Addis Ababa University

College of Health Science

Department of Medical Laboratory Science

Bacterial etiology of bloodstream infections, multidrug resistance and extended beta lactamase producing isolates among patients referred to Arsho advanced medical laboratory.

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This is to certify that the thesis prepared by **Misgana Abera**, entitled: **Bacterial etiology of bloodstream infections, prevalence of multidrug resistance and extended beta lactamase production among patients referred to Arsho advanced medical laboratory** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (diagnostic and public health microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of abbreviations

AAML Arsho Advanced diagnostic laboratory

ATCC American Type Culture Collection

AMR Antimicrobial resistance

AST Antibacterial susceptibility testing

BSI Blood stream infection

CLSI Clinical Laboratory standards Institute

CONS *Coagulase Negative Staphylococcus*

ESBL Extended spectrum beta-lactamase

MDR Multidrug resistance

MIC Minimum inhibitory concentration

MRSA Methicillin-resistant *Staphylococcus aureus*

PI Principal Investigator.

PLC Private limited company

SOP Standard operating procedure

Spp Species

V2C Viteck 2 compact

XDR Extensively drug resistance

Abstract

Background: Blood stream infection is one of the most important causes of morbidity and mortality globally. *Staphylococcus aureus*, *coagulase negative staphylococus*, *Streptococcus pneumonia* are major Gram- positive bacteria in causing blood stream infection. *Escherichia coli, Klebsiella pneumoniae, Pseudomonas* aeruginosa*, Acinetobacter baumannii* are major Gram-negative bacteria in causing blood stream infections. Development of multidrug resistance in major bacteria has increased the morbidity and mortality rate of blood stream infection.

Objective: To determine bacterial etiology of blood stream infection, the prevalence of multidrug resistance,XDR, Pan drug resistance and extended spectrum beta lactamase production of bacterial isolates.**Methods:**The present study was a laboratory based cross sectional study conducted at Arsho Advanced Medical Laboratory, Addis Ababa Ethiopia from Dec 2020 to June 2021.A total 0f 422 blood sample was collected and inoculated onto primary isolation following standards protocols. Species identification and antimicrobial susceptibility testing of bacteria were determined by automated Vitek 2 compact system (Bio Merieux,France) by using AST,GN72 card for gram negatives and AST,GP71 card for gram positive bacteria.

Results: Out of a total 422 samples processed, bacterial pathogens were isolated from 67(16%) samples. Among the isolates,40(59.7) were gram-negative and 27(40.3) were gram-positive bacteria. *Klebsiella spp* and *Coagulase negative staphylococci* were the dominat isolates. Of these isolates, 6 (60%) were ESBL positive for *K.pneumonae,1(50%) for K.oxytoca* and 4(66.7%) for *E.coli.*

Penicillin(90.2%) was the least effective antibiotic against Gram-positive bacteria while Ampicillin(87.5%) were the least effective antibiotic against Gram-negative bacteria.Piperacillin(92.5%) were the most effective antibiotic against Gram-negative and Tigecycline(91.7%) were the most effective antibiotic against Gram-positive bacteria. Out of 40 isolate of Gram-negative bacteria 21(52.5%) were MDR and from 27 isolates of Gram-positive bacteria16 (59.3%) were MDR.**Conclusion:** The magnitude of blood stream bacterial infection and the prevalence rate of multi-drug resistant bacterial strains causing blood stream infections were high. These findings were warranted the need for the continuous investigation of bacterial blood stream infection.

Keywords: Antimicrobial resistance, Multidrug resistance and Antimicrobial susceptibility.

1. Introduction

1.1 Background

Bacterial bloodstream infections are defined as the presence of viable bacteria in the bloodstream that can elicit an immune response. Bacteria may enter the bloodstream invasion, normally sterile parts of the body, in different ways. Bloodstream infections (BSIs) are characterized by high morbidity and mortality all over the world. BSI can be categorized as either community acquired or hospital acquired and can be the result of a broad variety of micro organisms (1)
Both bacteria and fungi can enter the normal sterile, bloodstream. These conditions are called bacteremia and fungemia respectively.*S.aureus*, *coagulase negative S.aures* and *E. faecalis* are commonest bacterial causes of BSI from gram positive organisms and *E.coli*,*K.pneumoniae,and Serratia* spp are from Enterobacteriaceae.*Pseudomonas* Spp and *A.baumannii* are the commonest from the non fermenter gram negative organisms and *non albicans Candida spp* followed by C. albicans are common from fungi (2).

Individuals with bacteremia may develop septicemia, a life threatening condition in which multiplying bacteria release toxins into the bloodstream and trigger the release of cytokines, causing fever, chills, malaise and lethargy, with difficulty in breathing especially in children. (3).BSIs ranging from mild infection to severe sepsis and septic shock causes high mortality, longer hospitalizations, and excessive cost to the patients and healthcare system(4).

Around 200,000 cases of bacteremia occur annually with mortality rates ranging from 20 - 50% worldwide (1).It causes millions of deaths globally each year. In case of neonatal sepsis it contributes nearly 13-15 % of all deaths and in developing countries where it contributes between 30-50 %(5).In sub Saharan countries including Ethiopia septicemia is an important cause of illness and death in children, the mortality rate approaches 53% which makes it a significant health problem in developing countries (6).

A minor injury occurring during tooth brushing, tooth extraction, abscesses, infected wound or boils, insertion of intravenous of bladder catheter, surgery and existing infections like lung infection, Urinary tract infection (UTI), gastrointestinal tract (GTI), burns or bedsores or from

areas of localized disease as in pneumococcal pneumonia, meningitis, pyelonephritis, osteomyelitis, cholangitis, peritonitis, enterocolitis and puerperal sepsis are the predisposing factor for bacteremia (7)

Antimicrobial resistance in bacterial pathogens is a worldwide challenge leading high morbidity and mortality in clinical settings. Multidrug resistant patterns in gram-positive and gram negative bacteria have resulted in difficult to treat or even untreatable infections with conventional antimicrobials .Since the early identification of causative microorganisms and their antimicrobial susceptibility patterns in patients with bacteremia and other serious infections is lacking in many health care institutions, broad spectrum antibiotics are liberally and mostly unnecessarily used. Such practice has, in turn, caused dramatic increases in emerging resistance and when coupled with poor practice of infection control, resistant bacteria can easily be disseminated to the other patients and the environment.(8)

Antibiotics resistance is a growing problem in developing countries such as Ethiopia. In Ethiopia the unregulated over-the-counter sale of these antimicrobials, mainly for self-treatment of suspected infection in humans, and to a lesser extent for use in animals without prescription, would inevitably lead to emergence and rapid dissemination of resistance (9)

Antimicrobial resistance occurs as a result of the selective pressure of antimicrobial usage and Extended-spectrum beta-lactamases (ESBLs) is also as an important cause of resistance in gram-negative bacteria. Beta-lactam antibiotics are among the safest and most frequently prescribed antimicrobial agents all over the world in treating Gram positive and gram negative infections. Production of beta-lactamases is the most common mechanism of bacterial resistance to these antibiotics. Extended spectrum beta lactamases (ESBL) are enzymes produced by Enterobacteriaceae that hydrolyze and inactivate most beta-lactams.They are frequently encoded by plasmids that carry genes conveying resistance to other antibiotic groups, such as amino glycosides and fluoroquinolones.According to the2013 report of the Centers for Disease Control and Prevention, ESBLproducing Enterobacteriaceae(ESBL-PE) were classified as a serious threat and the prevalence of ESBL infections keeps rising(10,11)

Blood culture is the gold standard to isolate the pathogen and knowledge about sensitivity pattern of the isolates remains the main stay of definitive diagnosis and management of BSI. Recently,

new techniques have become available for the rapid identification of pathogens from positive blood cultures. The VITEK 2 compact (bioMérieux, France) is a machine capable of running bacterial identification and drug susceptibility simultaneously. With regard to identification, VITEK 2 compact system utilizes 64 biochemical and substrates to cover a total of 115 Gram-positive and 135 Gram-negative taxain with an approximate turnaround time of seven hours. Identification of an isolate provides essential information on its pathogenic potential and is the most importance for the correct interpretation of antibiotic susceptibility testing Reduced turnaround times, better specimen management, enhanced quality control, reproducibility, precision, and the ability to track results are other benefits of the VITEK 2 compact system over conventional methods. The aim of this study is study prevalence of drug susceptibility profile of bacteria isolated blood samples collected from patients referred to Arsho Advanced Medical Laboratory by employing the fully automated VITEK 2 compact system (12)

1.2. Statement of the problem

Blood stream infections (BSI) caused by multidrug resistant (MDR) bacteria cause high morbidity and mortality. Around 200,000 cases of bacteremia occur annually with mortality rates ranging from 20 - 50% worldwide (1).It causes millions of deaths globally each year. In case of neonatal sepsis it contributes nearly 13-15 % of all deaths and in developing countries where it contributes between 30-50 %(5).In sub Saharan countries including Ethiopia septicemia is an important cause of illness and death in children, the mortality rate approaches 53% which makes it a significant health problem in developing countries (6).

This will leads to great economic loss encompassing use of more expensive antibiotics to treat infection as well as threat of resistance to them. The infections caused by MDR organisms are more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics .In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available by keeping in mind that high mortality and morbidity are associated with septicemia and right choice of empiric therapy is of importance. The increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community acquired BSI infections is making different classes of antimicrobial agents less effective resulting in emergence of antimicrobial resistance (7).

In our country, Ethiopia, it is wide practice that antibiotics can be purchased without prescription and without real etiological agent identification. This leads to misuse of antibiotics by the public thus contributing to increase spread of antimicrobial resistance of bacteria isolated from blood samples. . Hence, a study on the prevalence of blood stream infection, the distribution of etiological agents and their drug susceptibility patter by employing a fully automated VITEK 2 compact system for identification and AST simultaneously is of the highest priority.

1.3. Significance of the study

The antimicrobial susceptibility patterns of common pathogenic bacteria are essential to guide empirical and pathogen specific therapy; therefore, empiric antibiotic treatment is not effective in elimination of these pathogens much time in clinical practice. So identifying the various bacteria isolated from blood and studying their antibiotic susceptibility patterns in our study area will be one indicator in the appropriate treatment of patients to guide empirical and pathogen specific therapy for clinicians.

The results of this study could help to provide information on the magnitude of MDR and ESBL production of BSI causing organisms, help the physician in the selection of better antibiotic for treatment and helps to initiate epidemiological study on MDR and ESBL production in BSI causing organisms.

2. Literature Review

Multidrug resistant patterns in gram positive bacteria have resulted in difficult to treat or even untreatable cases and this cause the increase in mortality. A recent ECDC data from 2013 indicated a global increase in *methicillin-resistant staphylococcus aureus (MRSA*) (> 50%) isolated from blood, and a higher rate in vancomycin resistant *gram-positive coccus* in Europe. From gram negatives, *Enterococcus spp*. has shown increasing prevalence of acquired resistance to penicillin's, amino glycosides and vancomycin, which is observed in many countries (13).

A study conducted in Nepal on Etiology of bloodstream infection and antibiotic susceptibility pattern of the isolates shows that out of 1,205 blood samples, 15.4 %were culture positive. The most common bacteria isolated were: *Salmonella spp*. *Escherichia coli*, *Klebsiella pneumonia*e and *CONS*. Gram-negative bacteria were the predominant causes of BSIs.*Salmonella Typhi* was isolated in 71 % cases of bloodstream infection followed by *Salmonella Paratyphi A* in 16 %, *Escherichia coli* in 5.3 % and *Klebsiella pneumonia* in 0.5 %. The gram-positive organism responsible for causing BSI was *coagulase-negative staphylococcus* in 7 % cases. During ASTs, Gram-negative bacteria were sensitive to Chloramphenicol with only 0.5 % resistivity. *Salmonella Typhi* (85.6 % of isolates) showed resistance to Nalidixic acid. Gram-positive bacteria showed 100 % sensitivity towards Chloramphenicol and Gentamicin and were least sensitive to Amoxicillin. This study shows that *Salmonella spp*., was major cause of BSIs and recommend increase in antibiotic resistivity for BSI causing pathogens has necessitated continuous monitoring of the susceptibility of organisms towards antibiotics (14)

A study in Northern Vietnam on bacterial bloodstream infections etiology, drug resistance, and treatment outcome shows that from a total of 738 patients with BSI ,the predominant pathogens were *Klebsiella pneumoniae* (17.5%),*Escherichia coli*(17.3%),*Staphylococcu*s *aureus*(14.9%),*Stenotrophomonas maltophilia*(9.6%) and *Streptococcus* is(7.6%). The overall proportion of extended spectrum beta-lactamase (ESBL) production among *Enterobacteriaceae* was 25.1% and *of methicillin-resistance in S. aureus (MRSA*) 37%. This study shows that gram negative bacteria are the most common cause of both community and hospital acquired blood stream infection in adults presenting to their tertiary referral hospital, with high associated mortality. The extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* are prevalent in both community and health care setting (15).

A study from India carried out on prevalence & antimicrobial resistance pattern of extended spectrum B-lactamase producing *Klebsiella spp* isolated from cases of neonatal septicemia shows that from the 100 *Klebsiella* isolates, 58 were positive for ESBL production, which was much lower than 86.6 per cent reported in 2003. Almost all the isolates were sensitive to imipenam and meropenam.In this study drug resistance was found to be significantly more common in ESBL producing isolates than in non-ESBL producers and this study shows that there is a need of carefully formulated therapeutic strategies to control infections in NICUs and the high percentage of drug resistance in ESBL *producing Klebsiella spp* suggests that routine detection of ESBL is required by reliable laboratory methods (16).

Another prospective study conducted in India on resistant patterns of bacteria isolated from bloodstream infections at a university hospital shows from 168 isolated bacterial strains , The most frequently identified Gram-positive bacteria were *coagulase-negative staphylococci* 63.5%, *Staphylococcus aureus* 23.1%, *Enterococci* 5.8% and alpha hemolytic *streptococci* 5.8%. The most frequently Gram-negative bacteria identified were *Acinetobacter* species 31%, *Salmonella* typhi 24.1%, *Escherichia coli* 23.3% and *Pseudomonas aeruginosa* 13.8%. *Coagulase-negative staphylococci* showed maximum resistance to cefaclor 57.1% and ampicillin 46.9%. *Staphylococcus aureus* showed maximum resistance to amoxicillin 100% and ampicillin 91.7%. *Acinetobacter* species showed maximum resistance to amoxicillin 89.7%, amoxi-clavulic acid 87.1% and ampicillin 85.7*%. Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* showed maximum resistance to ampicillin, 46.4%, 92%, 93.8% and 100%, respectively (17**).**

A study carried out in Dhaka Bangladesh from 2005-2014 Bacterial etiology of bloodstream infections and antimicrobial resistance indicates overall, 13.6% of the cultured blood samples were positive and Gram-negative (72.1%) bacteria were predominant throughout the study period. *Salmonella Typhi* was the most frequently isolated organism (36.9% of samples) in this study and a high percentage of those strains were multidrug-resistant (MDR). However, a decreasing trend in the *S. Typhi* isolation rate was observed and, noticeably, the percentage of MDR *S. Typhi* isolated declined sharply over the study period. An overall increase in the presence of Gram-positive bacteria was observed, but most significantly we observed the percentage of MDR Gram-positive bacteria to double over the study period. Overall, Gram

positive bacteria were more resistant to most of the commonly used antibiotics than Gram-negative bacteria, but the MDR level was high in both groups.(18)

A hospital-based prospective cross-sectional study conducted in Tanzania on Bacteremia and resistant gram-negative pathogens among under-fives shows of the 1081 children admitted during the study period, The prevalence of bacteremia was 6.6%.*Escherichia coli* and *Klebsiella pneumonia* accounted for (33.3%)and(28.6%) of all the isolates respectively. Others gram negative bacteria; *Citrobacter spp* (9.5%), *Enterobacter spp* (4.25%), *Pseudomonas spp* (9.5%), *Proteus spp* (4.25%) and *Salmonella* spp (4.25%). These isolates were highly resistant to ampicillin (95%), cotrimoxazole (90%), tetracycline (90%), gentamicin (80%), augmentin (80%), chloramphenicol (65%), ceftriaxone (35%), cefotaxime (35%) ciprofloxacin (30%), amikacin (30%), ceftazidime (25%) and norfloxacine (10%)(19).

Another cross-sectional study conducted at Muhimbili National Hospital Dares Salaam, Tanzania between April and May 2018.on multi-drug resistant bacteria predict mortality in bloodstream infection shows ,There were 402 participants with blood culture included in the study, 11.4% (46/402) had culture-positive BSI. Gram-negative bacteria (74%) were the Common cause of BSI, with a predominance of *Enterobacteriaceae* (22), followed by *Pseudomonas aeruginosa* (11). The majority of bacteria (70.5%) isolated from patients with BSI Were Multi-drug resistant (MDR). Forty-six percent (46%) of *Pseudomonas aeruginosa* were resistant to meropenem while 68% (15/22) of Enterobacteriaceae were extended-spectrum β Lactamase producers.Carbapenemase production was detected in 27% (3/11) of *Pseudomonas Aeruginosa* and one *Proteus mirabilis*. Forty percent (40%) of *Staphylococcus aureus* were *Methicillin-resistant Staphylococcus aureus*.(20).

A study conducted in Unguja Zanzibar on Prevalence and Antimicrobial Resistance of Microbes Causing Bloodstream Infections shows Pathogenic bacteria were recovered from the blood of 14%of the patients (66/469). The most frequently isolated microbes were *Klebsiella pneumoniae, Escherichia coli, Acinetobacter spp. and Staphylococcus aureus.* Infections were community-acquired in 56 patients (85%) and hospital-acquired in 8 (12%) (Data missing for 2 patients). BSI caused by extended-spectrum beta-lactamase (ESBL) producing

Enterobacteriaceae (*E. coli, K. pneumoniae*) was found in 5 cases, of which 3 were community-acquired and 2 hospital-acquired. Three of these patients died. Six of 7 *Salmonella Typhi* isolates were multidrug resistant. *Streptococcus pneumoniae* was found in one patient only.(21)

A study from Egypt carried out on an emerging antimicrobial resistance in early and late onset neonatal sepsis, among the isolates gram positive cocci showed highest resistance to ampicillin (amoxicillin-sulbactam 100% and amoxicillin-clavulanate75%), cephalosporin's (Ceftazidime 94%, cefoperazone 100%,cefepime 86%, ceftriaxone 100%,cefuroxime 100%, cefoxitin 80%), carbapenems (Imipenem 84%, meropenem 86%),piperacillin-tazobactam (100%),and erythromycin (86%). Less resistance was evident to amino glycosides (Amikacin, 49%, gentamicin, 57%), quinolones (ciprofloxacin 77%, Levofloxacin 75%), clindamycin (53%), and rifampicin (49%). Least resistance among gram positive bacteria was found to vancomycin (18%) finally they concluded that there shall be global regulations to restrict the use of antimicrobials in the community as well as in the hospital setting (22).

A study on bacterial blood stream infections and antibiogram among febrile patients at Bahir Dar Regional Health Research Laboratory Center, Ethiopia shows of 561 blood specimens requested for blood culture, 220 blood cultures had aerobic bacterial growth. Gram negative bacterial isolates constituted 115 (52.3%) of the isolated bacteria. *Staphylococcus aureus* 50(22.7%), *coagulase negative staphylococci* 35(15.9%), *Klebsiella pneumoniae* 35 (15.9*%), Escherichia coli* 19 (8.6*%), Pseudomonas aeruginosa 15* (6.8%) and *Acinetobacter* species 13(5.9%) were the most dominant isolates. Overall, drug resistance for gram positive bacteria were 7 to 61% and for gram negatives 6.9 to 82.6%. Among the gram positive bacteria, high resistance levels were observed against penicillin (61%) and oxacillin (52.9%) (23).

A study on multi-drug resistance profile of bacteria isolated from blood stream infection at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia.Out of a total of 422 samples processed, bacterial pathogens were isolated from 64 (15.2%) samples. Among the isolates, 29 were Gram-positive and 35were gram negative bacteria. *Staphylococcus aureus* and *Klebsiella pneumoniae* were the dominant isolates. Penicillin (86.7%) was the least effective antibiotic against Gram-positive bacteria while ampicillin (85.7%) and amoxicillin clavulanic acid (77.14%) were the least effective antibiotic against Gram-negative bacteria. Clindamycin (80%) and amikacin

(97.1%) was the most effective antibiotic against Gram positive and Gram-negative bacteria, respectively. Out of 29 isolate of Gram-positive bacteria, 16 (55.2%) were multidrug resistant of which 11 (35.9.3%) were extensively drug resistant and 2 (6.9%) were pan drug resistant. Out of 35 isolates of Gram- negative bacteria, 26 (74.3%) were multidrug resistant of which 18 (54.4%) were extensively drug resistant (24).

A laboratory based prospective cross sectional study performed by Abera K. and his friends on Bacterial Profile of Adult Sepsis and their Antimicrobial Susceptibility Pattern at Jimma university specialized hospital, south west Ethiopia shows from a total of 95 adult septic cases involved in this research, 15 (15.8 %) were positive to eight different types of bacteria. Gram positive organisms were isolated in 53.3 % of these episodes with *Staphylococcus aureus* being the most frequent, while Gram negative accounted for the remaining 46.7 % with *Escherichia coli* being the commonest isolate among gram negative bacteria. The isolates shows high rates of resistance to most antibiotics tested in-vitro. The ranges of resistance to Gram positive bacteria were 0 % to 100 %, and to gram negative from 14.3 % to 85.7 %. In this study multi-drug resistance (resistance to three or more drugs) was observed in 80 % of isolates. Of this 87.5% and 71.4 % accounted for gram positive and gram negative bacteria respectively and ciprofloxacin is the effective drug against the tested gram positive and gram negative bacteria isolates (25).

A study done at Mekelle hospital Northern Ethiopian bacteriological profile and antimicrobial susceptibility patterns of blood culture isolates among febrile patients' shows out of the total 514 febrile patients, 144 (28%) culture positive were isolated. *S.aureus*54 (37.5%), *CONS* 44 (30.6%), *E. coli*16 (3.1%), *Citrobacterspp*. 9 (1.7%) and *S.typhi* 8(1.6%) were the most dominant isolates, collectively accounting for >90% of the isolates. Antimicrobial resistance pattern for gram positive and gram negative bacteria was 0–83.3% and 0–100%, respectively. High resistance was seen to Trimethoprim/sulphamethoxazole101 (70.1%), Oxacillin 65 (62.5%), Ceftriaxone 79 (58.9%) and Doxycycline 71(49.3%). Fifty-nine percent of the isolated bacteria in this study were multi drug resistant. Most bacterial isolates were sensitive to Gentamicin, Ciprofloxacin and Amoxicillin clavulanic acid. All gram positive isolates in this current study were sensitive to vancomycin. Prevalence of bacterial isolates in blood was high. It also reveals isolated bacteria species developed multi drug resistance to most of the antibiotics tested, which

high lights for periodic surveillance of etiologic agent, antibiotic susceptibility to prevent further emergence and spread of resistant bacteria pathogens (27).

3. Objectives

3.1. General objective:

- To determine bacterial etiology of blood stream infection, the prevalence of multidrug resistance,XDR, Pan drug resistance and extended spectrum beta lactamase production of bacterial isolates at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

3.2. Specific objectives:

- To determine the distribution of bacteria implicated in causing blood stream infection.
- To determine an overall antimicrobial susceptibility profile of bacterial isolates.
- To determine MDR, XDR, PDR and extended spectrum beta lactamase production of bacteria isolated from blood.

4. Hypothesis

The Prevalence of multi drug resistance and extended spectrum beta lactamase production of bacteria isolated in our laboratory could be similar with previous studies conducted in Ethiopia.

5. Materials and Method

5.1. Study Area

The study was conducted on different health facilities (hospitals, health service clinics) and Arsho branch laboratories and clinics. Patients referred to Arsho Advanced Medical laboratory (AAML) from different health facilities. But the test was carried out at AAML which is a private diagnostic laboratory found in Addis Ababa Ethiopia with nine branches located different part of the city. It is one of Accredited Laboratory by Ethiopian National Accreditation office with specific scopes since 2015.The laboratory is found at Kirkos Sub city, Woreda 02,House No 612.Curently the microbiology laboratory runs around 5-7 blood culture samples in average per day.

5.2. Study design and period

A laboratory based cross sectional study was conducted from Dec 2020 to September 2021 to identify the bacterial profiles and antimicrobial susceptibility pattern among patients come to the study area.

5.3. Population

5.3.1. Source population

All patients who was suspected for different illness and referred to the study site during the study period were the source of population.

5.3.2. Study population

All patients who were requested for blood culture test and volunteers to participate in during the study period were the study population.

5.4. Inclusion and Exclusion criteria

5.4.1. Inclusion criteria

All age and sex groups referred for blood culture test and that are volunteers were included.

5.4.2. Exclusion criteria

Patients those were not volunteer to participate in the study and who took antibiotics currently within the last 07 days were excluded

5.5. Study variables

5.5.1. Dependent variable

- Prevalence of isolated bacteria from blood culture
- Antimicrobial susceptibility pattern of bacteria

5.5.2. Independent variable

- Age
- Sex

5.6. Measurement and Data collection

5.6.1. Sample size calculation

The sample size was calculated based on single population proportion. Since there was limited published data available on prevalence of antimicrobial susceptibility pattern and extended beta

lactamase production from blood samples which were done by using an automated V2C system machine in Ethiopia. The value of p taken as 50% (0.50).Considering 95% confidence interval, 5% margin of error, and the sample size is calculated using the following standard formula.

The sample size $n= z(\alpha/2)^2 p(1-p)/d^2$

Where,

n = Sample size

α = level of significance

z = at 95% confidence interval Z value (a = 0.05) =>Z $\alpha/2=1.96$

p = prevalence

d = Margin of error at (5%) (0.05)

$n = (1.96)^2 0.5(1-0.5)/ (0.05)^2$

$n=384$

To minimize errors arising from the likelihood of noncompliance, ten percent of the sample size will be added to the normal sample. Accordingly the required sample size will be 422.

5.6.2. Sampling method

Convenient sampling techniques were used. Even if it is abiased;but it is an easily access able and convenient method.

5.6.3. Data collection procedure

Data was collected by using the structured data collection form to obtain information on socio demographic status, previous antibiotic usage. Informed consent was taken from each patient and verbal informed consent was taken on behalf of children and ICU patients from their parents or guardians (see more on annex 1-8)

5.7. Laboratory analysis

5.7.1. Specimen collection and culturing

Prior to blood collection, the skins of each study participants were disinfected with 70% alcohol and subsequently with Povidone-iodine. About 10 ml and 5 ml of venous blood in duplicates(x2) were collected aseptically from adults and children, respectively. Blood collected from each patient which collected from two different sites was inoculated into two blood culture bottles containing 50 ml and 25 ml of sterile brain heart infusion broth (Oxoid, Basingstoke, UK) aseptically. All blood culture broths were incubated at 370C; checked for sign of bacterial growth daily up to 7 days. Blood culture bottles that showed signs of growth were sub-cultured onto Blood agar base (Oxoid, Basingstoke, UK)) to which 5% sheep blood is added, Chocolate agar (Oxoid, Basingstoke, UK)) and, MacConkey agar (Oxoid, Basingstoke, UK)) in Bio safety cabinet.MacConkey agar plate was incubated aerobically at 37ºC for 18-24 while chocolate and blood agar were incubated in micro aerophilic (5-10% CO_2) incubator. Blood culture broth with no bacterial growth after 7 days were sub-cultured before being reported as a negative. Pure isolates of bacterial pathogen were preliminary characterized by colony morphology and Gram-stain. Identification and antimicrobial susceptibility testing of bacterial isolates were determined with automated VITEK 2 compact system following the instruction of the manufacturer.

5.7.2. Principle of VITEK 2 compact system

The VITEK 2 compact system is an automated microbiology bacterial identification and antimicrobial susceptibility system. Uses advanced colorimetry technology to determine individual biochemical reactions contained in a variety of microbe identification cards. After inoculation with a standardized suspension of the unknown organism, each self-contained cards is incubated and read by the instrument's internal optics. Comparison of results to known species specific reactions in the VITEK 2 database yields organism identifications. A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present (12).

5.7.3. Suspension Preparation for ID card and V2C AST-GN 72 and AST-GP 67 card

Was done by suspending in 3 ml of sterile saline (Aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube to achieve a turbidity equivalent to that of a McFarland 0.50 standard (range, 0.50 to 0.63), as measured by the DensiChek (bioMe´rieux) turbidity meter. These suspension were used for the inoculation of identification cards, while an AST card were inoculated after the bacterial suspensions was further diluted following the instruction of the manufacture. Then place the ID card into the test tube and then transfer the test tube into the Cassette. (12)

5.7.4. Inoculation

Identification cards were inoculated or filled with microorganism suspensions using an integrated vacuum apparatus. Each card has a pre-inserted transfer tube used for inoculation and has bar codes that contain information on product type, lot number, expiration date, and unique identifier that can be linked to the sample. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card were placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassettes can accommodate up to 10 tests (VITEK 2 Compact). The filled cassette were placed

manually (VITEK 2 compact) into a vacuum chamber station. After the vacuum applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells (12, 27).

5.7.5. Card sealing, Loading and Incubation

Inoculated cards were passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30cards. All card types were incubated on-line at 35.5 + 1.0ºC. Each card removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings based on their wave length, and then returned to the incubator until the next read time. Then data collected at 15-minute intervals during the entire incubation period (12, 27).

5.7.6. Bacterial Identification

Identification and antimicrobial susceptibility testing of isolated bacteria were determined with Automated VITEK 2 compact system using bacterial isolation and identification cards based on Manufacturer's instruction. The VITEK 2 compact system is an integrated modular system that consists of a filling, sealer unit, a reader-incubator, a computer control module, a data terminal, and a multi copy printer. The system detects bacterial growth and metabolic changes in the micro-wells of thin plastic cards by using a fluorescence-based technology. The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. (24)

Substrates and biochemical tests used for identification of gram negative bacteria were Ala-Phe-Pro-Arylamidase, Adonitol, L-Pyrrolydonyl-Arylamidase, L-Arabitol, D-Cellobiose, Beta-Galactosidase, H2S production, Beta-N-Acetyl-Glucosaminidase, GlutamylArylamidasepNA, D-Glucose,Gamma-Glutamyl-Transferase,Fermentation/Glucose, Beta-Glucosidase, D-Maltose, D-Mannitol, D-Mannose, Beta-Xylosidase, Beta-Alanine arylamidaseetc.. and Substrates and Biochemical tests used for identification of gram positive bacteria were DAmygdalin, Phaspatidiylinstolphospholipase,D-xylose,Urease,Ala-Phe-Pro-Arylamidase,Bgalactosidase,

Alphaglucosidase, cyclodextrine, Optochinresistance, Bacitracinresisistance, Llactatealkalization(11,28)

5.7.7. Drug susceptibility testing

Antimicrobial Susceptibility testing with the VITEK-2 compact system was performed using AST cards known as GN AST and GP AST cards. The cards were filled with inoculums in filling chambers .The VITEK-2 System automatically processes the antimicrobial susceptibility cards until MIC's are obtained. The VITEK-2 compact system subsequently corrects, where necessary for MIC's or clinical category in accordance with the internal database of possible phenotypes for microorganism antimicrobial agent combinations .Preparation of inoculums was done by transferring 280µL of culture suspension from the 0.5 McFarland culture suspension for gram positives and145 micro liter for gram negative for filling the identification cards(11,24).

Antibiotics with their different concentration used for determination of drug susceptibility profile in this investigation were Quinopristin/Dalfopristin, CefoxitinScreen, Benzyl penicillin, Oxacillin, Gentamicin, Ciprofloxacin, Levofloxacin, Inducible Clindamycin Resistance, Erythromycin,Clindamycin,Vancomycin and Tetracycline for gram positives ; Trimethoprim/Sulfamethoxaole,Ampicillin,Amoxicillin/ClavulanicAcid,piperacillin/ Tazobactam,Cefalotin,Cefazolin,Cefuroximeaxil,Cefoxitin,Cefpodoxime,Ceftazidime,Ceftriaxone,Cefepime,Gentamycine,Toberamycin,Ciprofloxacin,Levofloxacin and Teteracyclin for gram negatives.(11,28)

5.7.7.1. Extended spectrum beta-lactamase detection

Initial screening for ESBL was done by the diameters of zones of inhibition produced by Ceftazidime (30µg), Ceftriaxone (30µg) and Cefotaxime (30µg) found to be within the CLSI screening criteria. These breakpoints indicative of suspicion for ESBL production are: for CAZ≤22mm, CRO, ≤ 25 mm and for CTX≤ 27mm. Phenotypic detection of ESBL production was confirmed by double disk synergy test and combination (double disk potentiate) test according to CLSI 2020 (28) guidelines.

5.7.7.2 Double Disk Synergy Test (DDST)

The organism to be tested was spread onto a Mueller–Hinton agar plate. The antibiotic disks used were Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Aztreonam (30µg) and

Amoxicillin/ Clavulanic acid (20/10 µg). The four antibiotics were placed at distances of 20 mm (edge to edge) from the Amoxycillin/Clavulanic acid disk that was placed in the middle of the plate.After 24hr incubation, if an enhanced zone of inhibition between either of the cephalosporin antibiotics and the Amoxicillin/Clavulanic acid disk occurred, the test was considered positive.

5.7.8. Quality control

Standard Operating Procedures (SOP) were strictly followed verifying that the sample was properly collected; handled and transported at test site. Card and media meet expiration date and quality control parameters per CLSI guideline 2020.Visual inspections of cracks in media or plastic petridishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination was performed. QC was performed to check the quality of medium. Each new lots were quality controlled before use by testing the *E. coli* ATCC 25922 and/or *Staphylococcus aurous* ATCC 25923 standard strains. For ESBL, ESBL-positive *K. pneumoniae* ATCC 700603 and ESBL negative *E. coli* ATCC 25922 control strains were used in this study.

5.8. Data quality assurance

To maintain the quality of data the data collection form was pre-tested and collected data was checked carefully on spot and daily for their completeness, accuracy, and clarity. To assure the quality of laboratory results; Standard operating procedures (SOPs) of the microbiology laboratory of AAML were strictly followed in all steps of the Pre-analytical, analytical and post-analytical. In addition, well-trained and experienced laboratory professionals were participate in the laboratory analysis procedure.

5.8.1. Pre analytical phase

A Socio-demographic characteristic of patients was collected using structured data collection sheets after getting informed consent. All blood culture specimens were collected by well trained laboratory personnel by following standard operational Procedure. When specimens reach the laboratory, it was checked to ensure that the correct specimen had been sent and the name on the specimen was the same as that on data collection form. To avoid sample contamination leak proof and sterile sample container was used.

5.8.2. Analytical phase

All materials, equipment and Procedures was adequately controlled. All stains and reagents was clearly labeled, dated, and stored correctly. The preparation, fixation, staining and reporting of smears as detailed in the SOPs of the microbiology laboratory of AAML was strictly followed. At regular intervals and whenever a new batch of gram stain was prepared, control smears of appropriate organisms were stained to ensure correct staining reactions. For each item of equipment there was clear operating and cleaning instructions, and service sheets. The temperature of a refrigerator, incubator, and water-bath was monitored and documented Culture media was tested for Performance and sterility

5.8.3. Post Analytical Phase

Post-analytical phase the results were recorded with the patients' identification number. The terminology and format used in reporting was standardized. All reports were concise and clearly presented. Before leaving the microbiology laboratory, all reports were double checked for correctness. Purified bacterial cultures were stored in nutrient broth with 20% glycerol at –81°C by sub culturing every month. These cultures may be stocked in this condition for 5-10 years.

5.9. Data analysis and interpretation

Data entry and analysis will be done using SPSS 20 (Statistical Package for social sciences statistical software version 20) .The descriptive statistics was used to calculate and to see the relation between dependent variable and independent variables using frequencies and crosstabs. Finally, the results were presented on words, charts.

5.10. Ethical consideration

All ethical considerations and obligations were fully addressed, and the study was conducted after the approval of the Internal Review Board (IRB) of Arsho Advanced Medical Laboratory private limited company and Departmental Ethics and Research review committee" of the Department of Medical Laboratory Sciences, Collage Health Science, Addis Ababa University. Written informed consent was obtained from the participants before data collection. Each respondent was given the right to refuse to take part in the study and to withdraw at any time during the study period. All the information obtained from the study subjects were coded to maintain confidentially. When the participant was found to be positive for bacterial pathogen, they were informed and will receive proper treatment. An assent form would be completed and signed by a family member and/or adult guardian for participants under the age of 18 years.For those who admitted at health facility I have got the consent form from the person who have gave the care for them after they were asked and volunteer to participate.

5.11. Dissemination of results

The findings of this study will be forwarded to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University. And an attempt will be made to present the findings in different conferences and will be sent for peer-review journals for publication.

5.12. Operational Definition

Blood stream infections (BSIs)-is the condition in which pathogens have entered the bloodstream (1)

Multi Drug Resistance:MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories (29).

Antimicrobial resistance: Antimicrobial resistance is the ability of microbes to resist the effects of drugs that is, the germs are not killed, and their growth is not stopped. It happens when microorganisms such as bacteria change when they are exposed to antimicrobial drugs (30) .E**xtensively drug resistant (XDR)**: is defined as non susceptibility to at least
One agent in all but two or fewer antimicrobial categories (29).

Pan drug resistance (PDR): is defined as non Susceptibility to all agents in all

Antimicrobial categories (i.e.no agents tested as susceptible for that organism)(29).

Minimal Inhibitory Concentration (MIC): is the lowest concentration of antimicrobial agent that prevents visible growth of microorganisms in agar or broth dilution susceptibility test (29).

Non-susceptible: A bacterial isolate was considered non-susceptible to an antimicrobial agent when it tested resistant or intermediate when using clinical breakpoints as interpretive criteria provided by the Clinical and Laboratory Standards Institute (CLSI 2020) (29).

Susceptible: A bacterial isolate was considered susceptible to an antimicrobial agent when inhibited by usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used (29, 30).

Intermediate: implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used (29, 30).

Resistant: A bacterial Isolate was considered susceptible to an antimicrobial agent when not inhibited by the usually achievable concentrations of the agent with normal dosage (29, 30).

6. Results

6.1 Socio demographic characteristics

Four hundred twenty-two (n=422) eligible study participants were investigated during the study period. Of these participant 57% (n=242/422) of them were females and 43 % (n=180/422) were males. The majority of patients 37% (n=158/422) and 24% (n=100/422) were between 25-44 and 45-64 years of age respectively as shown below in Table 1, and the mean (std. deviation) ages of patients was 36.5(21.7).Among the total blood samples processed 84% (n=355/422) showed no growth for blood culture and 16% (67/422) show growth for gram positive and gram negative bacteria. From this 40.3% (n=27/67) were gram positive and 59.7 % (n=40/67) were gram negative bacteria. Females had a higher isolation rate than males 60% (n=24/40) versus 40 % (n=16/40) among the isolated gram negative bacteria and also females had higher isolation rate than males 66.7 %(n=18/27) versus 33.3 %(n=9/27) among isolated gram positive bacteria. The rate of isolation of gram negative and gram positive bacteria was highest in 25-44 years 27.5% (n=11/40) and in 45-64 years 37.0 % (10/27) respectively. As shown in **Table 1**.

Table 1: Frequency of gram-negative and gram positive bacteria isolates in relation to sex and age group at AAML from Dec 2020 to September 2021, Addis Ababa, Ethiopia (n=422).

Variables	Category	Total blood samples	No growth for bacteria N (%)	Growth of Gram negative N (%)	Growth of gram positive N(%)
Sex	Female	242(57%)	201(56.6)	24(60.0)	18(66.7)
	Male	180 (43%)	154(43.4)	16(40.0)	9(33.3)
	Total	422(100)	355(84)	40(9.6)	27(6.4)
Age group[1]	<1	17(4.0)	12(3.8)	5(12.5)	0(0.0)
	1-14	39(9.2)	29(8.2)	7(17.5)	3(11.1)
	15-24	31(7.3)	27(7.6)	1(2.5)	3(11.1)
	25-44	158(37.4)	141(39.7)	11(27.5)	6(22.2)
	45-64	100(23.7)	81(22.8)	9(22.5)	10(37.2)
	>65	77(18.2)	65(18.3)	7(17.5)	5(18.5)
	Total	422(100)	355(84.1)	40(9.5)	27(6.4)

[1]WHO age classification for health (33)

6.2. Prevalence of gram positive, gram negative bacteria and ESBL production

From the total bacterial isolates gram negative bacteria shown higher prevalence than gram positive bacteria. As shown in table 2 below *CONS* are the predominant isolated bacteria 16(59.3%) among gram positives followed by *S.aureus* 8 (29.6%), *E.faecalis* 3(11.1%), respectively.

Table 2: Frequency distribution of Gram positive bacteria isolated from blood specimens at AAML from Dec 2020 to September 2021 (n=27)

species	Frequency (n=27)	percentage
***CONS*(n=16)**		
S.lugdinesis	2	7.4%
S.warneri	4	14.8%
S.epidermidis	5	18.5 %
S.haemoliyticus	3	11.1%
S.hominis	2	7.4 %
Others		
S.aureus	8	29.6%
E.faecalis	3	11.1%
Total	27	100

Key;**S**-*Staphylococcus*

As shown in table 3 below *Klebsiella* spp 12(30%) are the most predominant one among the isolated gram negative bacteria followed by *Acinetobacter spp*10(25%), *E.coli 8*(20%), *E.cloacae* 3(7.5%) *P.aeruginosa 3*(7.5%),*Morexella* spp 2(5%),*Salmonella group A* 1(2.5%) and *pantoea* 1(2.5%) respectively. A total of 12 isolates of *Klebsiella* spp and 6 isolates of *E.coli* were isolated from blood. Of these isolates, 6 (60%) were ESBL positive for *K.pneumonae,1(50%) for K.oxytoca* and 4(66.7%) for *E.coli.*

Table 3: Frequency distribution of Gram negative bacteria and ESBL producing strains isolated from blood specimens at AAML from Dec 2020 to September 2021 (n=40)

Species	Frequency n(%)	ESBL N(%)
Klebsilla spp		
K.pneumonae	10 (25%)	6(60%)
K.oxytoca	2 (5%)	1(50%)
***Acinetobacter* spp**		
A.baumani	4(10%)	
A.iwoffii	5 (12.5%)	
A.xylosidans	1 (2.5%)	
Others		
E.coli	8 (20%)	4(50%)
E.cloacae	3 (7.5%)	
P.aeruginosa	3 (7.5%)	
Morexella spp	2 (5%)	
Salmonella groupA	1 (2.5%)	
Pantoea	1 (2.5%)	
Total	40(100)	

6.3. Antimicrobial susceptibility patterns of gram positive and gram negative isolates

The overall drug susceptibility profile of Gram positive and Gram negative bacteria against the seventeen antibacterial drugs tested were summarized under Table 4 and Table 5 for both gram positive and gram negative bacteria isolates. Gram positive bacteria have showed the highest sensitivity towards Tigicycline (91.7%) followed by Linezolid (89.9%) and minocycline (84.5).Penicillin have indicated the highest resistance rate (90.2%) followed by Oxacillin (47.8%) and Sulphamethazole/trimethoprim (46.1%).

The first commonly isolated gram positive bacteria *CONS* were exhibited high resistance for Penicillin (85.7%), Oxacillin (78.6%), Erythromycin (64.3%).The least resistance was observed in, Minocycline (7.1%).Whereas Tigicycline,Vancomycin ,Daptomycin have showed no resistance.

S aureus showed high resistance rates for Penicillin (75%), Trimethoprim/sulfamethoxazole (75%), Erythromycin (50%), Daptomycin(37.5%),Tetracycline and Ciprofloxacin (25%) each. Whereas Vancomycin, Minocycline, Linezolid showed no resistance. The least resistance was seen for Rifampicin, Clindamycin, Gentamycin, Daptomycin, Moxifloxacillin and Oxacillin which was *MRSA* (12.5%) each.

Similarly, three of the isolated *E.faecalis* have showed they were 100% resistance for Penicillin followed by Gentamycin, Ciprofloxacin, Levofloxacin, Moxifloxacin and trimethoprim/sulfamethoxazole (66.7%) each of them. No isolates of *E.faecalis* were found which resistant to Oxacillin,Vancomycin,Minocycline,Tigicycline and Rifampicin.The least resistance was Erytromycin,Clindamycin,Quinopristin/Dalfopristin,Linezoid and Tetracycline (33.3%) each.

Table 4: Percentage of antibacterial susceptibility pattern of gram positive bacterial isolated from blood culture at AAML from Dec 2020 to September 2021(n=27)

Speci	P	*PEN*	*OXA*	GEN	CIP	*LEV*											*TMP*
S aureus (n=8)	I	0	0	0	0	0	0	0	0	12.5	0	0	12.5	0	12.5	0	0	12.5
	R	75	12.5	12.5	25	25	12.5	50	12.5	37.5	0	12.5	0	0	25	0	12.5	75
	S	25	87.5	87.5	75	75	87.5	50	87.5	50	100	87.5	87.5	100	62.5	100	87.5	12.5
S.faecalis (n=3)	I	0	0	0	0	0	0	0	0	0	0	0	0	33.3	0	33.3	66.7	0
	R	100	0	66.7	66.7	66.7	66.7	33.3	33.3	33.3	33.3	66.7	0	0	33.3	0	0	66.7
	S	0	100	33.3	33.3	33.3	33.3	66.7	66.7	66.7	66.7	33.3	100	66.7	66.7	66.7	33.3	33.3
CONS(n=16)	I	0	0	14.3	7.14	7.14	7.14	0	0	0	0	0	7.14	21.4	14.3	0	0	0
	R	85.7	78.6	28.6	35.7	35.7	14.3	64.3	57.2	28.6	7.14	0	0	7.14	57.1	0	35.7	42.86
	S	14.3	21.4	57.2	57.2	57.2	78.6	35.72	42.9	71.4	92.9	100	92.86	71.4	28.6	100	64.3	57.14
Total [n=27]	I	0	0	3.6	1.8	1.8	1.8	12.5	0	3.1	0	0	17.4	13.7	19.2	8.3	16.7	3.1
	R	90.2	47.8	26.9	31.8	31.8	23.4	36.9	25.7	37.3	10.1	19.8	0	1.8	41.4	0	12	46.1
	S	9.8	52.2	69.5	66.4	66.4	25.2	50.6	74.3	59.6	89.9	80.2	82.6	84.5	39.4	91.7	71.3	50.8

PEN=Penicillin,OXA=Oxacillin,GEN=Gentamycin,CIP=Ciprofloxacin,LEV=Levofloxacin,MXF=Moxifloxacin,ERY=Erythromycin,CL=Clindamycin,QDA=Quinupristin/ Dalfopristin,LNZ=Linezolid,DAP=Daptomycin,VAN=Vancomycine,MNO=Minocyclin,TET=Tetracycline,TGC=Tigecycline,RIF=Rifampicin,TMP=Trimethoprim,P=pattern,I=intermediate,R=resistance,S=sensitive.

Among gram negatives Ampicillin shows high resistance(87.5%) followed by Cephalothin(80.5%) and Cefazolin(80%).High level of sensitivity was shown by Piperacillin/Tazobactom(92.5%),Levofloxacin(80%) followed by Gentamycin(77.5%) , Ciprofloxacillin(75%)and Tobramycin(70%).

The first commonly isolated gram negative organism *Klebsiella spp* shows high resistance for Ampicillin(100%) followed by Cephalothin,Cefazolin,Cefuroxime and,Ceftriaxone(91.7%)for each.also there is no *Klebsiella spp* isolates that resistant to Piperacillin/Tazobactam.

The second commonly isolated gram negative organism *Acinetobacter spp* shows high resistance for Ampicillin(90%).least resistance was seen Ceftazidime and Ceftriaxone(20%) and there was no resistance seen for Gentamycin, Levofloxacin,Piperacillin andTobramycin.

Among the isolated gram negative bacteria *E.coli* were the third predominant isolates with the high highly resistance for Ampicillin and Amoxacillin-Clavulinic acid (100%) for each. followed by Cephalothin,Cefazolin,Cefuroxime and Trimethoprim/sulfamethoxazole (87.5%) for each.The least resistance was shown for Gentamycin and Levofloxacin(50%).

The fourth isolated gram negative were *E.cloacae* showed high resistance 100% for Ampicillin,Amoxacillin/Clavulinic acid,Cephalothin,Cefazolin,Cefuroxime and Cefpodoxime.and high sensitivit100% were seen on Trimethoprim/sulfamethoxazole,Tobramycin,Piperacillin and Gentamycin.

The fifth isolated bacteria was *Pseudomonas spp* which shown high resistance for Ampicillin,Amoxacillin/Clavulinicacid,Cephalothin,Cefazolin,Cefuroxime,Cefoxiti,Ceftazidime, Ceftriaxone,Cefepime,Ciprofloxacin,Trimethoprim/Sulamthoxazole&Tetracycline(66.7%).The high sensitivity was seen on Piperacillin/Tazobactom,Levofloxacin,Gentamycin and Ciprofloxacin(100%) each.

The sixth isolated gram negative bacterial organism were *Moraxella spp* which was 100% resistance to Cephalothin,Cefazolin,cefuroxime,Cefoxitin and Ciprofloxacin .The high sensitivity 100% was shown in Cefepime,Gentamycin,Piperacillin/Tazobactom and Tobramycin.

The least isolated bacterial *were Salmonella group-A and Pantoea;*The high resistance (100%) showed for Ampicillin,Amoxacillin/Clavulinic acid,Cephalothin,Cefazolin,Cefuroxime,Cefoxitin,Cefpodoxime,Ceftazidime,Ceftriaxone and Cefepime;they were (100%) susceptible for the rest of antibiotics.*Pantoea* shown high resistance for Ampicillin and Amoxicillin/Clavulinic acid (100%) and also they were susceptible 100% for the rest of all antibiotics.

Table 5: Percentage of antibacterial susceptibility pattern of gram negative bacteria isolated from blood culture at AAML from Dec 2020 to September 2021 (n=40)

species		AMP	AMC	CEPH	CEFA	CEFU	CEFO	CEFP	CEFT	CEFT	CEFF	CIP	GEN	LEV	PIP	TOB	TET	TMP
***Klebsiella** spp(n=12)*	**I**	**0**	**8.3**	**0**	**0**	**0**	**0**	**8.3**	**0**	**0**	**0**	**0**	**0**	**16.7**	**8.3**	**25**	**8.3**	**0**
	R	**100**	**83.3**	**91.7**	**91.7**	**91.7**	**16.7**	**83.4**	**75**	**91.7**	**75**	**16.7**	**41.7**	**0**	**8.3**	**25**	**50**	**58.3**
	S	**0**	**8.3**	**8.3**	**8.3**	**8.3**	**83.3**	**8.3**	**25**	**8.3**	**25**	**83.3**	**58.3**	**83.3**	**83.3**	**50**	**41.7**	**41.7**
Acinetobacterspp(n=10)	**I**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**20**	**0**	**0**	**0**	**0**	**0**	**0**
	R	**90**	**90**	**60**	**60**	**50**	**70**	**60**	**20**	**20**	**30**	**10**	**0**	**0**	**0**	**0**	**40**	**30**
	S	**10**	**10**	**40**	**40**	**50**	**30**	**40**	**80**	**80**	**70**	**70**	**100**	**100**	**100**	**100**	**60**	**70**
E.coli(n=8)	**I**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12.5**	**0**	**0**	**0**	**37.5**	**0**	**0**
	R	**100**	**100**	**87.5**	**87.5**	**87.5**	**62.5**	**62.5**	**75**	**75**	**75**	**50**	**50**	**50**	**75**	**25**	**75**	**87.5**
	S	**0**	**0**	**12.5**	**12.5**	**12.5**	**37.5**	**37.5**	**25**	**25**	**25**	**37.5**	**50**	**50**	**25**	**37.5**	**25**	**12.5**
E.cloacae(n=3	**I**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	R	**100**	**100**	**100**	**100**	**100**	**66.7**	**100**	**33.3**	**33.3**	**33.3**	**33.3**	**0**	**33.3**	**0**	**0**	**33.3**	**0**
	S	**0**	**0**	**0**	**0**	**0**	**33.3**	**0**	**66.7**	**66.7**	**66.7**	**66.7**	**100**	**66.7**	**100**	**100**	**66.7**	**100**
***Moaxella** spp[N=2]*	**I**	**0**	**0**	**0**	**0**	**0**	**0**	**50**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	R	**50**	**50**	**100**	**100**	**100**	**100**	**50**	**50**	**50**	**0**	**100**	**0**	**50**	**0**	**0**	**50**	**50**
	s	**50**	**50**	**0**	**0**	**0**	**0**	**0**	**50**	**50**	**100**	**0**	**100**	**50**	**100**	**100**	**50**	**50**
P.aerugenosa [n=3]	**I**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	R	**66.7**	**66.7**	**66.7**	**66.7**	**66.7**	**66.7**	**66.7**	**66.7**	**66.7**	**66.7**	**0**	**0**	**0**	**0**	**33.3**	**66.7**	**66.7**
	S	**33.3**	**33.3**	**33.3**	**33.3**	**33.3**	**33.3**	**33.3**	**33.3**	**33.3**	**33.3**	**100**	**100**	**100**	**100**	**66.7**	**33.3**	**33.3**
***Salmonella** group A[n=1]*	**I**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	R	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	S	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

***Pantoea*(n=1)**	**I**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	R	**100**	**100**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	S	**0**	**0**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Total(n=40)	**I**	**0**	**2.5**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**7.5**	**0**	**5**	**2.5**	**15**	**2.5**	**0**
	R	**87.5**	**87.5**	**82.5**	**80**	**77.5**	**52.5**	**70**	**55**	**57.5**	**55**	**25**	**22,5**	**15**	**5**	**15**	**50**	**50**
	S	**12.5**	**10**	**17.5**	**20**	**22.5**	**47.5**	**25**	**45**	**42.5**	**45**	**75**	**77.5**	**80**	**92.5**	**70**	**47.5**	**50**

Ampicillin=AMP, Amox/clavulic acid =AMC ,Peperaciline/tazobactum=TZP,Cefalotin=CEPHA ,Cefazolin=CEFAZ,Cefoxitin=CEFOX,Cefpodoxime=CEFPO,Ceftazidime=CEFAZ,Ceftriaxone =CFTR,Cefepime=CEFEP,Gentamicin=GEN,Tobramicine=TOB,Ciprofloxacilin=CIP, Levofloxacin =LEV, Teteracycline=TET,Trimethoprime=TMP,P =pattern, I= intermediate R= resistance S=sensitive.

6.4. Multidrug resistance patterns for the isolated gram positive and gram negative bacteria.

Based on the finding of the study as shown in Table 6 below, the overall prevalence of multi drug resistance(resistant for more than three different classes of antibiotics) was 37(55.2%).The rate of gram positive bacteria was 16(59.3%) .Of the 40 isolates of the gram negative bacteria 21 (52.5%) were MDR.Among the gram positive bacteria *CONS and Enterococcus spp* isolates were highly multi-drug resistant 9(62.3%) and 2(66.7%) respectively, while in gram negatives; MDR were observed highly *in E.coli* and *Klebsiella Spp;*5(62.5%) and 6(50%) respectively. also there was 2(5%) of XDR isolates among gram negative bacteria of *Klebsiella spp*1(8.3%) and *E.coli1*(12.5%).There was no pan-drug resistance blood stream infection causing organism found in this study .

Table 6;Multidrug resistance pattern of gram positive negative bacterial isolates from blood samples at Arsho Advanced Medical Laboratory from Dec 2020 to September 2021

Isolated Gram positive bacteria	**Resistant to antibiotics N (%) in gram positive bacteria**				
	R0	R1	R2	MDR(≥R3)	XDR
***CONS*(n=16)**	1(6.3)	3(18.7)	2(12.5)	10(62.5)	0
S.aureus(n=8)	2(25)	0	2(25)	4(50)	0
E.faecalis(n=3)	0	1(33.3)	0	2(66.7)	0
Total	3(11.1)	4(14.8)	4(14.8)	16(59.3)	0
Isolated Gram negative bacteria	**Resistant antibiotics N (%) in Gram negative bacteria**				
***Klebsiella spp*(n=12)**	0	1(8.3)	4(33.3)	6(50)	1(8.3)
***Acinetobacter spp*(n=10)**	0	1(10)	4(40)	5(50)	0
E.coli(n=8)	0	1(12.5)	1(12.5)	5(62.5)	1(12.5)
E.cloacae(n=3)	0	0	1	2	0
***Pseudomonas Spp*(n=3)**	0	1	1	1	0
***Moraxella Spp*(n=2)**	0	0	0	2	0
***Salmonella group-*A(n=1)**	0	0	1	0	0
***Pantoea*(*n*=1)**	0	1	0	0	0
Total	0	5(12.5)	12(30)	21(52.5)	2(5)

RO-no resistant for any antibiotic,R1-resistant for 1class of antibiotic,R2-resistant for class 2 antibiotics,R3-resistant for 3 class antibiotics,>R3-resistant for more than 3 class of antibiotics.

NB:Class of antibiotics was made based on the CLSI 2020 . XDR: non-susceptible to 1 agent in all but 2 categories.

7. Discussion

Blood stream infection causing, ESBL-producing and antimicrobial resistance bacteria have become a serious worldwide problem. Dissemination of ESBLs compromises the activity of broad-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcomes for patients.(1,10,11)

The present study found that 67(16%) out of 422 total blood sample screened from suspected BSI cases were positive for the presence of bacteria (Table 1).This bacterial isolation rate is comparable with the previous studies done in Jimma Ethiopia 15.8% by Abera K and in Nepal 15.4% (14,25).Even if it was lower than reports from Mekelle Ethiopia 28% and Bahir dar Ethiopia 39.2% (23,26) this was may due to the patient status in which others only include inpatient and also isolated anaerobic bacteria. But it was higher than studies done in Tanzania 6.6%, Dhaka Bangladesh 13.6%, Muhimbili Dares salam 11.4, Unguja Zanzibar 14% (18, 19, 20,25). The difference between these studies might be due to difference in an infection control police in our country weak when we compare with other nations, the difference in the study participants, risk factors or extent of antibiotics use, Furthermore blood cultures were performed by using the more sensitive automated Vitek 2 compact system.

Gram negative bacterial strains have been the most prevalent causative pathogens of BSI in the present findings. This result is in agreement with the previous studies done in India shows 69% gram negative and 31% gram positives, Tanzania (69.7%, 30.3%) and Nepal (89.19%,) and 10.81%).In contrarily the cause of BSI in other studies conducted in Jimma, where Gram-positive microorganisms were 53.3% and gram negative microorganism were 46.7% and Mekelle Ethiopia 72.2% of the blood stream infections were caused by gram positive and 27.8% by gram negative bacteria. This variation might be due to epidemiological difference of the etiological agents, study settings (14, 16, 19, 25, and 26).

In this study gram negative bacterial isolates which was 59.7% ; where the most common one was *Klebsiella spp* 30%(n=12/40);from this *Klebsiella pneumoniae* was the leading gram negative species with frequency of 25%. Other Gram negative bacteria such as *Acinetobacter* and *E.coli* each accounts 25% and 20% respectively. Similar studies also *reported K,pneumoniae*

as the most frequently isolated organisms with the study conducted at Tikur Anbesaa hospital Addis Ababa,(24).In contrary other studies in Mekelle by Wassihun *E.coli*(10.4%) was the predominant gram negative bacteria followed by *S.typhi and Citrobacter spp* (5.6%) each. This variation may be due to the study settings (26).

Gram positive bacterial isolates were accounted for 40.3% in the present finding. *CONS* was the leading gram positive spp with the frequency of 59.3% (n=16/27) and other gram positive bacteria such as *S.aureus* and *Enterococcus* accounts 29.6% and 11.1% respectively. This is in agreement with the previous study conducted in Gondar Ethiopia 31.6%, India 63.5% and 33.3% (27, 16, and 15).Even if there is a variation in their prevalence and dissimilar to other studies that report *S.aureus* as major isolate. This variation might be due to most studies *CONS* were considered as contaminant.But now they are potentially important pathogens and their increasing incidence has been recognized. In recent years, *CONS* have become the major cause of nosocomial blood stream infections to some extent as results of the increasing use of intravascular devices and increased number of hospitalized immune compromised patients.

In this study the overall drug resistant rate of gram positive bacteria ranges from 0% for Tigecycline and 90.2% for Penicillin and of gram-negative bacteria ranged from 5% for piperacillin to 87.5% for Ampicillin. Similarly the overall antibiotic resistant rate of gram positive bacteria showed in India 0-100% (16).and gram negatives in Gondar 20-100% (27).The variation might be due to lack of antibiotic surveillance, antibiotics misuse, and weak infection control measures. About 85.7% strain of *CONS*, which was the most commonly isolated gram positive bacteria, was resistant to penicillin. This result was comparable with the result conducted in, Jimma and Addis Ababa (24, 25)

Overall multidrug resistance level was 55.2%.Of gram negative and gram positive bacterial isolates was 52.5% (n=21/40) and 59.3 %(n=16/27) respectively. The MDR level of the most frequently isolated pathogens; *Klebsiella* and *CONS spp* showed 50%(n=6/12) and 62.3% (n=9/14) lower than the previous studies done by AdaneBitew.et al 100%(n=13/13) and 75% (n=6/8) respectively. This difference might be due to the difference in study setting, previous antibiotic usage and also the awareness about the usage of drugs may be increased.

In the present study, the magnitude of ESBL-producing bacteria was 16.4%, which is lower than magnitudes reported by previous researchers in Northern Vietnam 25.1 %(15), in Dare salaam 68% (20). The difference may be due to the study participant and method difference.

In this study the magnitude of XDR bacterial was 5.0%, which is lower than the previous studies reported at Tikur Anbessa Specialized Hospital 54.4% (24). The difference may be due to Lack of antibiotic surveillance, antibiotics misuse, weak infection control measures, different study participants, awarness of the participants may also contribute to the magnitude of extensively drug resistance.

8. Strengeth and limitation

8.1. Strength

- Used the advanced VITEK 2 Compact system which was fully automated microbial identification and antimicrobial susceptibility system that provides highly accurate and reproducible results.

8.2. Limitation of the study

- Lack of patient's clinical history in their request paper which could have been a good variable for this study.
- Being the study as a single laboratory based it may lack representativeness.

9. Conclusion

In this study both Gram negative and Gram positive bacteria were responsible for blood stream infection. *Klebsilla* and *CONS spp* were among the most common Gram negative and Gram positive organisms identified causing blood stream infection, respectively. Multidrug resistance was detected in 55.2 % of isolates.

10. Recommendations

- A periodically surveillance of antimicrobial resistance pattern record and report is essential to develop treatment guideline.
- Establish antibiogram based on the susceptibility pattern for empiric therapy at national level is very crucial
- .Establishing health laboratories with modern methods such as VITEK 2 compact system for accurate identification of bacteria pathogens to the species level and determining drug susceptibility pattern of the etiologic agents for efficient management of bacterial infections should be considered for routine laboratory diagnosis.
- To prevent further emergence and spread of MDR bacterial pathogens, rational use of antibiotics and regular monitoring of antimicrobial resistance patterns are essential and mandatory in the management of blood stream infection.

11. References

1. Michel AF, Ronald NJ, Gary VD, Kari K, Bacterial pathogens isolated from patients with bloodstream infection frequencies of occurrence and antimicrobial susceptibility patterns from the sentry antimicrobial surveillance program, Antimicrobial agents and chemotherapy, 1998; 42: 7, 1762–1770.

2 Renu B, Abhijit B, Ketoki K, Vidya M and Amita G Blood Stream Infections, Biomed Research International Volume 2014 available fromhttp://dx.doi.org/10.1155/2014/645802.

3 Majida Q , Farooq A Prevalence of microbial isolates in blood cultures and their antimicrobial susceptibility profiles, Lahore Biomedica j ,27, 2011, .136-139.

4 Babay HA, Twum-Danso K, Kambal AM, Al-Otaibi FE. Bloodstream infections in pediatric patients. Saudi Med J 2005; 26:1555-61.

5 Sarkar SK, Battacharyya A, PariaK,MandalSM, A retrospective study on bacteria causing blood stream infection, antibiotics resistance and management, Indian journal of pharmaceutical sciences, 2018;80,3:547-551.

6 Aiker AM, Mturi N, Niugana P: Risk and cause of pediatrics hospital acquired bactermia Klifi district hospital, Kenya: prospective cohort study. 2011; 10, 37:2012–2017.

7 Prakash S, Shiva Raj K. C., Santosh L, Surya S and UpendraTS.Bacteriological Profile and antibiotic susceptibility pattern of blood culture isolates from patients visiting tertiary Care hospital in Kathmandu Nepal, Global Journal of Medical Research, Volume 16 Issue 1 Version 1.0 Year 201.

8 Murat Akova. Epidemiology of antimicrobial resistance in bloodstream infections. Virulence 2016; 7, 3, 252–266

9 Zenebe T, Kannan S, Yilma D, Beyene G: Invasive Bacterial Pathogens and their antibiotic susceptibility patterns in Jimma University specialized Hospital, Jimma, Ethiop J Health Sci 2001; 21,1:1–8.

10 Sibhghatulla S, Jamale F, ShaziS,Syed M. Danish R, Mohammad AK. Prevalence of multidrug resistant and extended spectrum beta-lactamase producing Pseudomonas aeruginosa in a tertiary care hospital. Saudi journal of biological sciences,2015;22:62-64

11 Centers for Disease Control and Prevention Antibiotic resistance threats in the United States, 2013.Available from:http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.

12 Pincus DH. Microbial identification using the bioMérieuxVitek 2 system: Inc. Hazelwood,MO, USA 2016.

13 Qiang Z , Epidemiology and microbiology of gram-positive bloodstream infections in a tertiary-care hospital in Beijing, China, Antimicrobial Resistance and Infection Control 2018 7:107

14 Prashubha B, Sarita M, Basudha S, NabeenD. Etiology of bloodstream infection and antibiotic susceptibility pattern of the isolates, Asian journal of medical sciences2016; 7:

15 Dat VQ, Vu HN, Nguyen H . Bacterial bloodstream infections in a tertiary infectious diseases hospital in Northern Vietnam: etiology, drug resistance, and treatment outcome. *BMC Infect Dis*. 2017; 17:493.

16 Amita J & Rajesh M. Prevalence & antimicrobial resistance pattern of extended spectrumb-lactamase producing Klebsiella spp isolated from cases of neonatal septicaemia,Indian J Med Res 125, 2007, 89-94

17 Alam M S, Pillai P K, Kapur P, Pillai K K. Resistant patterns of bacteria isolated from bloodstream infections at a university hospital in Delhi. J Pharm BioallSci 2011; 3:525-30

18 Ahmed D,NahidAMd, Sami BA,Halim F, Akter N, SadiqueT,et.al. Bacterial etiology of bloodstream infections and antimicrobial resistance in Dhaka, Bangladesh, 2017; 6:2

19 Christopher A, Mshana SE, Kidenya BR, Hokororo A, Morona D. Bacteremia and resistant gram-negative pathogens among under-fives in Tanzania. Ital J Pediatr. 2013; 39:27. doi:10.1186/1824-7288-39-27

20 Manyahi J, Kibwana U, MgimbaE, Majigom.Multi-drug resistant bacteria predict mortality

in bloodstream infection in a tertiary setting in Tanzania, 2020 https://doi.org/10.1371/journal.pone.0220424

21 OnkenA,SaidAK,Jørstad M, Pål A. Jenum MP, Bromberg .B.Prevalence and Antimicrobial Resistance of Microbes Causing Bloodstream Infections in Zanzibar,2015.e0145632. doi:10.1371/journal.pone.0145632

22 Lamiaa M, Nermin R, Dalia S, Dina A, Niveen S, Mona M and Hany .Emerging antimicrobial resistance in early and late-onset neonatal sepsis. Antimicrobial Resistance and Infection Control -2017:1-9

23 . Derese H, Bayeh A, Gashaw Y, Daniel M , Awoke D,Bacterial blood stream infections and antibiogram among febrile patients at Bahir Dar Regional Health Research Laboratory Center, Ethiopia,Ethiop. J. Sci. & Technol.2016; 9,2 103-112.

24. Seneshat E, Adane B, Tigist G, Dessie A, SolomoanG.Multi-Drug Resistance Profile of Bacteria Isolated from Blood stream infection at tikuranbessa specialized hospital, Addis Ababa, Ethiopia, ECmicrobiology14.3, 2018; 126-128

25. Abera K, Tesfaye K, Zewudneh S, Deresse D ,AndualemH.Bacterial Profile of Adult Sepsis and their Antimicrobial Susceptibility Pattern at Jimma university specialized hospital, south west Ethiopia,Health science journal 2016 ; 10.2:3

26..Wasihun et al.(2015) Bacteriological profile and antimicrobial susceptibility patterns of blood culture isolates among febrile patients in Mekelle Hospital, Northern Ethiopia.SpringerPlus 4:314 doi 10.1186/s40064-015-1056

27. Simgamsetty S. Ease with VITEK 2 systems, biomerieux in identification of non-lactose fermenting bacteria including their antibiotic drug susceptibility.Int J Res MedSci.2016; 4, 3:813-817

28. Clinical and Laboratory standards Institute (CLSI), Performance standards for Antimicrobial susceptibility Testing; CLSI supplement M100.2020.

29.Magiorakos A,Srinivasan A, Carey R, Carmeli Y, FalagasM,Giske C, et al. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an

international expert proposal for interim standard definitions for acquired resistance. European Society of Clinical Microbiology and Infectious Diseases-2011; 18:277-281.

30. WHO. Antimicrobial Resistance. Fact Sheet-2017. [Cited 2018 Nov 21]; Available from: http://www.who.int/mediacentre/factsheets/antibiotic-resistance/en/

31. Clinical and Laboratory Standards Institute (CLSI), Analysis and presentation of cumulative AST data; approved guideline M39-A4 .2014.

32.Silley P. Susceptibility testing methods, resistance and break points: what do these terms really mean? Rev. Sci. Tech. Off. Int. Epiz 2012; 31 (1): 33-41

33. World Health Organization. Provisional Guidelines on Standard International Age Classifications: Statistical Papers, WHO, United Nations, New York 1982; 74: 4-11.

Annexes

Annex 1: participants' information sheet [English version].

Principal Investigator: MisganaAbera, Addis Ababa University school of Allied Health Sciences.

Purpose: The purpose of this study is to assess the prevalence of multi drug resistance and extended beta lactamase production of isolated bacteria from blood culture.

Procedures to be carried on: you are invited to participate in the study after giving your consent and by giving the requested sample for investigation.

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study and there is no additional time required from you to stay during study.

Benefits of the study: There will be no financial benefit to you. But the result of the study will be used for to develop anti biogram that helps the patients avoiding empirical treatment.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you later on change your mind 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.

Annex 2. Informed consent [English version]

I, the undersigned individual, am oriented about the objective of the study. I have informed that all of my information will be kept confidential and used only for this study. Your signature below indicates that you have read /or listened, and understand the information provided for you about the study.

Before you sign, please understand purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have any question.

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.

Based on the above information I agree to participate in the research

Signature: ___________________ Date: __________________

Name of Data collector ___________________________ Signature ___________ if you have any question you can ask the principal investigator

Principal investigator MisganaAbera (BSc, MSc candidate)

Mobile: 0920123659

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Annex 3: Participant's information sheet [Amharic version]

ጥናቱን የምያጠናው :ምስጋና አበራ፡ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይን ስዲፓርትመንት

የጥናቱ አላማ፤ የጥናቱ ዓላማ መድሃኒት የተለማመዱ በደም ውስጥ የሚገኙ ባክቴሪያዎችን ስርጭት በአርሾ አድቫንስድ ሚዲካል ላቦራቶሪ ለብለድካልቸር ምርመራ ከተላኩ ናሙና ውስጥ በመለየት የፀረባክቴሪያ መድሃኒት የመቋቋም አቅማቸውን ማወቅ፤ አሁን ያሉበትን ደረጃ ማሳየት እና የመፍትሄ አቅጥጫ ማስቀመጥ።

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም፤ በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም ነገር ግን ከጥናቱ የሚገኘው ውጤት ለርስዎ ህክምና ተጨማሪ መረጃ ለማግኘት በተመሰሳይ ለመድሃኒት ልምምድ ያደረጉትን ካላደረጉት በመለየት ውጤታማ የሆኑትን መድሃኒቶች ይጠቁማል።

በጥናቱተ ሳታፊዎች ላይ ያለው ጉዳት፤

በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም።በጥናቱ ምክንያት የሚያባክኑት ተጨማሪ ጊዜም አይኖርም።

Annex 4. Informed consent [Amharic version]

የመረጃ ሚስጥራዊአጠባበቅ፣ የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል። በጥናቱ ላይ ያለመሳተፍ መብት አለዎት። ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል። በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማና ለህክምና ባለሙያዎች ብቻ የሚገለፅ ይሆናል። ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ። እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼያለሁ።

ፊርማ ----------------------------------

መረጃውንየሰበሰበው/ግለሰብስም----------------------------- ፊርማ -------------------------

የዋና ተመራማሪ አድራሻ፡ምስጋናአበራ አዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይን ስኮሌጅ፣ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት አዲስ አበባ፣ኢትዮጵያ

ኢ-ሜይል፣**misganaabera222@gmail.com**

ስ.ቁ +251920123659

Annex 5. Parental/Guardian consent form in English

I, the undersigned, have been told about this research. My child has to say to choose if I want to be in the study. I have been informed that other people will not know my child results as it coded

with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. 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.

Parent/guardian Signature: ____________________ Date: ___________________

Name of Data collector ____________________________ Signature ___________ if you have any question you can ask the principal investigator

Principal investigator Misgana Abera(BSc,MSc candidate)

Mobile 0920123659

E-mail.misganaabera222@gmail.com

Annex 6.Guardian /parental consent form in Amharic

የወላጅ/የአሳዳጊ/የሞግዚት የስስምምነት መጠየቂያ ቅጽ

እኔ ፊርማዬ ከዚህ በታች የተቀመጠው -የታማሚውወላጅ/አሳዳጊ/ሞግዚት ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ።

በጥናቱ ወቅትም ታማሚው መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ። በውጤቱ ከሚገኘዉ የህክምናአገልግሎት በቀርሌላታማሚው በግሉየ ሚያገኘዉ ጥቅም እንደሌለተረድቻለሁ።ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኛለ ጥያቄዎቼምበቂምላሽአግኝቻለሁ። የልጄ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደ ሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ ታማሚው ላይ እንደ ማያስከትል ተረድቻለሁ። በከዚ ህባሻገር ታማሚው በጥናቱ ውስጥ ለመካተት የእኔ ወላጅ አሳዳጊ/ሞግዚትፈቃድእንደ ሚያስፈልግ ተረድቻለሁ። በእኔ ፍቃደኝነት ታማሚው በጥናቱ እንደሚሳተፍ ከዚህበታች በፊርማዬ አረጋግጣለሁ።

የጥናቱተሳታፊ ወላጅ/አሳዳጊ/ሞግዚትፊርማ ______________________

መረጃውን የሰበሰበው ግለሰብስም--

ፊርማ -------------------------

የዋና ተመራማሪ አድራሻ

ምስጋና አበራ

አዲስ አበባ ዩኒቨርሲቲ፣የጤና ሳይን ስኮሌጅ፣የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት

አዲስ አበባ፣ኢትዮጵያ

ኢ-ሜይል.**misganaabera222@gmail.com**

ስ.ቁ +251-920123659

Annex7: Assent form for adolescent (12 -17 years old) study participants (English version)

I, the undersigned, have been told about this research. My parents or guardian have to say to choose if I want to be in the study. I have been informed that other people will not know my results as it coded with number rather than writing my name if I am in this study. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. 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 parents/guardian. By signing below I agree to participate in this research study.

Study participant Signature: ____________________ Date: ___________________

Name of Data collector ____________________________ Signature ___________ if you have any question you can ask the principal investigator

Principal investigator Misgana Abera (BSc,MSc candidate)

Mobile 0920123659

E-mail.misganaabera222@gmail.com

Annex 8: Assent form for adolescent (12-17 years old) study participants (Amharic version)

በአማርኛ የተዘጋጀ ዕድሜያቸዉ ከ 12 እስከ 17 ዓመት ለሆኑ ታዳጊ ወጣቶች የጥናት ተሳታፊዎች የተሳትፎ ማራጋጋጫቅጽ

ከዚህ በታች ስሜ የተገለጠው በዚህ ጥናት ውስጥ እንድሳተፍ ፍቃደኝነቴን ተጠይቂያለሁ። ወላጆቼም/አሳዳጊዎቼም በጥናቱ እንድሳተፍ ወይም እንዳልሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል በጥናቱ ወቅትም የእኔ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደ ማይታወቅ ተረድቻለሁ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ በግሌ የማገኘዉ ጥቅም እንደ ሌለ ተረድቻለሁ። ጥያቄ እንድ ጠይቅ ዕድል ተሰጥቶኛል ጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ። በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደ ሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በእኔ ላይ እንደ ማያስከትል ተረድቻለሁ። በከዚህ ባሻገር የኔ በጥናቱ ውስጥ ለመካተት የወላጆችም ወይም የአሳዳጊዎቸ ፈቃድ እንደ ሚያስፈልግ ተረድቻለሁ።በፍቃደኝነቴ በጥናቱ እንደምሳተፍም ከዚህበታች በፊርማዬ አረጋግጣለሁ።

የጥናቱ ተሳታፊፊርማ ----------------------------------ቀን------------------------------------

መረጃውን የሰበሰበው ግለሰብ ስም---

ፊርማ -------------------------

የዋና ተመራማዉ አድራሻ

ምስጋና አበራ

አዲስ አበባ ዩኒቨርሲቲ፣የጤና ሳይንስ ኮሌጅ፣የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት

አዲስ አበባ፣ኢትዮጵያ

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Annex 9: Laboratory data collection form

i. . Patient identification

Sample ID.______________________________

Age (years) ________________

Gender Male ☐ Female ☐

II. Laboratory Data

1. Date of specimen collection__________________

2. Gram stains result __________________________

3. Biochemical test____________________________

4. Organism isolated __________________________

5. Drug susceptibility pattern

    5.1. Sensitive to _____________________________

    5.2. Intermediate to ___________________________

    5.3 Resistance to _____________________________

III. Comments____

Name of principal investigator ____

Signature ________________ Date ______________

Annex 10.Procedure for blood collection

1. Palpate and identify appropriate vein

2. Disinfect the puncture site with iodine or alcohol (70%) beginning in the center and rubbing vigorously outward in concentric circles (approximately 50mm diameter).
3. Iodine should remain in contact with skin for about 1 minute or until dry to ensure disinfection.
4. Blood is obtained by inserting a needle into a vein in the arm.
5. **5 ml** of blood from a child is added to 25 **ml** of blood culture broth and **10 ml** of blood from an adult is added to **50 ml** of blood culture broth. It is important to use appropriate ratios of blood to culture broth for optimal bacterial growth.
6. Put the first collected blood in one bottle and repeat collecting of another blood from different site (example; left and right hand) with the same volume for the second bottle. These two bottles constitute one blood culture set.
7. Gently rotate the bottles to mix the blood & the broth (do not shake vigorously).
8. Label both bottles with, Patient name, unique identifier, Time of collection, and Initial of collector as well as site of collection.
9. Safely dispose of all contaminated materials.

Annex 11. Laboratory procedure for Gram staining technique

1. Labeling the slides clearly with patient code number.

2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.

3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.

4. Fix the dried smear by using heat or chemicals (methanol).

5. Cover the fixed smear with crystal violet stain for 30-60 seconds.

6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.

7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.

8. Wash off the iodine with clean water.

9. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with clean water.

10. Cover the smear with neutral red or safranine stain for 2 minutes.

11. Wash off the stain with clean water.

12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.

13. Examine the smear microscopically, first with the 40 X objective to check the staining and to

see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

• Gram positive bacteria -------------------dark purple

• Gram -negative bacteria -------------------pale to dark red

Annex 12.Laboratory procedure for media preparation.

A.SOP for preparation of Blood agar plate (BAP)

AIM of Blood Agar Plate: A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms. The medium is often used to investigate the forms of haemolysis from pathogenic microorganisms from clinical specimen. Blood Agar Base formulation has been used as a base for preparation of blood agar and to support good growth of a wide variety of fastidious microorganisms. Because it is a highly nutritious medium it can also be used as a general purpose growth media without adding blood. Blood Agar Base is suitable to isolate and cultivate a wide range of microorganisms with difficult growth.

Procedure for Preparation to make about 30-35agar plates

- Measure 500ml of distilled water using a measuring cylinder.
- Transfer the distilled water into a 1litre capacity conical flask.
- Weigh 20g of Blood Agar Base II powder using a weighing balance.
- And then add into the 500ml of distilled water and mix thoroughly.
- Boil until completely dissolved
- Autoclave at 121°C for 15 minutes.
- Allow to cool to 45-50°C in a water bath.
- Once the medium has been melted and cooled to 45-50 ºC
- Add 5-10% of defibrinated sterile sheep blood, in this case you can recuperate Haemophylus. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution.

- Aseptically add 25 ml of sterile defibrinated sheep blood with constant shaking.

- When mixing, avoiding froth formation.

- Gently pour 15-20 ml of the ready media on to the plates by using dispenser and allow setting.

- If air bubbles occurred, using a Bunsen burner gently invert and pass the flame over the poured blood agar in the plate to remove air bubbles. Leave standing for thirty minutes to solidify.

- Label on the bottom top of the blood agar plates the batch number & date prepared.

- Store the culture media plates upside down at 2-8^0C sealed in plastic bags to reduce chances of contamination.

Shelf life: up to sixteen weeks provided there is no change in the appearance of the medium to suggest contamination, haemolysis, or deterioration.

B.Sop for preparation of Chocolate (Heated Blood) Agar

AIM of Chocolate (Heated Blood) Agar: Chocolate agar is a non selective media which supplies the factors X and V required for the proper growth *of Haemophilus influenza*. It is also used to culture nutritionally demanding pathogens such as *Neisseria meningitidis* and *Streptococcus pneumoniae.* When Blood agar is heated, the red cells are lysed and the medium becomes brown in colour; it is referred to as chocolate agar. It is appropriate for isolating pathogenic bacteria in sputum, throat swabs, eye swabs, ear swabs, urogenital swabs, cerebrospinal fluid.

Procedure for preparation:

- Prepare as described for Blood agar except after adding blood, heat the medium in a 70° C water bath until it becomes brown in color. This takes about 10-15 minutes during which time the medium should be mixed gently several times.
- Allow the medium to cool to about 45°C,
- Remix and dispense in sterile petri dishes using a dispenser as described for blood agar.
- Leave standing for thirty minutes to solidify.
- Perform sterility testing as described for blood agar plate.
- Label the bottom of each plate with date of preparation and batch number.
- Store the culture media plates upside down at 2-8° C sealed in plastic bags to reduce chances of contamination.

Important: Care must be taken not to overheat or prolong the heating of the medium because this will cause it to become granular and unfit for use. Up to sixteen weeks provided there is no change in the appearance of the medium to suggest contamination or deterioration.

C.SOP for preparation of MacConkey Agar

Aim of MacConkey Agar is preferable for the isolation and differentiation of clinically important gram negative rods by inhibiting gram positive cocci.

Principle

MacConkey agar is selective and differential medium to distinguish gram negative Enterobacteriaceae and lactose fermenting bacteria from non lactose fermenters. MacConkey Agar is a selective and differential medium. It is only slightly selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially *Enterococci* and s*taphylococci*. Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

Procedure for preparation:

- Prepare as instructed by the manufacturer.
- Suspend 51.1g of powder in 1 liter of distilled or deionized water.
- Heat and boil until completely dissolved with frequent agitation.
- Sterilize in autoclave at 1210C for 15 minutes
- Cool to 45-50 0C
- Mix well and dispense by dispenser (15-20 ml) aseptically into sterile petri dishes.
- Leave standing for thirty minutes to solidify.
- Perform sterility testing as described before.
- Label the bottom of each plate with date of preparation and batch number.
- Store the culture media plates upside down at 2-80C sealed in plastic bags to reduce chances of contamination.
- Test Samples for performance, using stable, typical control cultures.

Annex13.SOP of Vitek 2 compact analyzer

Purpose

To describe the procedures for the preparation and identification of test microorganisms (test microbes and Quality Control Organisms) using the VITEK 2 Compact Instrument.

Procedure and Analysis

Follow the operational instructions below strictly for the proper use and required quality control activities on VITEK 2 Compact analyzer.

1. **Initiation of the V2C System**

- The V2C Instrument is always "on"; the instrument will say "Ready" or "Not Ready" on the digital screen. Once the computer is initialized, the instrument will say "Ready."The V2C will not run if it is not on ready mode.
- Select VITEK 2 Compact to initiate the system from the upper left side of the screen.
- After the system is initiated, log onto the system using the appropriate user name and password.
- The system is now initialized and ready for data entry.

2. Preparation of Organisms

A. QC organisms

- If starting from a frozen stock culture, remove the 0.5 mL cryovials from the -80°C freezer. Avoid repeated thawing and freezing of the frozen culture by aseptically removing a small portion (or loopful) of the frozen inoculums, then immediately return cryovials to -80°C freezer.
- Streak isolates the inoculum from a frozen stock culture or other source onto agar plate appropriate for the QC organism.
- Following this streak isolation, a second streak isolation on the appropriate media is recommended.

B.Non-QC organisms

- Use growth on tubes or plates to perform streak isolation on BAP or NA warmed to room temperature. A second streak isolation step is not required unless there is evidence of a mixed culture.

C.For cultures used on BCL and GN cards incubate cultures for 18-24 h at 36±1°C. For cultures used on GP cards, incubate cultures for 12-48 h at 36±1°C. For cultures used on ANC cards, incubate cultures under anaerobic conditions for 18-24 h (or until sufficient growth is obtained) at 36±1°C. All organisms to be identified must be pure cultures.

3. Perform Gram stain using an isolated colony from a pure culture plate from section 12.4b and document the Gram stain reaction.

4. Preparation of Inoculums

Select the appropriate card based on the Gram stain reaction and the organism's microscopic appearance. Allow the card(s) to come to room temperature before opening the package liner.

Aseptically transfer at least 3 mL of sterile saline into a clear polystyrene 12×75 mm test tube. Using sterile cotton swabs, prepare a homogenous organism suspension by transferring several isolated colonies from the plates to the saline tube. Adjust the suspension to the McFarland standard required by the ID reagent using a calibrated V2C Densi CHEK plus Meter, see below table.

Table 6; Suspension Turbidities Used for Card Inoculation.

Card	McF Range
GN	0.5-0.63
GP	0.5-0.63
ANC	2.7-3.3
BCL	1.8-2.2

Place the prepared suspensions in the cassette (see section 15, Instrument User Manual).

To use the Densi CHEK plus Meter to read samples:

i. Ensure the instrument is ON and set to the PLASTIC tube setting.

ii. Blank the Densi CHEK Plus by filling a test tube with sterile saline and inserting the tube into the instrument. Press the "0" key and slowly rotate the test tube. Ensure one full rotation is completed before the reading is displayed. The instrument will display a series of dashes followed by 0.00.

iii. To measure a sample, place a well-mixed organism suspension into the instrument and slowly rotate the test tube. Ensure one full rotation has completed before the reading is displayed. The instrument will display a series of dashes followed by a reading.

iv. Remove the test tube after completion of a reading. The instrument will automatically shut off when test tubes are not inserted after one minute.

- ❖ **NOTE:** If the instrument flashes 0.00 or 4.00, the suspension is either below 0.0 McF or above4.0 McF and is not within the reading range. Ensure suspensions are within the appropriate reading range to avoid compromised card results. If necessary, re-calibrate the Densi CHEK Plus instrument after processing each cassette.

5. Insert the straw (in the V2C card) into the inoculated suspension tube in the cassette.

- ❖ **NOTE:** The age of the suspension must not exceed 30 minutes before inoculating the cards.

6. Proceed to data entry.

7. Filling the Cards

- Place the cassette in the Filler box on the left side of the V2C unit and hit Start Fill button on the instrument. Filling the cards takes approximately 70 seconds for a cassette regardless of the number of cards in the cassette holder. The V2C instrument will beep when the filling cycle is complete.
- Discard individual cards that may have been exposed to multiple fill cycles.

- **NOTE:** The cassette must be placed inside the Loader Door within 10 minutes from the end of the filling cycle to avoid the cards being rejected.

- When the cards are finished filling, the Load Door is automatically unlocked. Place the cassette in the Load Door. The V2C Instrument will verify the scanned barcodes against the Virtual Cassette (the information scanned in by the analyst). Cards are sealed, straws are cut and the cards are loaded automatically into the carousel. The V2C will beep once all cards are loaded into the cassette.
- When the cards are loaded, remove the cassette and dispose of the tubes and straws in biohazard container.
- The V2C automatically processes the cards once all the cards are loaded.

- NOTE: Review the Navigation Tree. If the cassette status description in the Navigation Tree is red, the cassette needs more information to completely process the tests cards. Open up the red colored file and make sure all fields are defined.

8. Results

The VITEK system analyses the data results and determines the identity of the test microbes/QC organism based on colorimetric tests (biochemical reactions).Results are concurrently printed and the data sent to the Results View folder on the left side of the screen also called the Navigation Tree where the information is archived. A red cassette in the Navigation Tree is indicative of an error. If an error occurs during processing, refer to the Software User Manual.

Declaration

I assure that the work will provide on my own title research project has not been submitted Elsewhere for any degree or qualification in the study area. And also I the 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.

Misgana Abera (BSc.MSc candidate)

Signature________________ Date of submission_______________________

The work will provided in this thesis is the researcher's own work, therefore I will confirm that the research has been conducted under main supervision and completed as per the conditions of the technical and ethical requirements needed.

Advisor:

Dr. Adane Bitew([PhD. Associate Professor)

Signature________________ date _______________________