in alarming rate.

Therefore, this study was conducted to identify bacterial pathogens implicated in causing blood stream infection in Ethiopian HIV/AIDS patients and their antimicrobial susceptibility profiles will provide relevant information on the extent of the disease. It will help to identify infection control mechanisms and selection of appropriate antibiotics for empiric treatment.

The study outcome will also help the clinicians and policy makers to create awareness on how frequent bacterial pathogens are associated with HIV/AIDS patients and serve as a baseline information for further studies.

2. Literature review

Bloodstream infections are now considered as a major cause of illness of HIV infected individuals. The most important pathogens are *non-typhoid salmonella, Escherichia coli, staphylococci aureus coagulase negative staphylococci,* and Pathogens associated with intravenous catheter associated bacterial infection, skin soft tissue infection and endocarditis are associated with Gram positive bacteremia [15] and among HIV infected patients children are at high risk for blood stream bacterial infections. The study showed that in U.S (58%) were *streptococcus pneumonia* among HIV infected children [16].

The study by Furuno *et al.,* showed that among 131of *Staphylococci aureus* isolates, 88(66%) were methicillin resistance *Staphylococci aureus*, of which 47 (54%) were community associated methicillin resistant *Staphylococci aureus* at bacteremia and endocarditis among HIV patients in USA [17].

The study which was conducted in Cambodia, Thailand and Vietnam on the prevalence and the risk factor for blood stream infections on 2009 HIV infected out patients. 58 (2.9%) were blood culture positive, out of this Mycobacterium tuberculosis accounted for 31 (54%), followed by Fungi 13 (22%) and bacteria 9 (16%) [18].

The study which was conducted in Germany, showed that, 20% and 30% of community-acquired bacterial BSIs among HIV infected hospitalized patients in adults and children respectively. HIV positive patients are particularly at risk to develop non-typhoid salmonellae (NTS) bacteremia than HIV negative individuals. Based on this study bacterial BSIs in HIV positive patients more prevalent than HIV negative patients [19].

The study which was conducted in Capt Town, South Africa, pneumonia was most common cause of bacterial blood stream infection (67%). Among Gram positive the most common cause of bacteremia were *Streptococci pneumoniae* and *Staphylococci aureus* and *Klebsiella pneumoniae* was the most common gram negative organism. *Klebsiella pneumoniae* was resistant to many first and second line antibiotics and were all considered nosocomial. All *Staphylococci aureus* isolates were methicillin resistant, some of which were community-acquired among HIV infected hospitalized children patients [20].

According to study which was conducted in Nairobi, Kenya, showed that blood stream infections were caused by gram positive bacteria (43%), fungi (20%), gram-negative bacteria (15%), mycobacteria (15%), and mixed flora (7%) among HIV infected adults admitted to a Hospital [21].

The study which conducted in Kampala, Uganda, showed that (58%) were Gram negative and (26.3%) were *Staphylococci aureus* and (13.2%) were *Streptococci pneumonia* among Gram positive bacterial isolates was caused bacterial blood stream infections in HIV 1 infected children [22].

Another study on bacterial bloodstream infections in HIV infected adults attending Lagos Teaching Hospital in Nigeria, was conducted by Adeyemi *et al*. revealed that, (58%) were *coagulase-negative staphylococci* while, (23%) were Gram-negative *non-typhoidal Salmonellae* are the most common etiological agents of bacteremia among HIV-infected adults and this study also further demonstrated that isolates of bacterial pathogen were sensitive to vancomycin, cefotaxime, cefuroxime, and other new-generation antibiotic [23].

The study which was conducted in Nigeria, the prevalence and antibiotic resistance pattern of blood culture was performed on bacterial isolated obtained from human immune-deficiency virus (HIV) patients under HAART treatment. The prevalence of blood stream infection in HIV infected patients on HAART was found (22.9%) and their having the CD_4^+ T-cell counts was <300 cells/ul. Total number of 24 isolates were recovered with different species proportion: *Escherichia coli* being the most prevalent bacterial isolates 12 (50%), followed by *Staphylococcus aureus,* 8 (33.3%) and *Streptococcus pneumonia*, 4(16.7%). Among antibiotic susceptibility test revealed a high level of resistant was seen in co-trimoxazol (87.5%), followed by ciprofloxacin (83.3%), ampicillin (79.2%), levofloxacin (62.5%) while ceftazidine, ceftriaxone and gentamycin (54.2% each) showed the low level resistant [24].

A cross sectional study done by Gebremedhn et al., on prevalence and risk factor of methicillin resistance *Staphylococci aureus* among HIV patients in Mekelle, Ethiopia Out of 249 study participants, 81(32.5%) were *S. aureus* isolates, of which 6 (2.4%) were MRSA and among MRSA isolates were resistant to ciproflxacin and trimethoprim-sulphamethoxazole (16.7 %), clindamycin (33.3 %) and erythromycin (50 %) [25].

A study done by Alebachew *et al*., focused on the etiologic agents of bacterial sepsis and their antibiotic susceptibility patterns among patients living with Human Immunodeficiency Virus at Gondar, Ethiopia. The major isolates were 13 (13%) *Staphylococcus aureus* of which 5(38.5%) were MRSA, 8 (8%) *Coagulase negative staphylococci,* and 3 (3%) *Viridans Streptococci*. Majority of the isolates, 25 (80.6%), were multidrug resistant to two or more antimicrobial agents [13].

Scanty information is available in Ethiopia regarding the extent of bacterial blood stream infections. The current study will give enough information about prevalence of bacterial bloodstream infection, predisposal factors associated to bacterial infection and their antibiotic resistance pattern. Therefore, it will be used as a reference to other secondary studies and it play a significant value to establish appropriate treatment strategies and reduces drug resistant opportunistic infections in Ethiopian HIV/AIDS patients.

3. Objectives

3.1. General objective

To assess the prevalence of bacterial blood stream infections and their antimicrobial susceptibility pattern among HIV/AIDS patients in Felege Hiwot Referral Hospital ART clinic from Juley 15, 2016 to December 15, 2016 G.C.

3.2. Specific objectives

- To determine the distribution of bacteria implicated in blood stream infections in HIV/AIDS patients.
- To determine the major predisposing risk factors associated with blood stream infections.
- To evaluate the drug susceptibility profile of bacteria isolated from blood stream infections.

Hypothesis

- Distribution of bacterial blood stream infection is similar between HIV/AIDS patients with and without in different predisposing factors.

4. Material and Methods

4.1. Study Area

The study was conducted at Felege Hiowt Referral Hospital which is located in Bahir Dar Town, Northwest Ethiopia. Bahir Dar is 540km away from Addis Ababa, the capital city of Ethiopia. Felege Hiwot Referral Hospital was purposefully selected with the fact that it is one among the biggest tertiary level Referral Hospitals in the Region visited by around 7 million pre annual peoples from the surrounding Zones and nearby Regions both for inpatient and for outpatient treatment. The hospital has different departments including Diagnostic Laboratory and around 400 beds and 9 operating tables and ART-clinic with ART- Laboratory, ART-Pharmacy and around 17,569 HIV patients who ever enrolled in HIV patient care.

4.2. Study Design and period

A hospital based cross- sectional study was conducted to determined bacterial bloodstream infections among HIV/AIDS patients attending at ART-clinic from July15, 2016 to December 15, 2016 G.C.

4.3.Population

4.3.1. Source Population

The source of population was all HIV/AIDS patients who were visited ART-clinic during the study period.

4.3.2. Study Population

The study population was HIV/AIDS patients who suspected of bacterial bloodstream infections during the study period.

4.4. Measurement

4.4.1. Dependent Variable

- Bacterial isolates
- Susceptible and Resistance

4.4.2. Independent Variable

- Socio demographic factors: Age, sex, marital status, educational and occupational status.
- Use of intravenous catheter, Pregnancy, CD4 count and place of acquisition.

4.5. Inclusion and Exclusion

4.5.1. Inclusion

All HIV/AIDS patients who Visited ART-clinic and suspected to having bacterial bloodstream infections were included the study period.

4.5.2. Exclusion criteria

Patients receiving antibiotic treatment and neonates were excluded from the study.

4.6 Operational Definition:

Blood stream infection suspect patient is a patient who fulfils definition of SIRS. SIRS is the presence of 2 or more of the following:

a. Temperature <36^0C (96.8^0F) (hypothermia) or >38^0C (100.4^0F).
b. Heart rate >90 beats/min (tachycardia).
c. Respiratory rate >20 breaths/min (tachypnea).
d. WBC count <4000/mm^3 (leucopenia) or >12,000/mm^3 (leukocytosis) [26].

Blood stream infections were considered as health care associated if signs and symptoms became evident more than 48 hours after admission to the hospital and within 10 days after hospital discharge. BSIs were considered as community associated if signs and symptoms became within 48 hours after admission [27]. Intravascular catheter use were assessed by interview and chart reviews.

Definitions for multidrug resistant (MDR): based on European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), MDR was defined as acquired non susceptibility to at least one agent in three or more antimicrobial categories [28].

4.6. Sample size and Sample Techniques

Since studies on the prevalence of bacterial bloodstream infections in acquired immune deficiency syndrome patient and their *in vitro* susceptibility pattern are very limited in Ethiopia, the proportion of 50 % is used in the estimation of sample size as it represents the prevalence of bacterial pathogen in our setting. Therefore, at 95% confidence interval, 5% margin of error and 50% proportion, the sample size was calculated using the following standard formula:

$$\mathrm{No} = \frac{\left(\frac{Z\alpha}{2}\right) 2\,P\,(1 - P)}{(d)2}$$

$$N = \frac{(1.96)2(0.5)(1 - 0.5)}{(0.05)2}$$

$$N = \frac{3.8416 \text{ x } 0.5\ (0.5)}{0.0025}$$

$$No = \frac{0.96}{0.0025}$$

N=384

Where: n = Sample size

Z $(\alpha/2)^2$ = at 95% confidence interval Z value (α = 0.05) = 1.96

P = Population proportion (50%)

D = Margins of error (5%)

A total of 384 study subjects were included in the study and using a convenient sampling techniques from the study population visiting at ART-clinic.

4.7. Data Collection and Laboratory Methods

A pretested, structured questionnaire was used to collect sociodemographic information, clinical signs, or symptoms by clinician. Information collected including risk factors such as pregnancy status, the most recent CD4 count and hospitalization with and without intravenous catheter were assessed by interview and chart reviews. The pretest of the questionnaire was conducted in 10 patients at Felege Hiwot Referral Hospital and pretest results were not included in the final results of the research. We only used it to validate the questionnaire before conducting the actual data collection.

4.7.1. Sample collection and Handling

Blood for culture were collected under strict aseptic conditions using venipuncture technique. Careful skin cleaning using 2% iodine tincture and 70% alcohol was done before drawing blood in order to prevent contamination. A total of 10 ml of blood for adult and 2-5ml of blood for children were collected and transferred to blood culture bottles each contain 50ml of BHI for adult and 20ml of BHI supplemented for children [29].

4.7.2. Culture and Identification

All culture bottles were incubated aerobically at 37˚C for seven days for the presence of visible microbial growth by observing any one of the following indications: hemolysis, turbidity, gas production and coagulation of the broth. Positive culture bottles were sub-cultured on, Blood Agar Plate (BAP), Chocolate Agar Plate (CAP) and Mac Conkey Agar Plate (Oxoid). Blood Agar Plate and Chocolate Agar Plate were incubated in microaerophilic atmosphere on candle jar whereas Mac Concky Agar Plates were incubated at aerobically at 37^0C for 24-48 hours. All positive blood cultures were identified using their culture characteristics appearance on their respective media and Gram staining reaction. Bacterial isolates were confirmed by the pattern of standard biochemical reactions. Gram negative bacteria were identified by oxidase test, indole production, citrate utilization, motility, urease, oxidase, gas production, hydrogen sulfide production, carbohydrate fermentation, and lysine decarboxylases production tests [29].

Identification of Gram positive bacteria isolates: coagulase, catalase test and mannitol salt agar used for identification of *Staphylococcus species* and bacitracin (0.04 or 0.05 UI) and optochin (5μg) susceptibility tests for *streptococcus species* were done. Optochin disc (5 μg) was used for identification of *S. pneumoniae* (sensitive) from *viridans streptococci* and other alpha *haemolytic streptococcus* (resistant). Interpretation as sensitive if the zone diameter should be at least 10mm and resistant if zone of diameter less than 10mm [29].

Identification of *Haemophilus spices* by using chocolate agar plate and blood agar plate with XV factor. After overnight incubation at 35–37 ºC in a moist carbon dioxide atmosphere, capsulated *Haemophilus species* strains produce mucoid colonies. Blood culture broth which shows no microbial growth within 7 days was reported as culture negative, only after results were sub-cultured on blood agar, Mac Conkey, and chocolate agar [29].

4.7.3. Antimicrobial Susceptibility Test

According to the criteria of the Clinical and Laboratory Standard Institute (CLSI) of disk diffusion method, antimicrobial susceptibility testing was performed for all isolates. Three to five pure bacterial colonies of bacteria from culture media were taken and transferred to a tube containing 5 mL of saline and mixed gently to a homogenous suspension and incubated at 35^0C until the turbidity of the suspension becomes adjusted to a McFarland 0.5 and the excess suspension was removed by gentle rotation of sterile swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar (Oxoid) and for fastidious organisms, antimicrobial sensitivity test was done on MHA with 5% sheep blood. Antimicrobial sensitivity test for *Haemophilus species* by using Haemophilus test medium (HTM) incubate at 37^0 C, 5% co_2 for 16-18 hours were done [30].

The inoculated plates were left at room temperature to dry for 3-5 minutes and a set of 14 antibiotic discs (Oxoid) were placed 24 mm apart from each other on the surface of a Mueller-Hinton plate. Each disk was pressed down to ensure complete contact with the agar surface. The drugs for disc diffusion testing were in the following concentrations: ampicillin (10 μg), amoxicillin-clavulanic acid (30μg), ceftriaxone (30μg), ciprofloxacin (5μg), chloramphenicol (30 μg), cotrimoxazole (25 μg), erythromycin (15 μg), gentamicin (10 μg), oxacillin (1 μg), penicillin (10 IU), Piperacillin (100 μg) , tetracycline (30 μg), Clindamycin (2 μg), Cefoxitin (30 μg) and vancomycin (30 μg). Penicillin, vancomycin, erythromycin, oxacillin, Clindamycin and cefoxitin were tested only for Gram positive bacteria. The plates were then incubated at 35ºC for 24–48 hours. Diameters of the zone of inhibition around each disc were measured to the nearest millimeters using the ruler, and the isolates were classified as sensitive and resistant according to the standardized table supplied by the CLSI [30].

Detection of methicillin resistance *Staphylococcus*: Cefoxitin disc (30 μg) was used for the detection of Methicillin resistance *staphylococci aureus* based on CLSI guideline incubated at 35^0c for 16 to 18 hours for S. aureus (MRSA) and 24 hours for Coagulase Negative Staphylococci (CONS). Interpreted as Methicillin resistance S. aureus (MRSA) if the zone diameter ≤ 21mm and MR-CONS if the zone diameter ≤ 24mm [30].

4.8. Quality control.

Standard reference strains of *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923) and *Pseudomonas aeruginosa* (ATCC-27853) were used as a quality control throughout the study for culture and antimicrobial susceptibility testing.

4.9. Data analysis and Interpretation

Collected quantitative data were coded; double entered in EPI INFO to computer, processed, edited, and analyzed using SPSS version 20 (Statistical Package for social sciences) statistical software for analysis. During analysis of frequencies of the different variables were determined, Cross-tabulations and odd ratio were used to compare frequencies. Descriptive statistics were used to describe the study participants in relation to relevant variables. Chi-square and Logistic regression were employed to compare bacterial blood stream infection with demographic variables and other factors. P-value of <0.05 was considered as statistical significant.

4.10. Ethical Consideration

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. Then a letter informing to Felege Hiwot Referral Hospital, Bahir Dar about the study were written from ethical Committee of department of Medical Laboratory and permission were obtained from Felege Hiwot Referral Hospital to access data from study population. All eligible subjects were informed as their participation was voluntary. The aim of this study was only to collect necessary information which was helpful to assess prevalence of bacterial blood stream infections. All the information obtained from the study subjects were coded to maintain confidentially.

4.11. Result Dissemination

After presenting the results of this research to the School of Medical Laboratory Sciences, AAU, and other concerned bodies, efforts will be made to present the findings of the study report at appropriate seminars and workshops. The final research report will be disseminated as hard copies to selected recipients including School of Medical Laboratory sciences, College of Allied Health Science, AAU, Felege Hiwote Referral Hospital and an attempt will be also done to publish it in scientific journal.

5. Result

5.1. Demographical Characteristics

This study including 384 HIV/AIDS patients who suspected of having bacteremia, 157(41%) were male and 227(59%) were female. The age range of participants were 1-75 year with the mean and SD of 36 ± 11.8. Among total numbers of HIV positive patient participants, 26(6.8%) were 1-14 years of age group, 20(5.3%) were 15-24 years of age group, 252(65.6%) were 25-44 years of age group, 83(21.8%) and 3(0.8%) were 45-64 and >65 years of age respectively. However, blood culture positive isolates was higher in the 1-14 years of age 53.8% (n=14/26) than blood culture isolates from other age group. In the total numbers of blood culture positive in the years of 1-14 age group participant, 38.5% (10/26) were caused by gram negative bacteria. Out of this, 34.6% (9/26) of 1-14 years of age group were blood culture positive caused by *Klebsiella pneumoniae* [Fig.1 & 2]. There was significant association between blood stream infections and age group among HIV positive patients (Adjusted odd ratio [OR] 4.7, P=0.027) [Table 1]. In the present study, were 191(49.7%) married, 276(72.7%) were literates and 236(61.5%) were Employer (government and self-employed) [Table 1].

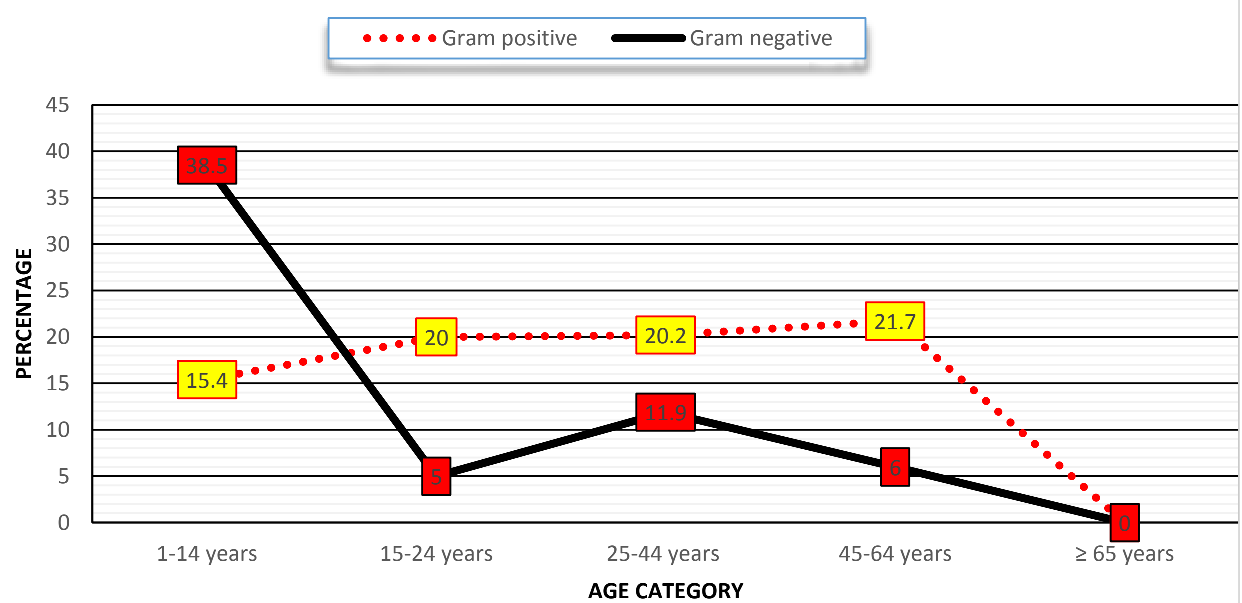

Fig.1: Distribution of Gram positive and Gram negative pathogen associated with age group category among HIV/AIDS patients.

TABLE 1: Demographical Characteristics of Bacterial Bloodstream infection among HIV/AIDS patients in Feleg Hiwote Referral Hospital July, 15 2016 to December 15,2016G.C.

Characteristic	Bacterial status		Total N (%)	P value	OR(95% CI)
	Presence N (%)	Absence N (%)			
Sex					
• Male*a	50(31.8)	107(68)	157(41)		
• Female	73(32.1)	154(67.8)	227(59)	0.949	1(0.66-1.6)
Age					
• 1-14	14(53.8)	12(46.2)	26(6.8)	0.027*	4.7(1.2-18.4)
• 15-24*a	5(25)	15(75)	20(5.3)		
• 25-44	81(32.1)	171(67.8)	252(65.6)	0.504	1.4(0.5 - 4)
• 45-64	23(27.7)	60(72.3)	83 (21.8)	0.807	1.2(0.4 - 3.5)
• >65	0 (0)	3 (100)	3(0.8)	0.999	0.0
Marital status					
• Un-married	34(36.2)	60(63.8)	94(24.5)	0.561	1.5(0.4 - 6)
• Married	57(29.8)	134(70.2)	191(49.7)	0.856	1.13(0.3-4.43)
• Widow*a	3(27.3)	8(72.7)	11(2.9)		
• Divorced	29(33)	59(67)	88(22.9)	0.705	1.3(0.32-5.31)
Educational level					
• Illiterate*a	32(30.7)	72(69.3)	104(27.3)		
• Literate	90(32.6)	186(67.4)	276(72.7)	0.732	1.08(0.7-1.8)
Occupational					
Farmer *a	11(29.7)	26(70.3)	37(9.6)		
Student	16(32)	34(68)	50(13)	0.821	1.1(0.44-2.8)
House wife	20(39.2)	31(60.8)	51(13.2)	0.359	1.5(0.62-3.8)
Employer*c	72(30.6)	164(69.4)	236(61.5)	0.924	1(0.5-2.2)
Other *b	4(40)	6(60)	10(2.6)	0.538	1.6(0.4-6.7)
Total	123(32)	261(68)	384(100)		

Note: *a: Take as reference value to categorical group, *b : Jobless and children under 4 years, *c : government and self-employed , X^2=chi squares, Age are categorized based on WHO guideline , * : miltivariant binary logistic regression, OR= odd ratio, CI= confidence Interval.

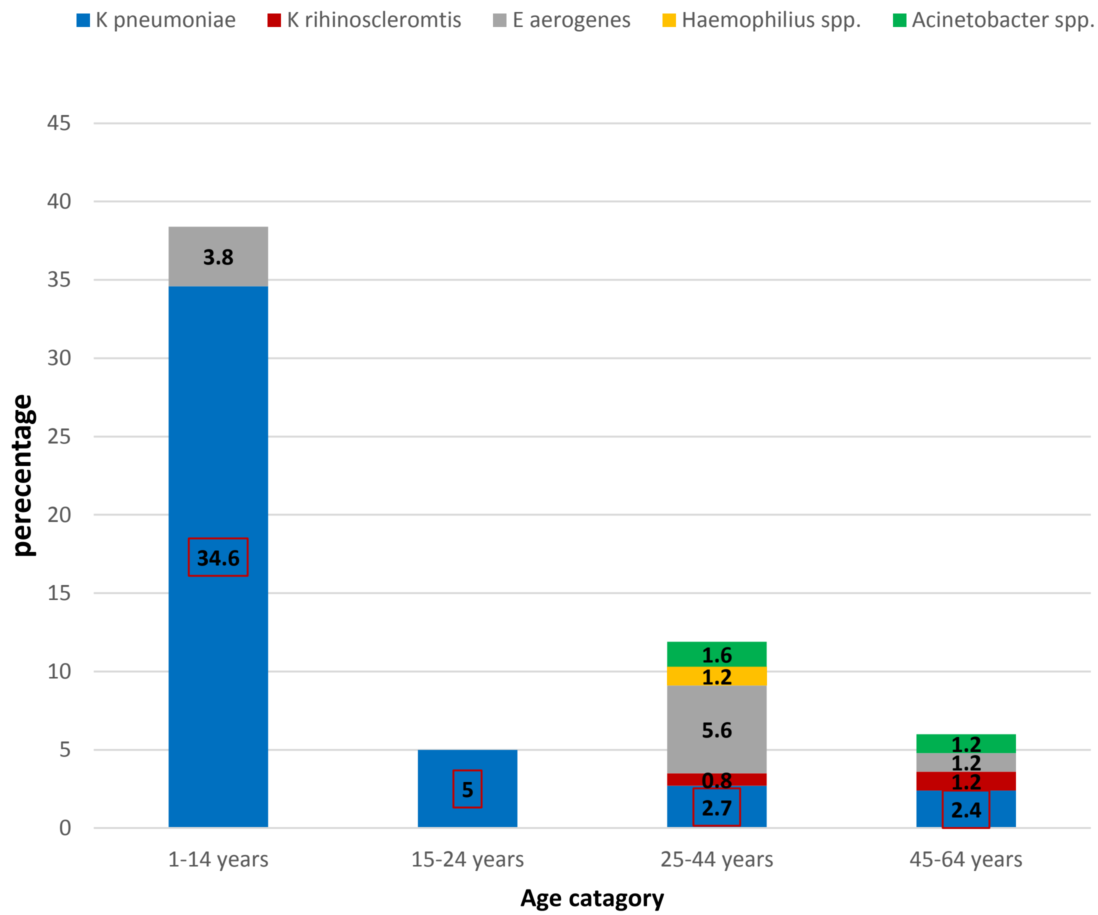

Fig 2. Disribution of gram negative bacteria isolates in relation to age category among HIV positive patients.

5.2. Bacterial Isolate

Among 384 collected samples from study participants, 123(32%) were positive for blood culture. Gram positive isolates 62.6% (n=77/123) were predominantly recovered from those positive blood cultures [Table 2]. *Staphylococcus aureus* 49.4% (n=38/77) was the most frequently isolates microorganism followed by *coagulase Negative Staphylococcus Species* 35% (n=27/77) while *Enterococcus species* and *Streptococcus pneumoniae* 5.2% (n=4/77) and 10.4% (n=8/77) respectively, were the lowest percentage of pathogens among Gram positive bacteria [Table 2].

Table 2: Prevalence of Bacterial Blood steam infection among HIV/AIDS patient at Felege Hiwot referral Hospital July 15, 2016 to December 15, 2016 G.C.

Pathogens	Frequency (N)	Percentage (%)
Gram Positive (n=77)		
S. aureus	38	49.3
CONS	27	35
S. pneumoniae	8	10.4
Enterococcus Spp	4	5.2
Total (n=77/123)	77	62.6
Gram Negative (n=46)		
K. pneumoniae	19	41.3
K rihinoscleromtis	3	6.5
E aerogenes	16	34.8
Haemophilus Spp.	3	6.5
Acinetobacter Spp.	5	10.9
Total(n=46/123)	46	37.4
Overall (n=123/384)	123	32%

Note: CONS= Coagulase Negative Staphylococcus.

Among the Gram Negative Bacteria isolates 37.4% (n=46/123), *Klebsiella pneumoniae* were the most leading pathogen 41.3% (n=19/46) and followed by *Enterobacter aerogenes* with a magnitude of 34.8% (n=16/46), *Acinetobacter Species* 10.9% (n=5/46), *Klebsilla rihinoscleromtis* 6.5% (n=3/46) and *Haemophilus species* 6.5% (n=3/46) had the lowest percentage pathogens [Table2].

5.3. Predisposal Factor

Among three hundred eighty four HIV positive participants, 81(21%) were CD4 count of <200 cells/μL and 303 (79%) were CD4 count of >200 cells/ μL. However, percentage of blood culture isolates among CD4 count of <200 cells/ μL were 76.5% (n=62/81) and among CD4 count of >200 cells/ μL were 20% (n=61/303). The study confirmed that the prevalence of bacterial isolates from blood samples was higher in patients having CD4 count of <200 cells/μL than CD4 count of >200 cell/ μL. There was significant association between blood stream infection and CD4 count among HIV positive patients (Adjusted odds ratio [OR] 7.6, P = 0.000) [Table 3].

Our study revealed that from the total of 384 hospitalized HIV/AIDS patients, 60(15.6%) were clinical suspected as health care associated BSIs, of which 73.3% (n=44/60) were positive blood culture while 26.7% (n=16/60) were negative. In the other hand, out of total 384 HIV positive patients, 324 (84.4%) were clinical suspected as community associated BSIs, of which 24.4% (n=79/324) were positive blood culture, while 75.6% (n=245/324) were negative. However, percentage of positive blood culture was higher in health care associated BSIs than community associated BSIs. Multivariate logistic regression revealed, there was not significant association of bacterial blood stream infections acquired from hospitalized or community (odds ratio [OR] 2.6, P=0.297) [Table 3].

Another predisposing factors considered in this study was the use of intravenous catheter. Among intravenous catheter used of hospitalized HIV/AIDS patients, 68.6% (n=48/70) were blood culture positive and 31.4% (22/70) were negative. Whereas among HIV/AIDS patients who without intravenous catheter, 23.9% (n=75/314) were positive blood culture and 76.1% (239/314) were negative. However, percentage of bacterial isolates was higher in HIV/AIDS positive patients with intravenous catheter than without catheter. There was not significant association of blood stream infection in HIV/AIDS patients with intravenous catheter [Adjusted odds ratio (AOR) = 1.6, p = 0.551] [Table 3].

In current study was showed that, among the total numbers of HIV positive pregnant woman's, 16.7% (n=2/12) were positive for blood culture and 83.3% (n=10/12) were negative. There was not significant association of blood stream infection and HIV positive pregnant woman's [Adjusted odds ratio (AOR) = 2.8, P = 0.246] [Table 3].

Table 3: Predisposal Factors Associated with Bacterial Blood stream infection among HIV/AIDS patients at ART-clinic of Felege Hiwot Referral Hospital, July 15 2016 to December 15 2016 G.C.

Risk associated factor	**Bacterial status**		**Total**	**Adjusted**	
	Yes N (%)	No N (%)	N (%)	P-value	OR (95% CI)
Intravenous catheter					
• Used	48(68.6)	22(314)	70(18.2)	0.551	1.6 (0.32-8.3)
• Not used*a	75(23.9)	239(76.1)	314(81.8)		
Place of acquisition					
• HCA	44(73.3)	16(26.7)	60(15.6)	0.297	2.6 (0.43-15.9)
• CA	79(24.4)	245(75.6)	324(84.4)		
Pregnancy status (n=223)					
• Pregnant*a	2(16.7)	10(83.3)	12(5.4)		
• Non pregnant	72(33.5)	140(66.5)	211(55.6)	0.246	2.8(0.5 - 15.22)
CD_4^+ cell/ μL					
• <200 cell/ μL	62(76.5)	19(23.5)	81(21)	0.000	7.6(3.2- 18.3)
• >200 cell/ μL*a	61(20)	242(80)	303(79)		
Total	123(32%)	261(68)	384(100)		

Note: *a: Has taken as reference value to categorical group, X^2= chi-square, OR= Odds Ratio, CI= confidence Interval, HCA=Health care associated, CA= community associated.

5.4. Antibiotic Susceptibility Pattern

The current study was showed that in the overall resistance rate for different antimicrobial agent Gram positive bacterial isolates was higher than gram negative among HIV/AIDS patients [4, 6& 7]. Among one hundred three bacterial isolates, (54.5%) of Gram positive and (17.3%) of Gram negative bacteria were resistant for at least five and above antimicrobial agent among HIV/AIDS patients.

5.4.1. Antibiotic susceptibility profile of Gram positive bacteria

More than 55% of Gram positive bacterial isolates were resistant for penicillin, cotrimoxazol, and tetracycline while *Staphylococcus aureus* (73.7%) and Coagulase negative *Staphylococci* (70%) were Oxacillin resistance whereas, (100%) of *Enterococcus species & Streptococcus pneumonia* for vancomycin while (100%) of *Enterococcus species* for Chloramphenicol and ciprofloxacin (93.5%) showed better effect on Gram positive bacteria [Table 4].

Out of the total thirty eight *staphylococcus aureus isolates,* 28(73.7%) were Methicillin resistance *Staphylococcus aureus* (MRSA) and out of twenty seven Coagulase Negative *Staphylococcus* isolates, 18 (66.7%) were Methicillin resistance Coagulase Negative *staphylococcus* (MR-CONS) [Table 5].

Among the total number of MRSA isolates 25% (n = 7/28) were health care associated while 75% (n=21/28) were community associated blood stream infections. The total number of MR CONS isolates 61.1% (n =11/18) were health associated and 38.9% (n=7/18) were community associated blood stream infections among HIV/AIDS positive patients [Table 5].

Table 4: Antibiotic Susceptibility Pattern of Gram Positive Bacterial isolates among HIV infected patient at Felege Hiwot Referral Hospital.

Gram positive	Antibiotic susceptibility Pattern									
	CIP N (%)	COT N (%)	CLI N (%)	E N (%)	OXA N (%)	FOX N (%)	VA N (%)	TE N (%)	P N (%)	C N (%)
S aureus(N=38)										
• *S*	36(95)	12(32)	27(71)	12(31.5)	9(24)	10(26.3)	-	9(23.6)	2(5.2)	-
• *I*	1(2.5)	1(2.6)	0(0)	4(10.5)	-	-	-	2(5.2)	-	-
• *R*	1(2.5)	25(65.4)	11(29)	22(58)	29(76.3)	28(73.7)	-	27(71)	36(94.8)	-
CONS(N=27)										
• *S*	25(92.5)	11(40.7)	19(70.4)	10(37)	8(30)	9(33.3)	-	7(26)	2(7.4)	-
• *I*	0(0)	2(7.4)	0(0)	3(11.1)	-	-	-	1(3.7)	-	-
• *R*	2(7.5)	14(51.8)	8(29.6)	14(51.9)	19(70)	18(66.7)	-	19(70.3)	25(92.6)	-
Entrococcus spp. (N=4)										
• *S*	4(100)	2(50)	-	3(75)	-	-	4(100)	0(0)	0(0)	4(100)
• *I*	0(0)	0(0)	-	0(0)	-	-	0(0)	1(25)	0(0)	0(0)
• *R*	0(0)	2(50)	-	1(25)	-	-	0(0)	3(75)	4(100)	0(0)
S pneumonia (N=8)										
• *S*	7(87.5)	4(50)	-	5(62.5)	-	-	8(100)	2(25)	1(12.5)	5(62.5)
• *I*	0(0)	0(0)	-	0(0)	-	-	0(0)	0(0)	0(0)	3(37.5)
• *R*	1(12.5)	4(50)	-	3(37.5)	-	-	0(0)	6(75)	7(87.5)	0(0)
Total (n=77)										
• *S*	72(93.5)	29(37.6)	-	30(39)	-	-	69(90)	18(23.4)	5(6.5)	-
• *I*	1(1.3)	3(3.9)	-	7(9)	-	-	2(2.6)	4(5.2)	0(0)	-
• *R*	4(5.2)	45(58.4)	-	40(52)	-	-	6(7.7)	55(71.4)	72(95.5)	-

Note: CIP = Ciprofloxacin COT= Cotrimoxazol, E= Erythromycin, P=penicillin, VA=Vancomycin, OXA=Oxacillin, CLI= Clindamycin, FOX= cefoxitin, CN=Genetamycin, TE= Teteracycline, S= sensitivity, I=Intermediate, R= Resistance, CONS= Coagulase Negative Staphylococci.

Table 5: MR Staphylococci in health care associated and community associated blood stream infections among HIV/AIDS patient at Felege Hiwot referral Hospital July 15, 2016 to December 15, 2016 G.C.

MR Staphylococci	**Place of acquisition**	
	HCA BSIs N (%)	CA BSIs N (%)
MRSA(n=28)	7(25)	21(75)
MR CONS(n=18)	11(61.1)	7(38.9)
Total(n=46)	18(39.1)	27(59.8)

Note: MRSA = Methicillin resistance *Staphylococcu aureus*, MR CONS= Methicillin resistance Coagulase Negative *staphylococci*, HCA BSIs = health care associated blood stream infections, CA BSIs = Community associated blood stream infections.

5.4.2. Antibiotic susceptibility profile of Gram negative bacteria isolates

More than 90% of Gram negative bacterial isolates were resistant for Ampicillin and Tetracycline. Ciprofloxacin (93.5%), ceftriaxone (98 %) and chloramphenicol (93.5%) were good active agent on Gram negative Bacteria. Among Gram negative bacterial isolates greater than 85% of *K. pneumoniae* and more than 80% of *E. aerugenes* were the most common resistant for ampicillin and tetracycline [Table 6].

Table 6: Antibiotic Susceptibility Pattern of Gram Negative Bacterial isolates among HIV infected patient at Felege Hiwot Referral Hospital

Gram negative bacteria	**Antibiotic Susceptibility Pattern**								
	CIP N (%)	COT N (%)	AMP N (%)	AMC N (%)	CRO N (%)	CN N (%)	TE N (%)	C N (%)	PI N (%)
K pneumoniae (n=19)									
• S	19(100)	12(63)	2(10.5)	14(73.6)	19(100)	8(42)	1(5)	16(84.2)	-
• I	0(0)	0(0)	0(0)	1(5.3)	0(0)	1(5.3)	0(0)	0(0)	-
• R	0(0)	7(37)	17(89.5)	4(21)	0(0)	10(52.6)	18(95)	3(15.8)	-
K.rihinoscleroma (n=3)									
• S	2(66.7)	2(66.7)	1(33.3)	2(66.7)	3(100)	3(100)	0(0)	3(100)	-
• R	1(33.3)	1(33.3)	2(66.7)	1(33.3)	0(0)	0(0)	3(100)	0(0)	-
E.aerognosa (n=16)									
• S	14(87.5)	6(37.5)	1(6.3)	10(62.5)	15(93.7)	6(37.5)	1(6.25)	16(100)	-
• I	0(0)	0(0)	0(0)	0(0)	0(0)	1(6.25)	2(12.5)	0(0)	-
• R	2(12.5)	10(62.5)	15(93.7)	6(37.5)	1(6.3)	9(56.25)	13(81.3)	0(0)	-
Haemophilus Spp (n=3)									
• S	3(100)	1(33.3)	0(0)	2(66.7)	3(100)	2(66.7)	0(0)	3(100)	-
• R	0(0)	2(66.7)	3(100)	1(33.3)	0(0)	1(33.3)	3(100)	0(0)	-
Acinetonbacter Spp (n=5)									
• S	5(100)	5(100)	0(0)	3(60)	5(100)	3(60)	0(0)	5(100)	3(60)
• R	0(0)	0(0)	5(100)	2(40)	0(0)	2(40)	5(100)	0(0)	2(40)
Total (N=46)									
• S	43(93.5)	26(56.5)	4(8.7)	31(67.4)	45(98)	22(47.8)	2(4.3)	43(93.5)	-
• I	0(0)	0(0)	0(0)	1(2.1)	0(0)	2(4.3)	2(4.3)	0(0)	-
• R	3(6.5)	20(43.5)	42(91.3)	14(30.4)	1((2.2)	22(47.8)	42(91.3)	3(6.5)	-

Note: CIP= Ciprofloxacin, COT=Cotrimoxazol, CN=Genetamycin, AMP = Ampicillin, AMC= Amoxicillin-Clavulanic acid, TE= Teteracycline, CRO= Ceftriaxone, C= Chloramphenicol, Pi=Piperacillin, S= sensitivity, I=Intermediate, R= Resistance.

The majority of the isolates 40.7% (n=50/123) from blood samples were multidrug resistant bacteria since they are at least to five or more types of antibiotic agents [Table 7]. The most leading multidrug resistant (MDR) Gram positive bacterial isolates, 92.1% (n=35/38) were *Staphylococcus aureus*, *Coagulase negative Staphylococcus* were 77.8% (n= 21/27) while 68.4% (n= 13/19) were *Klebsiella pneumoniae* among Gram negative bacterial isolates [Table 8].

Table 7: Antibiotic Resistance Profile of Bacterial isolate among HIV patient in Felege Hiwot Referral Hospital from July15, 2016 to December 15 2017G.C.

Bacterial Isolate	**Class of Drug**					
	R0 N (%)	R1 N (%)	R2 N (%)	R3 N (%)	R4 N (%)	R5 and above (%)
Gram Positive						
S. aureus(n=38)	0(0)	1(2.6)	0(0)	7(18.4)	4(10.5)	26(68.4)
CONS(n=27)	0(0)	2(7.4)	3(11.1)	4(14.8)	5(18.5)	13(48.1)
S pneumoniae (n=8)	0(0)	1(12.5)	1(12.5)	4(50)	0(0)	2(25)
Enterococcous Species (n=4)	0(0)	1(25)	0(0)	1(25)	1(25)	1(25)
Total (n=77)	0(0)	5(6.5)	4(5.2)	16(20.8)	10(13)	42(54.5)
Geram Negative						
K pneumoniae (n=19)	2(10.5)	0(0)	6(31.5)	4(21)	2(10.5)	5(26.3)
K rihinoscleromtis (n=3)	0(0)	0(0)	0(0)	3(100)	0(0)	0(0)
E aerogenes (n=16)	0(0)	0(0)	3(18.8)	7(43.7)	3(18.8)	3(18.8)
Haemophilus spp (n=3)	0(0)	1(33.3)	2(66.7)	0(0)	0(0)	0(0)
AcictobacterSpp (n=5)	0(0)	3(60)	1(20)	1(20)	0(0)	0(0)
Total (n=46)	2(4.3)	4(8.7)	12(28.2)	15(32.6)	5(11)	8(17.3)
Overall(n=123)	2(1.6)	9(7.3)	16(13)	31(25.2)	15(12.1)	50(40.7)

Note: R0 = nonresistance, R1= Resistance for 1 antibiotic, R2 = Resistance for 2 antibiotic, R3 = Resistance for 3 antibiotics, R4 = Resistance for 4 antibiotic and R5 = Resistance for 5 and above.

Table 8: Multidrug resistance profile of frequently isolate bacteria among HIV/AIDS infected patients in in Felege Hiwot Referral Hospital from July15, 2016 to December 15 2017G.C.

Bacterial species	Antimicrobial agents	Number of resistant isolates N (%)
Staphylococcus aureus **MDR = 92.1%(35/38)**	E P & TE	3(0.8)
	E P Oxa & TE	2(0.5)
	CLI P & Fox	1(0.3)
	CLI Cot E P & Fox	2(0.5)
	CLI Cot E P Fox & TE	5(1.3)
	CLI Cot E & P	1(0.3)
	Cot P Fox & TE	7(1.8)
	Cot P & Fox	2(0.5)
	CLI E P Fox & TE	2(0.5)
	Cot P & TE	3(0.8)
	Cot E P & TE	1(0.3)
	Cot E P Fox & TE	1(0.3)
	Cot E P & Fox	2(0.5)
	Cip Cot P Fox & TE	1(0.3)
	P Fox & TE	2(0.5)
CONS **(n=21) MDR= 77.8% (21/27)**	Cot E & TE	1(0.3)
	Cot E P Fox & TE	3(0.8)
	Cot E P & Fox	1(0.3)
	Co P Fox	1(0.3)
	Cot P & TE	1(0.3)
	CLI E P Fox & TE	5(1.3)
	CLI Cot P Fox & TE	1(0.3)
	CLI P Fox & TE	1(0.3)
	CLI Cot E P & TE	1(0.3)
	E P Fox & TE	2(0.5)
	E P & Fox	1(0.3)
	Cip Cot P Fox & TE	2(0.5)
	P Fox & TE	1(0.3)
K pneumoniae **(n=13) MDR= 68.4% (13/19)**	Amp Cn & TE	3(0.8)
	Cot Amp & TE	1(0.3)
	Amp Amc & TE	1(0.3)
	Amp Amc & Cf	1(0.3)
	Amp Amc TE & Cro	1(0.3)
	Cot Amp TE & Cf	1(0.3)
	Cot Amp Cn TE & Cro	1(0.3)
	Amx Cot Amp Cn & TE	3(0.8)
	Amx Cot Amp Amc Cn & TE	1(0.3)

Note: CIP = Ciprofloxacin COT = Cotrimoxazol, CN = Genetamycin, AMP = Ampicillin, AMC= Amoxicillin-Clavulanic acid, TE = Teteracycline, CRO = Ceftriaxone, C = Chloramphenicol, Cf = Cephalothin, E = Erythromycin, P = penicillin, VA = Vancomycin, OXA= Oxacillin, CLI = Clindamycin, FOX= cefoxitin, CN = Genetamycin, TE= Teteracycline, CONS = Coagulase Negative *Staphylococci*.

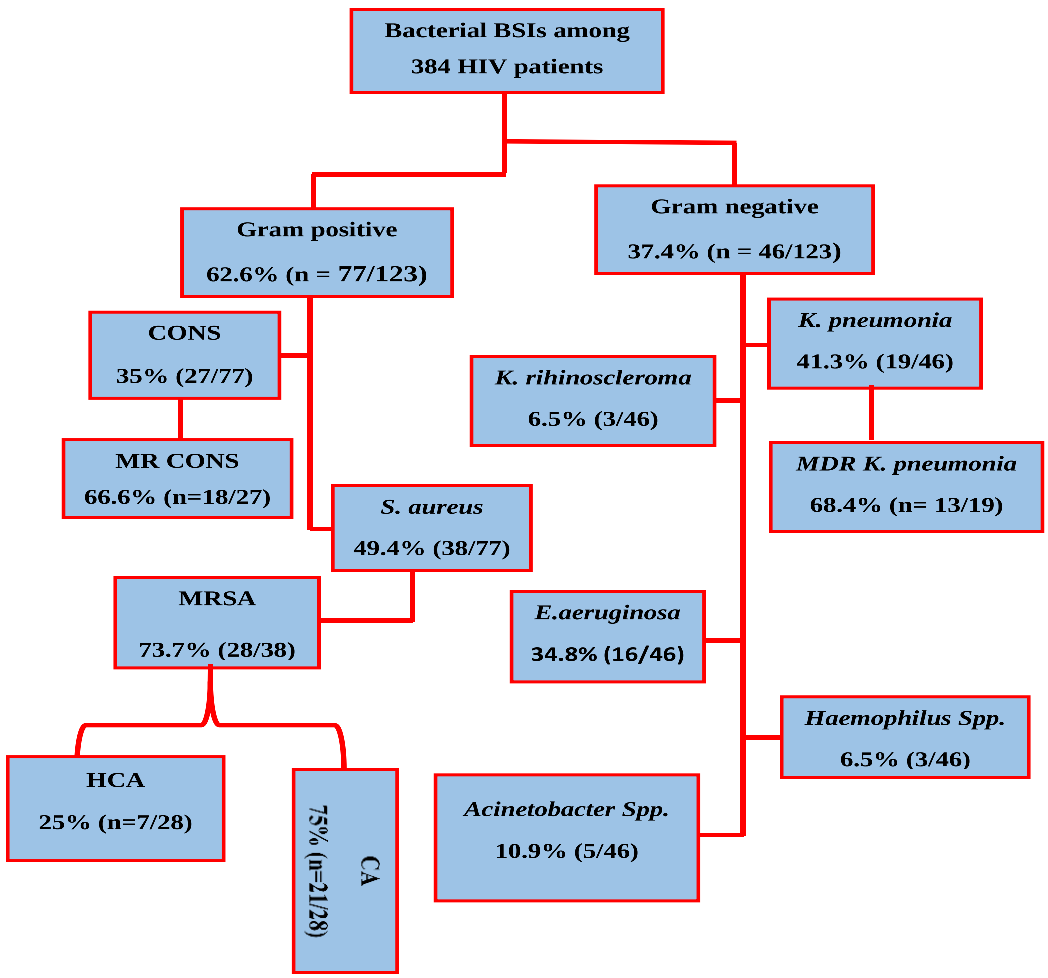

Fig.3 A flow chart showing the distribution of bacterial isolates among HIV positive patients. CONS=Coagulase negative streptococci, MRSA (methicillin resistance Staphylococci aureus, CA (community associated), HCA (health care associated).

Fig.3: A flow chart showed that summarized of the distribution of bacterial pathogens, major predisposing factor and prevalence of multidrug resistant profile of bacterial pathogens which causing blood stream infections among HIV/AIDS patients in Felege Hiwot Referral Hospital.

6. Discussion

The current study showed a prevalence of 32% of bacterial blood stream infections among HIV/AIDS patients attending ART clinic at Felege Hiwot Referral Hospital. However, this percentage was much lower than previous reports from USA (82%) among hospitalized HIV/AIDS patients [3] and from china (41%) among post-operated HIV/AIDS patients [31]. On other hand, this magnitude was significantly higher than a report by Oluyege *et al*., (22.9%) among bacterial sepsis in human immunodeficiency Virus Patients [24] and slightly higher than a report from Gondar, Ethiopia bacterial sepsis and their antibiotic susceptibility Patterns among HIV/AIDs patients by Alebachew *et al.,* (31%) [13].

In present study showed that, (31.8%) of male and (32.1%) of female patients who blood samples were blood culture positive among hospitalized HIV/AIDS patients at FHRH. However, this percentage was slightly higher than previous reports from Gondar, Ethiopia (30.8%) and (31.1%) of bacterial blood culture positive isolates from female and male respectively [13]. In other hand, this percentage was much lower than a report in USA, (43.1%) and (56.8%) of bacterial blood culture positive of female and male respectively [3].

In our study, prevalence of blood stream infections in (1-14) years of age group (53.8%) was higher than from other age group category among HIV/AIDS patients and there was significant association between blood stream infections and in age group of (1-14) years among HIV/AIDS positive patients (P=0.027) [Table1].This might be due to sever immunocompromised, malnutrition and decrease in neutrophil number and function all contributed to increased susceptibility to bacterial agent in the HIV infected children [32, 33]. The studies which was reported, bacterial blood stream infection were higher prevalence in children under five years among HIV infected patients in sub-Saharan Africa [34, 35]. However, in the current study prevalence of bacteremia (53.8%) was significantly higher than study which was conducted in Capt Town, South Africa, (24.8%) were bacteremia among HIV infected children [36] and from Nigeria, (21%) were positive blood culture among HIV positive children [37].

Our study also confirmed that a percentage of bacteremia among $CD4^+$ count of <200 cells/μL of blood (76.5%) was higher than bacteremia among $CD4^+$ count of >200 cell/μL of blood (20%) [p=0.000][Table 2]. However, this percentage was higher than a study was reported in China, (66.7%) were positive blood culture isolates among $CD4^+$ count of <200 cells/μL in HIV/ADS patients [31] and from USA, prevalence of bacteremia among HIV/AIDS patients with $CD4^+$ count of <200 cells/μL were (21.4%) [17]. the current study was showed that a significant relationship between low CD4+ count of <200 cell/μL of blood and bacterial bloodstream infections among HIV/AIDS patients. The lowest CD4+ count of a patient indicated the higher the risk of opportunistic infections. This is why HIV treatment (ART) is especially important in patients with low CD4+ count [38, 39].

The studies on bloodstream infections among HIV/AIDS patients have identified venous catheter as a risk factor. The long- term venous catheter related blood stream infections appeared to be 5 to 10 fold higher among HIV positive patients [40] and (67.8%) of Gram positive and (12.9%) of Gram negative bacterial isolates from patients with central venous catheter related to blood stream infection [2]. In our study, Bloodstream infection cause due to intravenous catheter used was (68.6%). However, it was much higher than bacterial bloodstream infection without intravascular catheter among HIV patients were (23.9%) [Table 3].

In the present study, percentage of health care associated blood stream infections (73.3%) was significantly higher than community associated blood stream infections (24.4%) among hospitalized HIV positive patients at FHRH. This might be due to compromised immunity, prolonged hospitalization and use of invasive devices were increasing the risk of acquiring health care associated BSIs among HIV positive patients [41, 42]. However prevalence of community associated BSIs (73.3%) in our study was significantly higher than the study revealed in Germany, (20%) and (30%) were community associated BSIs in adult and Children respectively among hospitalized HIV/AIDS patients [19]. On other hand, this prevalence slightly lower than a reported in Thailand, (78.5%) were community associated BSIs among HIV infected patients [43].

In the current study was showed that, prevalence of health care associated BSIs (24.4%) was much lower than a study was reported from Brazil, (57.5%) were health care associated BSIs among HIV positive patients [42]. In other hand, this percentage marginally higher than a study was conducted in Thailand, (21.5%) were health care associated among HIV/AIDS patients [43].

In our study, Gram positive bacteria isolates (62.6%) was more common than Gram negative bacterial isolates (37.4 %). This might be due to the fact that the percentage of Patients with severe infection caused by Gram Positive has been increasing in recent years in the septicemia and severe systemic infection. [44, 45]. The study revealed that prevalence of Gram positive bacteria isolates (72.5%) were higher proportion than Gram negative (22.2%) among HIV/AIDS infected patients in Madrid, Spain [46] and Gram positive bacterial isolates (83.4%) was more common than Gram negative (16.6%) among HIV/AIDS positive patients in Gondar, Ethiopia [13].

In the present study, among Gram positive bacterial isolates, (49.3%) were *Staphylococcus aureus* followed by coagulase negative *Staphylococcus Species* were (35%) while (41.3%) were *Klebsiella pneumoniae* common among Gram negative organism. However, this percentage was significantly higher than a reported from Nigeria (11.88%) were *Staphylococcus aureus* and (13.86%) were *Klebsiella pneumoniae* among bacteremia in children infected with HIV/AIDS [37] and from Cambodia (19.3%) were *Staphylococcus aureus* in the blood stream infections among HIV positive patients[47].

The antibiotic susceptibility test results on the current study showed that among the total number of bacterial isolates, (40.7%) were resistant to five and above types of antimicrobial agent. However, this percentage was significantly higher than a report in Gondar, Ethiopia, (29%) bacterial isolates were resistant to five and above antimicrobial agents among HIV/AIDS patients [13]. The reason for higher resistant isolates among HIV/AIDS patients might be multifactor: One of this is inappropriate use of broad spectrum antibiotics such as: frequent administration of cotrimoxazole as prophylaxis.

The current study also reported that (58.4%) of gram positive and (43.4%) of gram negative bacterial isolates were resistant for cotrimoxazole. Although it is very difficult to generalize with this sample size, this study showed the highest resistant profile of many bacterial isolates to cotrimoxazole. Therefore, large scale comparative study in the future will answer this critical question. However, this percentage was much lower than a study by Alebachew *et al.,* (65.3%) of gram positive and (100%) of gram negative bacterial isolates were resistance for cotrimoxazole [13] and Another study also was conducted in Mwanza, Tanzania, higher resistant bacterial isolates among HIV patients with and without prophylaxis regiment to antimicrobial agent cotrimoazole was (81.3%) and (75%) [48].

In the present study, more than 93% of Gram positive and Gram negative bacterial isolates showed good sensitivity pattern to Ciprofloxacin and 100% of *Enterococcus Species* and *Streptococcus pneumonia* were active agent to vancomycin [Table.5 and 6]. In other hand, Gram Negative bacteria showed good sensitivity pattern to ceftriaxone (98%) and chloramphenicol (93.5%) [Table 6]. However, this percentage was slightly comparable to a study in Gondar, Ethiopia, (100%) of Gram negative bacterial and greater than (75%) of Gram positive bacterial were sensitive to active agents of ciprofloxacin among HIV/AIDS infected individual [13] and greater than (80%) bacterial isolates were susceptible to ciprofloxacin among HIV 1 infected children in Kampala, Uganda [22] and from India among blood culture Isolates ceftriaxone and ciprofloxacin were very effective with low resistance of (8%) and (30.3%) respectively [49].

In our study confirmed, out of the total numbers of *Staphylococcus aureus* isolates, (73.7%) were Methicillin resistance *Staphylococcus aureus* (MRSA) and (66.7%) were Methicillin resistance *Coagulase Negative staphylococci* (MR-CONS) among *Coagulase Negative Staphylococci isolates* was most common pathogen caused bloodstream infection. However, this percentage of Methicillin resistance *Staphylococcus aureus* was significantly higher than a reported in Bahir Dar, Ethiopia, (16.8%) were MRSA among HIV infected patients [50] and from Italy, (32%) of MRSA caused bacteremia among HIV/AIDS infected patients [51].

7. Limitation

- Limitation of this study was not considered some bacteria such as *Mycobacterium* and anaerobic bacteria which could be causative agents of bacterial bloodstream infection among HIV/AIDS infected person due to lack of appropriate Laboratory setup for isolation and characterization of these bacteria.
- This study was not including some parameters such as mortality rate and predisposing factors (like living style, personal hygiene and so on.) among HIV positive patients.
- Lack of same reagents and antibiotics disc to undergo some vital test.

8. Conclusions

This study has shown that, prevalence of bacterial bloodstream infection, major predisposing factor and their antimicrobial susceptibility pattern among HIV/AIDS patients.

- Prevalence of bacterial blood stream infections (32%) among HIV/AIDS patients was higher in this study and the most common pathogens for bacteremia was *S. aureus*, *CoNS* and *K. pneumoniae*. Prevalence of bacterial blood stream infection more common in years of 1-14 age group (53.8%) than other age group on this study.
- Majority of positive blood culture isolates from hospitalized HIV/AIDS patients with intravenous catheter, hospital acquired and low $CD4^+$ lymphocyte count.
- Ciprofloxacin and vancomycin was found to be active antibacterial agent for gram positive bacteria while ceftriaxone, ciprofloxacin and chloramphenicol were more effective for gram negative bacteria.
- Most of the isolates from bacterial blood stream infections among HIV/AIDS patients (40.7%) were multidrug resistant for five and above types of antibiotic agents including cotrimoxazole, which is the most commonly prescribed prophylaxis agent in ART clinics.

9. Recommendation

Based on the finding of this study we recommend the following:

- Blood culture tests should be considered for better management of bacterial blood stream infection among HIV/AIDS patients.
- The prevalence of bacterial blood stream infection increasing due to intravenous catheter used, hospitalized patients and low $CD4^{+}$ lymphocyte count. So that health workers and clinicians should be take special care for HIV/AIDS patients.
- Ciprofloxacin and vancomycin (for *Enterococcus spp.* and *S. pneumoniae*) can be used against gram positive bacteria and chloramphenicol and ciprofloxacin for gram negative bacteria among HIV/AIDS patients. So that clinicians can use as treatment for blood stream infections in HIV/AIDS patients.
- Majority of bacterial isolates was resistant for at least five and above antimicrobial agent among HIV/AIDS patients. We recommend shall be need further assessment regarded to mortality rate, loss of cost and time due to blood stream infection caused by multidrug resistant pathogens among HIV/AIDs patients.
- Continuous antimicrobial susceptibility surveillance should be implemented in this study area with large scale study among Ethiopian HIV/AIDS patients especially, focusing on cotrimoxazole.

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ANNEXES

ANNEX I: Different Laboratory Procedures.

Blood for culture were collected under strict aseptic conditions by using venipuncture Technique. Carful skin cleaning using 2% tincture of Iodine and 70% alcohol was done before drawing blood to prevent contamination. A total of 10 ml of blood for adult and 2-5ml of blood for children were collected and transferred to blood culture bottles each contain 50ml of BHI for adult and 20ml of BHI supplemented (Oxoid) for children.

Blood Culture Sample collection, storage and transportation

Procedure

1. Patient Identification and laboratory Specimen Labeling
2. Precautions when performing the venipuncture: If patients with a bleeding disorder (Hemophilia or von Willebrand's Disease).
3. Properly position the patient: Hospitalized patients should lie in bed in comfortable position.
4. Assemble supplies: use 2% tincture of Iodine and 70% alcohol for blood culture, Gauze, Tourniquet, cline glove, Syringes used with butterfly and blood bottle.
5. Infection control for blood collection: Always wear gloves and observe standard precautions when collecting biological specimens.
6. Apply the tourniquet: A tourniquet is used to occlude venous flow and cause the vein to swell, making the vein more prominent.
7. Select the vein site: The larger median cubital and cephalic veins (in the antecubital area) are used most frequently.
8. Cleanse the venipuncture site: Clean puncture site first by using 2% iodine tincture and clean Iodine by using 70% alcohol when specimen collect for blood culture.
9. Perform the venipuncture: Using an evacuated system and needle with safety-guard method.
10. Release the tourniquet: Release tourniquet as soon as blood enters tube, within one minute.
11. Remove the needle: Place clean gauze over venipuncture site and remove needle quickly with one smooth motion.
12. If syringe was used, transfer specimen to blood bottle and apply direct pressure to puncture site for 30-60 seconds or until bleeding stops.

13. Label the specimen containers and handle blood according to specimen requirements.
14. Prepare the specimen containers for transport to the Lab: Transport the labeled blood specimen containers with the required request forms to the laboratory using a sealed specimen transport bag.

A. Culture media preparation

I. Materials required: Culture media, Petri dishes, inoculating loops, Bunsen burner, Autoclave, Refrigerator, Measuring cylinder, Boiler, Balance and Flask.

II. Media Preparation

1. Weighing, dissolving and boiling. The media is weighed based on the manufacturer's direction, then dissolving is mediated by mixing very well until all the powder is mixed totally and then boiled to get homogenous solutions.
2. Sterilization: It is done by autoclaving at 121^{0}C for 15 min.
3. Dispensing: It is done after cooling and solidifying the media.
4. Quality control testing: Is done by using control organism i.e. by inoculating that grows and which do not grow on the prepared media and looking its change, or by incubating 4-5% of prepared media for sterility testing overnight on incubator and check for the growth.
5. Labeling and storage. All culture media must be clearly labeled with its name, preparation, and expiration date and stored at 2-8^{0}C.

B. Culture and Identification Procedure.

1. All culture bottles were incubated aerobically at 37˚C for seven days for the presence of visible microbial growth by observing any one of the following Hemolysis, turbidity, gas production and coagulation of the broth.
2. Positive culture bottles tested for growth were sub-cultured on, Blood Agar plate (BAP), Chocolate Agar Plate (CAP) and Mac Conkey Agar Plate (Oxoid).
3. Blood Agar Plate and Chocolate Agar Plate incubated in Microaerophilic atmospheric on candle jar but Mac concky Agar plate were incubated at aerobic at 37 ^{0}c for 24-48 hours.
4. All positive blood cultures were identified by their characteristic appearance and Gram staining reaction and confirmed by the pattern of biochemical reactions.

5. Gram negative bacteria were identified by indole production, citrate utilization, motility, urease, oxidase, gas production, hydrogen sulfide production, carbohydrate fermentation, and lysine decarboxylases production tests.
6. Gram positive bacteria: for *staphylococcus spp*. coagulase, catalase test, and Mannitol Salt Agar and for *streptococcus Spps*, bacitracin and opt chin susceptibility tests.
7. Blood culture broth which shows no microbial growth within 7 days was reported as culture negative, only after results were sub-cultured on blood, MacConkey, and chocolate agar.

C. Gram staining: It is a differential staining technique used to characterize bacteria as Gram positive and Gram negative.

Required materials and reagents

- Crystal violet, Gram's Iodine, Acetone alcohol, Safranin, Immersion oil Microscope, Bunsen burner, Cotton, Forceps, Microscope slide, staining bottles.

Procedure

1. The fixed bacterial smear is subjected to crystal violet.
2. After a minute of exposure to the staining solution, then the slide is washed in water.
3. The smear is treated with few drop of Gram's Iodine and allowed to act for a minute.
4. The slide is again washed in water and then decolorized in absolute ethyl alcohol or acetone.
5. The smear is finally treated with few drops of counter stain such as dilute carbol fuchsin, neutral red or Safranin.

Result

- Gram positive bacteria – dark purple
- Gram negative bacteria – pale to dark red

Identification of *Streptococcus pneumoniae* by using optochin disc test: Streptococcus pneumoniae are sensitive to optochin (ethylhydrocupreine hydrochloride). Placing a disc (5 µg) on culture plate and culturing the plate aerobically (not in CO2) can help to provide a rapid presumptive identification of *S. pneumoniae*. The zone of inhibition should be at least 10 mm. Most *viridans streptococci* and other alpha haemolytic streptococci are resistant to optochin.

Interpretation

If the zone of inhibition is less than 10 mm consider as resistant and if zone o inhibition is greater than 10mm consider as sensitive.

Identification of *Haemophilus species* using X, V, XV disc:

Procedure

1. Prepare a saline suspension (approx. 0.5 McFarland turbidity) of the test organism from a primary culture. Using a swab, inoculate the suspension on a plate of blood agar base or nutrient agar known to lack factors X and V.
2. Place discs 10–20 mm in from the side of the plate and set up a control plate using a known strain.
3. Incubate the plates overnight in a moist carbon dioxide atmosphere at 35–37 ºC.
4. Examine the plates for growth around each tablet or disc.

Interpretation

- *H. influenza:* Growth around factor XV and slight growth between X and V
- *H. parainfluenzae*: Growth around factor XV and factor V.

D. Biochemical Testing

1. **Catalase Test**: To differentiate *staphylococci* from *streptococci species*.
    - Required materials: Test tubes, H_2O_2 and glass rod.

   Procedure

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

Results

- Active bubbling . . . . Positive catalase test (*Staphylococcus species*).
- No bubbles . . . . Negative catalase test (*Streptococcus species*).

2. **Coagulase Test:** To identify S. aureus from other *staphylococcus species (CONS).*

Slide Coagulase Test procedure

1. Place a drop of distilled water on each end of a slide or on two separate slides.
2. Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.
3. Add a loop full of plasma to one of the suspensions and mix gently.
4. 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)

Results

- Clumping within 10 secs . . . . *S. aureus*
- No clumping within 10 secs . . . . No bound

3. **Indole Test:** Testing for indole production is important in the identification of *enterobacteria*. Most strains of *enterobacteria species* break down the amino acid tryptophan with the release of indole.

Detecting indole using tryptone water

1. Inoculate the test organism in a bijou bottle containing 3 ml of sterile tryptone water.
2. Incubate at 35-37^{0}C for up to 48 h.
3. Test for Indole by adding 0.5 ml of Kovac's reagent. Shake gently. Examine for a red color in the surface layer within 10 minutes.

Results

- Red surface layer . . . . Positive indole test.
- No red surface layer . . . .   Negative indole test.

4. **Motility Testing**

Procedure

1. Use straight inoculating pick
2. Make a stab inoculation from stock culture, stab as straight as possible (in and out)
3. Incubate at an appropriate temperature for 24 to 48 hours.
4. Observe the culture holding it up to a light source.

Result: A positive motility test is indicated by red growth along the inoculation line.

5. **H_2S Test:** H_2S is produced by bacterial anaerobic degradation. Hydrogen sulfide is a by-product when carbon and nitrogen atoms in the amino acid are consumed as nutrients by the cell.

Required

- TSI slant, inoculating loop, incubator.

Procedure

1. The TSI agar inoculated by stabbing the butt and streaking the slant
2. Incubate at 35-37°C for 18 to 24 hrs.
3. Look for the black precipitate

Result

- Black precipitate indicates the presence of Hydrogen Sulfide producing bacteria

6. **TSI test**: Used to determine if the bacteria can ferment glucose and/or lactose and if it can produce H_2S or other gases.

Procedure

- Inoculate the bacterial colony first by stubbing in to the TSI agar butt (bottom of the tube) through the center then streak the slant in a wavy pattern

Result

- A yellow slant and butt on TSI indicates the organism ferment Sucrose and/or Lactose, A yellow butt shows that the organism ferment glucose. Black precipitate indicates hydrogen Sulfide production. Whereas production of gases indicated by cracks or bubble in the media.

7. **Citrate Utilization Test:** Used for identification of *enterobacteria*. The test is based on the ability of an organism to use citrate as its only source of carbon.

Citrate method using Simmon's citrate agar

Procedure

1. Prepare slopes of the medium in bijou bottles as recommended by the manufacturer (store at 2-8°C).

2. Using a sterile straight wire, first streak the slope with a saline suspension of the test organism and then stab the butt.
3. Incubate at 35^0C for 48 hours. Look for a bright blue color in the medium.

Results

- Bright blue . . . . Positive citrate test
- No change in color of medium. . . . . Negative citrate test

8. **Urease test:** using identification of enterobacteria by testing of urease enzyme activity.

Procedure

1. Inoculate heavily the test organism in a bijou bottle containing 3ml
   Sterile Christensen 'smodified urea broth.
2. Incubate at 35–37°C for 3-12 h (preferably in a water bath for a quicker result).
3. Look for a pink color in the medium.

Results

- Pink color . . . . Positive urease test
- No pink color . . . . Negative urease test.

9. **Oxidase Test**: used to assist in the identification of Pseudomonas, *Neisseria, Vibrio, Brucella*, and *Pasteurella* species, all of which produce the enzyme cytochrome oxidase.

Procedure

1. Place a piece of filter paper in a clean petri dish and add 2 or 3 drops of freshly prepared oxidase reagent.
2. Using a piece of stick or glass rod, remove a colony of the test organism and smear it on the filter paper.
3. Look for the development of a blue-purple color within a few seconds as shown in color.

Results

- Blue-purple colour . . . . Positive oxidase test (within 10 seconds)
- No blue-purple colour . . . . . Negative oxidase test (within 10 seconds)

Note: Ignore any blue-purple colour that develops after 10 seconds.

E. **Antimicrobial Susceptibility Test procedure:** According to the criteria of the Clinical and Laboratory Standard Institute (CLSI) of disk diffusion method, antimicrobial susceptibility testing was performed for all isolates.

Procedure

1. Select at least 3-5 pure colonies of bacteria from on agar plate culture
2. Taken and transferred to a tube containing 5 mL of saline and mixed gently to a homogenous suspension.
3. Incubated at 35°C until the turbidity of the suspension becomes adjusted to a McFarland 0.5.
4. By using sterile swab and the excess suspension was removed by gentle rotation of the swab against the surface of the tube.
5. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar (Oxoid) and for fastidious organism's sensitivity was done on MHA with 5% sheep blood.
6. The inoculated plates were left at room temperature to dry for 3-5 minutes and a set of 14 antibiotic discs (Oxoid) were placed 24 mm apart from each other on the surface of a Mueller-Hinton plate.
7. Each disk was pressed down to ensure complete contact with the agar surface.
8. Incubated at 35°C for 24-48 hours and diameters of the zone of inhibition around the disc measured using the ruler and the isolates were classified as sensitive, intermediate and resistant.

Antimicrobial susceptibility test for *Haemophilus species* by using Haemophilus Test Medium (HTM):

Preparation of HTM

1. Prepare a fresh hematin stock solution by dissolving 50 mg of hematin powder in 100 mL of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved.
2. Add 30 mL of the hematin stock solution and 5 g of yeast extract to 1 L of MHA and autoclave.
3. After autoclaving and cooling, add 3 mL of an NAD stock solution (50 mg of NAD dissolved in 10 mL of distilled water, filter sterilized) aseptically.

ANNEX II: Participant Information Sheet (English and Amharic version)

Name of the investigator: Mohabaw Jemal. I am a laboratory technologist postgraduate student at Addis Ababa University.

Name of Institute: Addis Ababa University, School of Allied health science, Graduate studies in clinical laboratory science diagnostic and public health microbiology specialty.

Topic of the Research: Bacterial bloodstream infections in acquired immunodeficiency syndrome patients and their *in vitro* antimicrobial susceptibility pattern at Felege Hiwot Hospital, Bahir Dar, Amhara, National Regional State, North west Ethiopia.

Research objective: the general objective of this study is to assess the prevalence of bacterial blood stream infections and their antimicrobial susceptibility pattern among HIV/AIDS patients at Felege Hiwot Referral Hospital, Bahir Dar, Amhara, National Regional State, North west Ethiopia.

Introduction

First of all we would like to thank you in advance for your cooperation and consent in participation in this study. Please read 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.

Aim of the study is to assess bacterial bloodstream infections among HIV/AIDS patients and their *in vitro* antimicrobial susceptibility pattern. It is one of the major health problems in our country, the result of the study can be helpful in planning and intervention to solve the problem. 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

As a participant of this study, you are expected to give blood. Being asked to give sample does not neccessarly 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 to participate

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. But you may fill minor temporary pain during sample collection.

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 blood stream infection. 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 are:

Investigator: Mohabaw Jemal (BSc), DMLS; AAU, +251918723323

Email- muhabawjemal11@gmail.com

Advisor: Dr. 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 III. Participant Consent Form.

I undersigned to confirm that, as I give consent to participate after a clear understanding of the objectives of the study and with recognition of my right to withdraw from the study if I change my mind. The proposal has been explained to me in the language I understand.

I put my signature below indicates that I have read /or listened, and understand the information provided for you about the purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy.

I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study.

Agree to participate? Yes --------------------- No ----------------------

Participant's signature ___________________ Date______________________

Investigators signature __________________ Date______________________

ANNEX IV. Information sheet for parents/guardian Assent Form (English version)

I undersigned the purpose of this study. My child has to say to choose if I want to be in the study. I have been informed there is no harm except little discomfort during sample collections. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child. By signing below I agree to let my child to participate in this research study. My signature below indicates that I have read /or listened, and understand the information provided for you about the purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy.

I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study.

Agree to participate? Yes --------------------- No ----------------------

Participant's signature ___________________ Date______________________

Investigators signature __________________ Date______________________

Questionner English version

1.1.Verbal consent letter!

Introduction

Well come to the interview

My name is Mohabaw Jemal, I came from Addis Ababa University School of Allied Health Sciences, Department of Medical Laboratory Sciences. My study aim is investigating principally the status of bacterial blood stream infections and their *in vitro* antibiotic susceptibility pattern among HIV/AIDS patients at Felege Hiwot referral Hospital in Bahir Dar.

You are selected to be one of the participants in the study and you will be kindly requested to give information. Giving possible information on the questions doesn't have any harm to you and any others. Any information that we collect about you on this research will be kept in secret.

Information about your identity will be put away after re-coding your file; and kept in a secured place. Only the principal investigator will be able to link your identity with the code number.

At the end of the study we will write a report about the results of the study through publication or any other means. We assure you that the reports will not bear any information of your personality like name and identity. Since participation in this study is entirely voluntary, you can refuse to participate in this study at any time. Your refusal to participate will not affect any of your benefits.

Interviewer ------------------------------ date of interview ---------------------

Identification

Code No: ________ Card No. ________ Ward: ___________

Hospital/ health center ID: __________ Address: woreda: ________ Keble: _______

Tell: ______ Sample ID: _________

PART ONE: Patient Identification and Demographic data

Quest No	**Questions**	**Response**	**Code**	**Remark**
01	Age	1 -14 15-24 25-44 45-65 >65	1 2 3 4 5	
02	Sex	Male Female	1 2	
03	place of residence	Urban Rural	1 2	
04	Occupation	Farmer Students Employer(Gov't) House Wife Other	1 2 3 4 5	
05	Educational status	Illiterate Primary Secondary College/university/Diploma	1 2 3 4	

Part Two: Clinical Data

Quest No	**Questions**	**Code**	Code	Remark
01	1. Have you now admitting in hospital?	Yes No	1 2	

	1.1. How long period of time admitting in the hospital?	< 2 day >2 days	1 2	
	1.2.Have you ever take any medicine through intravenous by using intravenous catheter?	Yes No	1 2	
02	2. Have you ever had previously admitting in hospital or health centre?	Yes No	1 2	
	2.1.How long time after discharge from the hospital?	< 10 days >10 days	1 2	
	2.2.Have you ever take any medicine through intravenous by using intravenous catheter during hospital admitted ?	Yes No	1 2	
03	**<u>For Females</u>** 3. Are you pregnant?	Yes No	1 2	
04	4. CD4 count cells/µL	ħ ‹200 ħ ›200	1 2	

Investigators signature __________________ Date______________________

የአማርኛ ቅጂ

ለጥናቱ ተሳታፊ የኤቻ.አይ.ቨ ህሙማን ላይ ለቃለ መጠይቅ ሚታደል

የበጎፈቃደኝነት የስምምነት ቅጽ

የዋና ተመራማሪው ስም: ሞሃባው ጀማል

የተቋሙ ስም: አዲስ አበባ ዩኒቨርሲቲ የድህረ ምረቃ ትምህርት ቤት፤ የጤናና ሳይንስ ኮሌጅ፤ የህክምና ላቦራቶሪ የትምህርት ክፍል.

የጥናቱ ርዕስ: በባህረ ዳረ ከተማ በፈለገ ህይዎት ሆስፒታል በኤቻ.አይ.ቨ ህሙማን ላይ ባክቴሪያል የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ::

መግቢያ:

ይህ የማብራሪያ እና የስምምነት ቅጽ አሁን እርስዎ እንዲሳተፉበት የምንጠይቅዎትን የምርምር ጥናት በተመለከተ ነው፡፡ እባክዎ በዚህ ጥናት ለመሳተፍ ከመወሰንዎ በፊት ይህንን ቅጽ መረጃ ሰብሳቢዎቹ በሚያነቡልዎት ጊዜ በጥንቃቄ በማዳመጥ ጥያቄዎች ካለዎት ይጠይቁ፡፡ በዚህ ጥናት መሳተፍ ከጀመሩ በኋላም በማንኛዉም ጊዜ ጥያቄዎች ካለዎት ማንሳት ይችላሉ፡፡ ጥናቱን የሚያካሄደው በአንድ ህብረተሰብ ጤና አጠባበቅ ተመራቂ ተማሪ እና በአንድ የአዲስ አበባ ዩኒቨርሲቲ የጥናቱ አማካሪ ነው፡፡

የጥናቱ ዓላማ: ባክቴሪያል ብለድ ስትሪም የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ በሚል ርዕስ ነዉ፡፡

የጥናቱ ዘዴ፡ የጥናቱን ዓላማ ለማሳካት የሚያስፈልገው የደም ናሙና በሙያው በሰለጠነ የላቦራቶሪ ባለሙያ አማካኝነት ከበሽተኛው ይወሰዳል፡፡ ናሙናው የሚወሰደው በባህረ ዳረ ከተማ በፈለገ ህይዎት ሆስፒታል በኤቻ.አይ.ቨ ህሙማን ላይ ነው፡፡ የተወሰደው የደም ናሙና በበፈለገ ህይዎት ሆስፒታል ላቦራቶሪ ውስጥና በክልልሎ ላቦራቶሪ በደም ላይ የባክቴሪያ ፍተሻ ይዳሰሳል፡፡ የተገኘው የባክቴሪያ ዓይነትም በየትኛው የመድሃኒት ዓይነት ሊጠፋ እንደሚችል አብሮ ይመረመራል፡፡ የደም ናሙና የተወሰደላቸው የኤቻ.አይ.ቨ ህሙማን ሰለ ማህበራዊና ግላዊ ሁኔታ ፤ ስለ ጤና ነገር ይጠየቃሉ፡፡ መልሱም በዚሁ ዓላማ በተዘጋጁ ፎርሞቹ ላይ ይሞላል፡፡ ስምዎን መግለጽ የግድ አይደለም፡፡ መልስዎትም በሙሉ በሚስጥር ይጠበቃል፡፡ ለሌላ ተቋም ወይም ግለሰብም ተላልፎ አይሰጥም፡፡ የእርስዎ መረጃ ከላይ ለተጠቀሰው ዓላማ ብቻ ይውላል፡፡

ጥናቱ የሚያደርሰው ችግር: ከዚህ ጥናት ጋር በተያያዘ በናሙና አወሳሰድም ወቅትም ሆነ የውጤት ትንተናው በኤቻ.አይ.ቨ ህሙማን ላይ የሚያደርስ ምንም ችግር የለም፡፡ ሁሉም ነገር በጥንቃቄና በሚስጢር የሚያዝ ይሆናል፡፡

ጥናቱ የሚያስገኘው ጥቅም : በጥናቱ በመሳተፍዎ ምክንያት የሚያኙት ምንም ዓይነት የገንዘብ እንዲሆም የተለየ ጥቅማጥቅም አይኖርም:: ውጤቱን መሰረት ባደረገ ባክቴሪያል ብለድ ስትሪም የተባለዉን በሽታ በኤቻ.አይ.ቨ ታማሚዎች ላይ የሚደረሰዉን የጤና ጉደለት፤ ሥርጭት እና የሚበጀውን መድሃኒት ለመምረጥ፤ለማወቅ ይጠቅማል፡፡ በመሆኑም ቤተሰብዎት እና ህብረተሰቡ ከዘህ ሂደት ተጠቃሚ ይሆናል፡፡

ሚስጥር ስለመጠበቅ: ከዚህ ጥናት የሚሰበሰብ መረጃ ሁሉ በሚስጥር ይጠበቃል፡፡ በተደረገው ጥናት ውስጥ እርስዎ ስለመሳተፍዎ ማንም የሚያውቅ አይኖርም፡፡ የጥናት ውጤትዎም ሥምዎ ባልተፃፈበት ሁኔታ በመለያ ምልክት ይቀመጣል፡፡ ለመሆኑ መለያ አይኖረውም፡፡ መረጃው ከዋና ተመራማሪው በስተቀር ማንም ሊያገኘው ይችልም፡

በጥናቱ ያለመሳተፍ ወይም ራስን ከጥናቱ የማግለል መብት: ጥናቱ የሚካሄደው ፈቃደኝነት ላይ በተመሰረተ መልኩ ነው፡፡ በጥናቱ መሳተፍም ሆነ ያለመሳተፍ መብት አለዎት፡፡ ወይም ደግሞ በማንኛውም ጊዜ ከጥናቱ የመውጣት መብት አለዎት፡፡ የመጠይቆችንም ጥያቄ አለመመለስና የደም ናሙናውንም ያለመስጠት መብት አለዎት፡፡

ማበረታቻ: በጥናቱ በመሳተፍዎ ምክንያት የሚያኙት ምንም ዓይነት ማበረታቻ አይኖርም፡፡ የመሳተፍም ሆነ ያለመሳተፍ ምርጫዎ የተጠበቀ ነው፡፡

ተጨማሪ መረጃዎችን ለማግኘት ከፈለጉ

ሞሃባው ጀማል: +251918723323 Email- muhabawjemal11@gmail.com

1. ለአዋቂዎች የኤቻ.አይ.ቪ ህሙማን የስምምነት ቅፅ

ስለጥናቱ አስፈላጊ የሆኑትን መረጃዎች አንብቤ ወይም ተነቦልኝ አላማውም በባህረ ዳረ ከተማ በፈለገ ህይዎት ሆስፒታል በኤቻ.አይ.ቪ ህሙማን ላይ ባክቴሪያል የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ የሚጥና ሲሆን ለጥናቱ አስፈላጊ የሆኑትን መረጃዎች እንደምሰጥ ተረድቻለሁ፡፡ ከጥናቱ ጋር የተያያዙ ጥያቄዎችንም ጠይቄ ማብራሪያዎች ተሰጥተውኛል፡፡ የጥናቱ ተሳታፊ ለመሆን ሙሉ ፍቃደኛ ነኝ፡፡ የሚሰራው ጥናት ጠቃሚ ስለሆነ የምጠየቃቸውን መረጃዎችና የደም ናሙና ለመስጠት ፍቃደኝነቴን ነኝ፡፡

የተሳታፊ ፊርማ፡ -------------- የተሳታፊ አድራሻ፡------------------------ ቀን፡-------------------------------

2. የወላጀ/የአሳዳጊ የስስምምነት መጠየቂያ ቅጽ

እኔ የልጁ አስታማሚ ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡ "ብለድ ስትሪም ኢንፌክሽን የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ" ሲሆን በጥናቱ ልጁ እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና ሲወሰድ ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት ልጁ ላይ እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም የልጁ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ ልጁ በግሉ የሚያገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ የልጁ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በልጁ ላይ እንደማያስከትል ተረድቻለሁ፡፡ በከዚህ ባሻገር የልጁ በጥናቱ ውስጥ ለመካተት የእኔ የወላጁ አሳዳጊ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ልጁ በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

ይህን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለዉ አድራሻ ይጠቀሙ፡፡

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የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል፤ የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ

ኢ-ሜይል፤ muhabawjemal11@gmail.com

ለተጨማሪ መረጃ፡ አዲስ አበባ ዩኒቨርሲቲ ፤ የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ፡፡ ስልክ+251112755170

የአማርኛ ቅጅ ቃለመጠይቅ

በባህረ ዳረ ከተማ በፈለገ ህይዎት ሆስፒታል በኤቻ.አይ.ቪ ህሙማን ላይ ባክቴሪያል የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ የሚደርገ ጥናት::

ከህመምተኞችጋር የተደረገ ቃለመጠይቅ

የተጠያቂ መለያ ቁጥር :----------------------------

ጤና ይስጥልኝ፡፡ ሞሃባው ጀማል እባላለሁ፡፡ የመጣሁት ከአዲስአበባ ዩኒቨርሲቲ የህክምናና ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ የትምህርት ክፍል ሲሆን የዚህ ጥናት ዋና ዓላማ በባህረ ዳረ ከተማ በፈለገ ህይዎት ሆስፒታል በኤቻ.አይ.ቪ ህሙማን ላይ ባክቴሪያል የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ የሚደርገ ጥናት ነው፡፡ የሚሰጡኝ መረጃ ሚስጢራዊነቱ ሙሉ በሙሉ የተጠበቀ ነው፡፡ የእርሶ ስምም ሆነ መለያ መረጃዎች አይመዘገቡም፡፡ እርስዎ በጥናቱ የመሣተፍም ሆነ ያለመሳተፍ መብት ያለዎት ሲሆን ጥያቄዎችን ያለመመለስም ሆነ የማቋረጥ መብትዎ የተጠበቀ ነው፡፡ ነገርግን እርሶዎ የሚሰጡኝ መረጃ ለጥናቱ ጠቃሜታው የጎላ ነው፡፡

በጥናቱ ለመሳተፍ ፍቃደኛ ነዎት? ሀ) አዎን   ለ) አይደለሁም

ክፈል አንደ: ማህበራዊና ግላዊ ሁኔታ

ቁጥር	ጥያቄ	መላሽ	ኮድ
01	እድሜ	1 -14 15-24 25-44 45-65 >65	1 2 3 4 5
02	ጾታ	ወንድ ሴት	1 2
03	የመኖሪያቦታ	ከተማ ገጠር	1 2
04	የሥራ አይነት	ገብርና ተማሪ የመንግስት/በገል ተቀጣሪ የቤት እመቤት ሌላ	1 2 3 4 5
05	የትምህርትሁኔታ	ማንበብም /መጻፍም ማይችል 1ኛ ደረጃ 2ኛ ደረጃ ኮሌጅና ከዚያ በላይ	1 2 3 4

ክፍል ሁለት: የጤና ሁኔታ

ቁጥር	ጥያቄ	መላሽ	ኮድ
01	3. በዚህ ሰዓት በበሺታው ምክኒያት ሆስፒታል ውስጥ ተኝተዋል ?	አዎን አይደልም	1 2
	1.2. ምን ያህል ጊዜ ሆስፒታል ውስጥ ቆይተዋል	ከሁለት ቀን በላይ ከሁለት ቀን በታች	1 2
	3.2.በተኞበት ሰዓት በተለያዬ የሰዉነት ክፈለዋት ላይ በደም ስር የሚሰጥ እንደ የውሃ ምግብ ወይም መዳሃኒት ተሰጠዎታል?	አዎን አይደልም	1 2
02	4. ከዚህ በፊት በሆስፒታል ወይም በጤና ጣቢያ ውስጥ ተኝተዉ ነበር?	አዎን አይደልም	1 2
	4.1.በሆስፒታል ውስጥ ተኝተዉ ከወጡ ምን ያህል ጊዜ ወነህ/ሺ?	ከ 10 ቀን በታች ከ 10 ቀን በላይ	1 2
	4.2.በተኞበት ሰዓት በተለያዬ የሰዉነት ክፈለዋት ላይ በደም ስር የሚሰጥ እንደ የውሃ ምግብ ወይም መዳሃኒት ተሰጠዎታል?	አዎን አይደልም	1 2
03	5. የጾታ ሁኔታዋት ሴት ከሆነ "አሆን የረግዝና ሁኔታዉት ምን ደን ነዉ?"	ያረገዙ አይደለሁም	1 2
04	6. የ የሲዲ ፎር ህዋሲያን መጠን	ከ ‹200 ከ ›200	1 2

አ ስ ተ ያ የ ት ------------------------- የ ጥና ቱ ተ ቆ ጣጣሪ ፊ ር ማ ------------------------ ቀ ን ----------------

ANNEX V: Assurance of Principal Investigator

I, undersigned agree to accept all responsibilities for the scientific and ethical conduct of the research project. I will provide timely progress report to my advisor and seek the necessary advice and approval from my primary advisors in the course of the research. I will communicate timely to my advisors all stakeholders involved in the study including any source of funding for this research.

Name of the Investigator: Mohabaw Jemal (BSc in MLT, MSc candidate)

Signature: ____

Approval of the primary Advisor

Name of the Primary Advisor: ___________________________

Signature: ____

Date: ____

Name of the Second Advisor: _____________________________

Signature: ____

Date: ____

Place: Addis Ababa University School of Allied health Sciences, Department of Medical Laboratory Science. Addis Ababa, Ethiopia

Date of submission______/______/_______