Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Stream Infection Suspected Blood Samples Referred to Adama Public Health Research and Referral Laboratory, Oromia, Ethiopia

Roman Abaje

MSc Thesis Submitted to the Department of Applied Biology,School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

October, 2023

Adama, Ethiopia

Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Stream Infection Suspected Blood Samples Referred to Adama Public Health Research and Referral Laboratory, Oromia, Ethiopia

Roman Abaje

Advisor: Hunduma Dinka (Professor), ASTU

Co-Advisor: Takele Oli (BSc, MSc), APHRRL

MSc Thesis Submitted to the Department of Applied Biology, School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

October, 2023

Adama, Ethiopia

Declaration

I hereby declare that this Master Thesis entitled "**Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Stream Infection Suspected Blood Samples Referred to Adama Public Health Research and Referral Laboratory, Oromia, Ethiopia**" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citations.

Roman Abaje _____ _____

Name of student Signature Date

Recommendation

We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled "**Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Stream Infection Suspected Blood Samples Referred to Adama Public Health Research and Referral Laboratory, Oromia, Ethiopia**" prepared under our guidance by Roman Abaje submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Biotechnology. Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures

Professor Hunduma Dinka (PhD)	_____	_____
Major advisor	Signature	Date
Takele Oli (Bsc, MSc)	_____	_____
Co-advisor	Signature	Date

ACKNOWLEDGEMENTS

Above all I would like to express my special thanks to my Almighty God for giving me health and strength in my career. Secondly, I would like to thank and express my deepest appreciation to my advisor Professor Hunduma Dinka, Adama Science and Technology University, Department of Applied Biology, School of Applied Natural Science for his unlimited guidance and kindly supporting me throughout my research writing and critical evaluation of this paper. Also I would like to thank my co-advisor Takele Oli for giving me direction, suggestions in this research work**.**

I would like to express my sincere gratitude to the APHRRL for allowing me to do my MSc research work by providing laboratory facilities, necessary reagents and consumables. My heartfelt appreciations go to APHRRL staffs and all my best friends, who contributed in the successful accomplishment of this thesis work.

Last but not least, my especial thanks go to my beloved family and friends for their encouragement and support in every aspect of my life.

LIST OF ABBREVIATIONS AND ACRONYMS

AMC	Amoxicillin
AMK	Amikacin
APHRRLC	Adama Public Health Research and Referral Laboratory Center
AST	Antimicrobial Susceptibility Test
ASTU	Adam Science and Technology University
BHI	Brain Heart Infusion
BLH	Black Lion Hospital
BSI	Blood Stream Infection
CAZ	Ceftazidime
CHL	Chloramphenicol
CIP	Ciprofloxacin
CIP	Ciprofloxacin
CoNS	Coagulase Negative Staphylococcus
CRE	Carbapenem-Resistant Entrobacteriaceae
CRO	Ceftriaxone
CRO	Ceftriaxone
CTX	Cefotaxime
DIC	Disseminated Intravascular Coagulase
DNA	Deoxyribo Nucleic Acid
EARS-Net	European Antimicrobial Resistance Surveillance Network
FEP	Cefepime
GEN	Gentamaycin
GNB	Gram Negative Bacilli

I	Intermediate
IPM	Imipenem
KB	Kirb-bauer
MDR	Multidrug Resistant Organism
MHA	Mueller Hinton Agar
ORSA	Oxacillin-Resistant Staphylococcus Aureus
OXA	Oxacillin
PCR	Polymerase Chain Reaction
PEN	Penicillin
R	Resistant
S	Susceptible
SOP	Standard Operating Procedures
Spp.	Species
SPSS	Statistical Package for Social Sciences
TSB	Tryptose Soya Broth
TSI	Triple Sugar Iron
VRE	Vancomycin-Resistant Enterococcus

TABLE OF CONTENTS

Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ANNEXES

ABSTRACT

Blood stream infection (BSI) is characterized by the presence of microorganisms in blood circulation and is one of the leading causes of morbidity and mortality worldwide. It can range from self-limiting infections to sepsis, multiple organ failure, and even death. In Ethiopia; few studies are conducted on BSI and antimicrobial resistance profiles of bloodstream infection causing bacterial species. However, there is no data regarding the bacterial profile and antimicrobial susceptibility for BSI suspected blood samples referred to APHRRL. Therefore, the objective of this study was aimed to study bacterial profile and antimicrobial susceptibility patterns on BSI suspected blood samples referred to APHRRL. For these purpose a cross-sectional study on 252 blood samples suspected of BSI referred from August2022 to May 2023 to APHRRL was conducted. The samples were processed following standard microbiological techniques to identify the bacteria. Antibiotic susceptibility testing was done on pure culture isolates by disc-diffusion method for the commonly used antimicrobials in the study area. Of these samples, 150 (59.52%) were males and 102 (40.48%) were females with age ranges from two days to 84 years old. Out of 252 blood culture results, 88 (34.9%) (60 males and 28 females) were culture positive. The most prevalent bacterial isolates were *Cougulase Negative Staphylococcus* 21(23.9%) followed by *Staphylococcus aureus* 17(19.3%), *Klebsiella species* 13(14.8%), *Enterobacter species* 10(11.4%), *Escherichia coli* 7(8%) and *Streptococcus species* 7(8%), which together accounted for >85% of the isolates. Forty eight percent bacterial isolates constituted gram-negative, while 51.3% were gram-positive bacteria species. In the present study, the ranges of resistance for gram positive and negative bacteria were from 41.2-100%, and 25-100%,respectively. High levels of resistance to the majority of tested antimicrobial were observed in the isolates. From the total of 12 antimicrobials used in this study, 11 of them were found to be resistant to all the isolates. The present study revealed that both grams positive and negative bacteria were responsible for the cause of BSI and found to be not active against the commonly prescribed antimicrobial in the study area. As a result, careful selection of antimicrobials during prescription should be taken into consideration because the majority of isolated bacteria currently identified in use in the study area showed high rates of resistance to the most common antimicrobials.

Keywords: *Antimicrobial, Blood-stream, Disc-diffusion Gram-negative, Infection, Resistance, Susceptibility*.

1. INTRODUCTION

The term "bloodstream infection" (BSI) generally refers to the growth of a microorganism in a blood of a patient who has clinical signs of infection and has had contamination ruled out (Iqbal *et al*., 2020). BSI is potentially fatal infection that worsens rapidly due to the spread of microorganisms and their toxins in the blood (Negussie *et al*., 2015). BSI continues to be one of the most common infections in pediatric age groups, as well as one of the leading causes of morbidity and mortality in neonates and children worldwide. The severity of the infection is high in infants and children due to their weak immune system. It can range from self-limiting infections to life-threatening sepsis, multiple organ failure, disseminated intravascular coagulation that necessitates immediate and aggressive antimicrobial treatment (Laupland, 2013).

BSI is the eighth leading cause of mortality, and accounts for 10-20% of all nosocomial infections. BSI is a major cause of illness and death in children in Sub-Saharan Africa, including Ethiopia; the mortality rate approaches 53%, making it a significant health problem in these countries (Aiken *et al*., 2011).Garget al. (2007) revealed that, about 20–50% incidence of bacteremia in patients. Hence, it requires timely detection and identification of blood borne pathogens with urgent rational antimicrobial therapy.

Klebsiella pneumonia, Escherichia coli, Enterobacter spp., Staphylococcus aureus, Coagulase Negative Staphylococcus (CoNS), Streptococcus pneumoniae, Streptococcus pyogens, and Streptococcus pyogens, *Salmonella Typhi and Acinetobacter species* are the most common microorganisms isolated in BSIs, as are fungi such as *Candida albicans* and other *Candida* species with regional variation in distribution (Iqbal *et al*., 2020; Habyarimana *et al*., 2021). For instance, *Salmonella enterica* is a common pathogen isolated from blood samples in both African and Asian regions, but their serotypes differ significantly. In Africa, *Salmonella paratyphi* is the dominant organism in the *Salmonella* group, whereas *Salmonella typhi* is the most frequently isolated organism in Asia. Aside from their isolation rate and antibiotic use, the susceptibility pattern varies significantly (Deen *et al*., 2012).

Despite the advances in molecular-based diagnosis of bacterial sepsis, a blood culture has a high positive predictive value and is an important component for an accurate diagnosis of BSIs and for an appropriate choice of antibiotics, which is important for subsequent management

(Negussie *et al*., 2015). Many factors should be considered when choosing empiric therapy, including the pathogen that is most likely to be the cause of the infection based on age, gender, risk factors, and antimicrobial susceptibility pattern. Thus, rapid detection and identification of clinically relevant microorganisms in blood cultures is critical, and determining antimicrobial susceptibility patterns for rapid antimicrobial therapy administration has been revealed to reduce morbidity and mortality associated with BSIs (Banik *et al*., 2018).

1.1.Statement of the problem

Nowadays, antimicrobial resistance is a worldwide issue. A mean mortality rate of 18.1% was reported in a meta-analysis on community acquired BSI in Africa (Reddy *et al*., 2011). Many bacterial pathogens have developed resistance to the majority of antibiotics, making it difficult for health care professionals to prescribe appropriate antimicrobial therapy. This is the main problem in developing countries like Ethiopia, where there are no national guidelines for antibiotic use, there are no good laboratory facilities to perform antimicrobial drug susceptibility tests, and antimicrobial are overused or misused without any professional or rational prescription. In addition to these, antimicrobials are usually prescribed before the results of a blood culture are available. As a result, it has been demonstrated that implementing continuous monitoring trends in the microbiology of BSI pathogens is critical in guiding and selecting the appropriate antimicrobials for empiric antimicrobial treatment strategies and infection control programs (Taiwo *et al*., 2008).

The prevalence of antimicrobial resistance in patients with septicemia is increasing, and it varies in accordance with geographical and regional location even among hospitals in the same city. This requires the knowledge of common bacterial pathogens prevalent in that area based on blood culture results, to help clinician choose the right antibiotic therapy. Variability between hospitals in different countries is substantial and requires continuous analysis of local trends (Nazir *et al*., 2018).

Some studies documented data on bacterial BSIs and their antimicrobial resistance profiles of bloodstream causing bacterial species in Ethiopia (Dagnew *et al*., 2013;Wasihun *et al*., 2015). However, data on bacteriological profile and antimicrobial susceptibility test on BSI suspected blood samples referred to APHRRL are not documented. Therefore, for the effective

management of BSI in population, study of bacteriological profile along with the antimicrobial sensitivity pattern plays a great role. Such data are not only necessary for the clinicians to be aware of the emerging resistant strains of pathogens that are a threat to the community but also provide platform to initiate effective empirical therapy.

1.2.Significance of the study

Bacterial sepsis continues to be a leading cause of morbidity and mortality, especially in underdeveloped nations, despite improvements in detection and treatment. The BSI-causing agents and their patterns of antimicrobial sensitivity change throughout time and place to place. The presence of bacteria in the blood plays a critical role in the diagnosis of a febrile patient: it confirms the presence of infection, gives the clinician confidence in the empirical therapy they've chosen, and gives them the most recent knowledge of the local etiologic patterns and antibiotic sensitivities, which will help them manage the patient. Studies on bacterial profiles and their susceptibility patterns are mostly limited in Ethiopia, with the exception of a few papers on blood culture isolates from the hospitals of Tikur Anbessa, Gondar, Mekelle, Arba Minch and Jimma. The results of this study will benefit the community of the study area. Additionally, hospitals, health centers, private clinics, pharmacies, and laboratories can provide early treatment by keeping records of the results, identifying the pathogens circulating in the area, and knowing their antimicrobial resistance status. Moreover, the results of this study will offer decision-makers recommendations for patient management based on the regional pattern of antimicrobial susceptibility. Finally, the result of this study will also be used as a base for designing and doing other researches.

1.3. Objectives

1.3.1. General objective

- To determine the bacterial profile and antimicrobial susceptibility pattern of blood stream infection suspected blood samples referred to APHRRL.

1.3.2. Specific objectives

- To identify etiological agents of blood stream infection suspected from referred blood samples to APHRRL, and
- To determine the antimicrobial susceptibility pattern of the identified agents responsible for BSI from blood samples referred to APHRRL

1.4.Research Hypothesis

Null hypothesis: Blood samples that are referred to APHRRL do not contains bacterial isolates that are not resistant to frequently prescribed and commercially accessible antimicrobials

Alternative hypothesis: Blood samples that are referred to APHRRL contain bacterial isolates that are resistant to frequently prescribed and commercially accessible antimicrobials.

2. LITERATURE REVIEW

2.1. Overview of blood stream infection (BSI)

Bloodstream infections (BSI) are infectious diseases characterized by presence of viable microorganisms, bacterial or fungal in the bloodstream that causes significant patient morbidity and mortality globally (McNamara *et al*., 2018). Although it is still common in developed countries, the burden is disproportionately high in least developed countries (LDCs) and developing countries (Lochan *et al*., 2017). These infections are frequent and present life-threatening conditions in hospital and confirmed by the positivity of one or more blood cultures that elicit or have elicited an inflammatory response defined by changes in clinical, laboratory, and hemodynamic properties and lead to morbid consequences. The presence of microorganisms in the circulation, on the other hand, poses a threat to most organs if untreated, bloodstream infections can cause shock, disseminated intravascular coagulation (DIC), multiple organ failure, and death (Vasudeva *et al*., 2016). In this sense, BSI and sepsis are two sides of the same coin, because sepsis is an infectious syndrome caused by an infectious disease, whereas BSI is a sepsis caused by viable microorganisms circulating in the bloodstream (Viscoli, 2016).

Bloodstream infections are generally classified into three types based on their mode of occurrence: in immune-competent hosts with intact defenses, in patients at the end of life, and in patients with pathological conditions that put them at risk of infection (Viscoli, 2016).

BSI is the leading cause of morbidity and mortality in people of all ages, particularly in individuals in which their immunity level is low or compromised (Deku *et al*., 2019).Although it is still common in developed nations, the burden is high in the least developed and developing countries. The emergence and global spread of multidrug-resistant (MDR) organisms, such as oxacillin-resistant *Staphylococcus aureus* (ORSA), vancomycin-resistant *Enterococcus* (VRE), species has been a major source of concern.VRE and MDR Gram-negative *bacilli* (GNB), carbapenem-resistant *Enterobacteriaceae* (CRE), and MDR non fermenters such as *Pseudomonas aeruginosa* and *Acinetobacter species*. Several studies have shown that BSI caused by MDR organisms has a high mortality rate (Hurley *et al.*, 2003; Stewardson *et al*., 2016).

2.2. Etiology and epidemiology of blood stream infection

2.2.1. Etiology of BSI

Protozoa, fungi, viruses, and bacteria can all cause BSI. Bacteria make up the majority of BSI among the four types of pathogens. BSI can be divided into two stages, such as sepsis and septic shock, depending on the severity and extent of organ failure. The most significant and prevalent types of Gram-positive bacteria that can enter the circulation include *Staphylococcus aureus*, *CoNS, Enterococci*, and alpha-hemolytic *Streptococci*. Gram-negative bacteria like *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi*, and *Acinetobacter species* are other significant types. These bacteria have a variable geographic distribution depending on where they are found (Beshah *et al*., 2022).

Many studies have found that the presence of microorganisms in blood circulation causes blood stream infections. Gram-negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella species, Neisseria meningitidis*, and *Haemophilus influenza* may be present, as well as Gram-positive bacteria such as *coagulase-negative staphylococci* (*CONS),Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogen* have been identified as the causative agents the infection (Dagnew *et al.*, 2013). Additional Fungi including *Candida albicans* and other *Candida* species have been reported as a etiological agent of BSI (Habyarimana *et al.*, 2021).

Table 1: Rank order of pathogens causing bloodstream infection worldwide by 4-year period

Source (Diekema *et al*., 2019)

	Pathogen (%) in 4 year period				
Rank	**2001-2004**	**2005-2008**	**2009-2012**	**2013-2016**	**All years**
1	*S. aureus (22.7)*	*E. coli (20.0)*	*E. coli (21.3)*	*E. coli (24.0)*	*S. aureus (20.7)*
2	*E. coli (20.2)*	*S. aureus (19.4)*	*S. aureus (18.8)*	*S.aureus (18.7)*	*E. coli (20.5)*
3	*K.pneumoniae(6.6)*	*K.pneumoniae (7.8)*	*K.pneumoniae (8.5)*	*K.pneumoniae (9.9)*	*K.pneumoniae (7.7)*
4	*E. faecalis (5.6)*	*P. aeruginosa (5.4)*	*E. faecalis (5.3)*	*P.aeruginosa (5.4)*	*P. aeruginosa (5.3)*
5	*P. aeruginosa (5.4)*	*E. faecalis (5.1)*	*P.aeruginosa (5.2)*	*E.faecalis (5.0)*	*E. faecalis (5.2*
6	*S.epidermidis (3.9)*	*S.epidermidis (3.4)*	*E.faecium (3.8)*	*S.epidermidis (4.1)*	*S. epidermidis (3.8)*
7	*S.pneumoniae (3.5)*	*E. cloacae (3.3)*	*S.epidermidis (3.1)*	*E.faecium (3.4)*	*E. cloacae (2.9)*
8	*E. cloacae (3.1)*	*E. faecium (3.1)*	*E. cloacae (2.8)*	*E. cloacae (2.1)*	*S.pneumoniae (2.8)*
9	*E. faecium (2.2*	*A.baumanniia (2.4)*	*A.baumannii (2.4)*	*A.baumannii (2.0)*	*E. faecium (2.8)*
10	*P. mirabilis (1.7)*	*S.pneumoniae (2.2)*	*S. pneumoniae*	*S.pneumoniae (1.9)*	*A. baumannii (2.0)*

S= Staphylococcus andStreptococcus, E=Escherichia and Entrococcus, k=Klebssiella, P= pseudomonas and Proteus, A=Acinetobacter.

2.2.2. Epidemiology of BSI

The epidemiology and pathogen profile of the microorganisms that cause blood stream infection vary greatly from country to country. Several factors influence the epidemiology of BSIs, including demographic changes, the emergence of multi-drug resistant pathogens, and advances in medicine, with an increasing number of immune-compromised patients and the use of invasive devices (Mehl *et al.,* 2017).For example, study conducted in developing countries has been shown that *Salmonella enterica* serotype *Typhi* was the most common bacterial pathogen, isolated in adults (30%) and in children (25%)in both African and Asian countries. *Staphylococcus aureus, Escherichia coli*, and other Gram-negative organisms were also commonly isolated in adults, as were *Streptococcus pneumonia* and *Haemophilus influenzae* in children (Deen *et al*., 2012). However, BSI is caused primarily by the organisms *Escherichia coli, Staphylococcus aureus*, and *Streptococcus pneumoniae*, according to population-based studies from Australia, Canada, Denmark, Finland, Iceland, New Zealand, Sweden, and the United States (Laupland, 2013).

A recent meta-analysis research found that the incidence of community-onset BSI varied by continent, with rates of 14.6% (range, 3.4 to 38.2%) in Africa, 7.3% (range, 2.0 to 48.4%) in Asia, 2.9% (range, 2.1 to 19.2%) in Europe, and 7.3% (range, 2.9 to 15.6%) in the Americas (Marchello *et al*., 2020). Additionally, the most common causes of morbidity and mortality for adults in sub-Saharan Africa are blood stream infections. According to reports, 39% of hospital fatalities in sub-Saharan nations are attributable to BSI. According to studies, the frequency of BSI in countries in Eastern Africa ranges from 11 to 28 % 13-15. In Ethiopia, a heterogeneous study population's combined prevalence of BSI was considerably lower, at 27.78% (Lewis *et al*., 2019; Wasihun *et al*., 2015).

From 2002 to 2009, data from the European Antimicrobial Resistance Surveillance Network (EARS-Net, formerly EARSS, which includes 198 laboratories in 22 European countries) revealed that *Escherichia coli* and *Staphylococcus aureus* were the most common BSI-causing pathogens (Tian *et al.,* 2019). Similarly, data from South Korea's surveillance network in 2016-2017 revealed that *Escherichia coli* and *Staphylococcus aureus* were the most common BSI-causing pathogens (Lee *et al.*, 2018),while data from Japan revealed that E. coli, *Staphylococcus aureus*, *Streptococcus species*, and *Klebsiella species* were the most common BSI-causing pathogens (Hattori *et al.*, 2018).

2.3. Diagnosis of blood stream infection

Bloodstream infection is globally a major public health concern that leads to significant morbidity and mortality. As a result, early identification of the pathogen responsible for the disease allows clinicians to implement appropriate antibiotic therapy in a more well-timed manner. Prompt antibiotic treatment not only increases the chances of patient survival, but it also significantly reduces hospital stay length and associated healthcare costs. Currently, blood cultures (BCs) are considered to be the reference standard for diagnosing BSIs and are still indispensable for the diagnosis of BSI. The blood culture with biochemical and susceptibility testing has been the gold standard and most commonly used method for diagnosing bloodstream pathogens, it is being supplemented or replaced by more advanced methods, such as molecular tests, which can reduce turnaround time from several days to a few hours (Dunbar *et al.*, 2022). However, the implementation of these rapid diagnostic test may increase clinical microbiology laboratory cost (Walker *et al.*, 2016).

Continuously monitoring trends in the microbiology of BSI pathogens and their antibiotic susceptibility patterns is therefore important to guide empiric antibiotic treatment strategies and prevention programs, such as infection control measures. However, BSIs remain undiagnosed due to limited diagnostic laboratory facilities resulting in poor clinical outcomes and the increasing risk of antimicrobial resistance (Habyarimana *et al*., 2021). Global public health is now seriously threatened by antimicrobial resistance (AMR). For the purpose of preventing the emergence and spread of AMR, it is crucial to identify antimicrobial drug resistance accurately and quickly. This is followed by proper antimicrobial treatment and antimicrobial stewardship (Gajic *et al*., 2022).

Although monitoring of resistance at the national and international levels is very helpful for public health, doctors' practical need for awareness of local resistance rates is much greater. An antibiogram is a practical and accessible way to measure the infections and susceptibilities prevalent at a given institution. The idea that local (hospital or institutional) antibiograms should be created each year specifically for each hospital and even ward is therefore becoming more and more prevalent. This rule is especially relevant to some hospital sections with high rates of resistance, like intensive care units. This is also crucial for secondary and tertiary hospitals that serve chronically ill patients who have already received many courses of antibiotics, increasing the antimicrobial selection pressure (Klinker *et al*., 2021).

Bacteria are pathogenic microorganisms that can cause a wide range of illnesses in people, from minor to fatal. For patients suffering from these conditions to receive effective therapy, accurate disease-causing bacteria agent detection is necessary. Gram Positive Bacteria and Gram Negative Bacteria are the two categories under which bacteria are categorized. We can distinguish between the two types of bacteria, check for their presence or absence, and determine whether they are gram positive or gram negative thanks to a range of inherited biochemical tests (AL-Joda and Jasim, 2021).

The core of clinical microbiology is the identification of bacteria and the characterization of their antibiotic susceptibility pattern. Up to the genus and species level, bacteria can be identified by their growth needs, colony characteristics on standardized culture media, and a number of biochemical and serologic properties. Biochemical assays for microbiological identification use various metabolic behaviors demonstrated by various bacterial species. In order to distinguish

between gram positive and gram negative microorganisms, several biochemical assays are utilized. Gram negative bacteria are identified using biochemical tests such as the oxidase test, urease test, indole test, sulfur test, and methyl red/voges-proskauer test while Gram positive bacteria are identified using biochemical tests such as the catalase test, coagulase test, starch hydrolysis test, and nitrate test (Janda and Abbott , 2002;Kootallur, 2011).

2.4. Treatment of blood stream infection

Blood culture to isolate pathogens and determine drug sensitivity of isolates remains the mainstay of BSI diagnosis and management. The rapid administration of antimicrobial therapy to patients with BSI requires early identification of the bacteria causing BSI and their antimicrobial susceptibility pattern (which should be susceptible in vitro to the antimicrobials chosen). It has been demonstrated that this improves treatment outcomes (Hailu *et al.*, 2016).

Despite the fact that antimicrobials are currently the only way to treat bacteremia, many bacterial pathogens have become resistant to antibiotic regimens, posing a serious public health concern with global economic and social implications (Birru *et al.*, 2021). Antibiotic resistance is becoming increasingly prevalent in developing countries like Ethiopia. Without a prescription, the unregulated over-the-counter sale of these antimicrobials in Ethiopia, primarily for self-treatment of suspected infections in humans and, to a lesser extent, for use in animals, would inevitably result in the emergence and rapid spread of resistance (Zenebe *et al.*, 2011).

Early and adequate treatment is critical, and should be based on guidelines and direct examination of samples taken from the infectious source. Other major determinants of first choice antimicrobials in healthcare-associated and nosocomial BSIs include previous antibiotic therapy knowledge and a history of multi-drug resistant organism (MDRO) carriage. The first antimicrobial dose should be adjusted based on pharmacokinetic knowledge. In general, a high dose is advised at the start of treatment. If MDRO is suspected, combination antibiotic therapy is required because it broadens the treatment spectrum (Timsit *et al.*, 2014).

Certain species of bacteria respond better to particular antimicrobials than others. However, a broad-spectrum antibiotic that can kill several distinct types of bacteria should be used initially because it takes time to identify the bacterium that causes an infection. The effectiveness of antimicrobial for sepsis depends on many factors, including the type of infection, the risk of

contracting specific bacteria, the resistance profile, and even access to medications. Ceftriaxone, cefepime (Maxipime), ceftazidime, vancomycin, ciprofloxacin (Cipro), and levofloxacin have been used often among the antimicrobials prescribed by the doctor for the treatment of BSI (Buckman *et al*., 2018).

2.5. Antimicrobial resistance status in Ethiopia

Antimicrobial resistance has increased dramatically over the last few decades, with serious consequences. The nature, magnitude, and solutions to this problem are studied and described in the Western world, but this knowledge base is lacking in developing African countries. There is insufficient data on mortality related to pathogen distribution and resistance pattern. As a result, guidelines for empiric treatment of severe bacterial diseases cannot be provided in the absence of such reports. While updated studies on the outcome of sepsis in Africa are almost non-existent, however there are a few reports on bacterial culture results (Willmann *et al.*, 2013).

Despite obvious needs, Ethiopia's resource situation has prevented antimicrobial resistance from being prioritized as a major public health concern. In 1988, the pediatric department of Black Lion Hospital in Addis Ababa (BLH) reported multi-resistant *Klebsiella species* with resistance rates to chloramphenicol, gentamicin, and co-trimoxazole of 96%, 89 %, and 86%, respectively (Moss, 1992). In the early 1990s, newly introduced third generation cephalosporins (3GC) cured 76% of severe bacterial infections that were resistant to standard antibacterial treatment(Oli *et al*., 1990). Resistance to ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, and co-trimoxazole was found in 86%, 83%, 3%, 86%, 9%, and 43% of 76 gram-negative blood culture (GNB) isolates from pediatric patients at the same hospital in 2006 (Shitaye *et al.*, 2010).

Two recent reports on the sensitivity patterns of urinary tract pathogens in Ethiopia indicate alarming levels of resistance among GNB. According to these studies, from 2009 to 2014, the proportion of strains resistant to ceftriaxone and ciprofloxacin increased from 33 to 48% and 24 to 36%, respectively (Kindie *et al.*, 2014).

According to the study of Negussie et al.(2015) reports from blood tested 27.9% were found to be positive. Of this 51.8% were Gram negative and 46.4% Gram positive bacteria constituted. The most common pathogen found was *Staphylococcus aureus* (23.2%) followed by *Serratia marcescens* (21.4%), *CoNS* (19.6%), *Klebsiella species* (16%), and *Salmonella species* (5.4%).

Whereas, according to the study carried out by Dagnew *et al*. (2013), the predominant bacteria isolated from blood culture were *Coagulase negative Staphylococcus* (CoNS) (42.3%), followed by *S. aureus* (23.9%) and *Klebiesella species* (12.7%).The majority of bacterial isolates tested positive for resistance to Ampicillin, Penicillin, Co-trimoxazole, Gentamicin, and Tetracycline, all of which were commonly used in the study. Hence, the emergence of multi-drug resistant isolates calls for infection prevention measures as well as additional large-scale studies to determine appropriate empiric antibiotic choice (Negussie *et al*., 2015)

In Ethiopia, Ampicillin and gentamycin was the most frequently recommended combination therapy (21.9%), followed by the single antibiotic prescriptions (10.8%) of benzylpenicillin and cloxacillin (2.6%) (Unicef, 2015). According to a cross-sectional study report from Bishoftu general hospital in Ethiopia, gram-positive and gram-negative bacteria were found to have resistance rates of 65%–85% to ampicillin and ceftriaxone in a different study from Eastern Ethiopia (Dire Dawa), whereas gram-positive bacteria were found to be 93% responsive to gentamycin and 71% sensitive to ciprofloxacin(Teklea*bet al*., 2016).A study carried out in the Gondar University Hospital in northwest Ethiopia found that MDR was present in 85.7% of gram-positive isolates and 92.1% of gram-negative isolates. Among the isolates of *S. aureus*, 41.2% of them were methicillin resistant (MRSA), and *K. ozaenae* displayed extremely high levels of resistance (90-100%) against ampicillin, amoxicillin, and chloramphenicol(Dagnew*et al*., 2013).

3. MATERIALS AND METHODS

3.1. Description of Study area

The present study was conducted at Adama Public Health Research and Referral Laboratory (APHRRL), originally known as the Adama Regional Laboratory, was founded in 1995 EC by the Oromia Health Bureau with aid from the CDC-Ethiopia in Adama town, located next to the Adama Hospital and Medical College. Adama is a town in the central Oromia Region of Ethiopia located in the East Shewa Zone about 84 km southeast of the capital, Addis Ababa. The city is between the base of an escarpment to the west, and the Great Rift Valley to the east.The annual average minimum and maximum temperature of Adama town is 18 and 32 respectively. Geographically, the town is located at longitude 39° 27' E and latitude of 8° 54' N at an altitude of 172.According to 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA) Adama town has a total population of 220, 212 of whom 108,872 (49.44 %) are male and 111,340 (50.56%) females (CSA, 2007).

APHRRL is dedicated to good laboratory and clinical practice as well as the requirements of ISO 15189:2012, and performs its work in the most professional and ethical way. APHRRL is serving the Oromia region through carrying out different research projects and also processing different samples referred to the center from different hospitals of the city particularly, Adama Medical Hospital, Adama General Hospital, Adama Comprehensive Specialized Hospital Medical College (ACSHMC), Aklesia Memorial General Hospital (AMGH), Rift Valley Hospital, Adama Hospital Medical College (AHMC), and Medin Beza Hospitals (Riyad, 2021). Laboratory procedures including media preparation, biochemical tests, blood culture and antimicrobial susceptibility test for the referred samples are carried out at APHRRL.

3.2. Study design

A cross sectional study design was applied on blood samples suspected of BSI that were referred to APHRRL from August 2022 to May 2023 to conduct bacteriological profiling and antimicrobial susceptibility patterns test.

3.3. Sample size determination

In this study the required sample size was calculated using a single population proportion formula (Lwanga, 1991)with 18.2% prevalence from a previous study conducted in septicemia suspected patients attending Gondar University hospital, Gondar, Ethiopia (Dagnew *et al*., 2013). The considered confidence interval was 95% (z =1.96) with marginal error of 5% (d = 0.05). Thus, to determine the sample size, the single population proportion formula was as follow:

$$\mathbf{n = (Z)^2 P (1-P) \backslash d^2}$$

Where:

^{Z}=z-score (at 95% confidence interval); P= proportion; d= marginal error

Thus, $n = 1.96^2 * 0.182(1-0.182)/0.05^2$

As a result, the initial sample size was 229, and after computing a 10% non-response rate the ultimate sample size was 252.

3.4. Blood sample analysis and socio-demographic information collection

For this cross sectional study, blood samples from clinically suspected cases of BSI referred to APHRRL was identified and used to isolate bacteria and determine antimicrobial susceptibility patterns. All socio-demographic information such as age, sex, and sample identification number were recorded on registration book. Finally, all information on socio-demographic variables, blood culture results, antibiotic susceptibility pattern and sender hospitals or institutions were collected manually by using a pre-prepared data abstraction format from the registration book on which laboratory findings after investigation of patient`s blood are recorded(Annex 1 and 2).

3.5. Blood culture and biochemical characterization for bacterial identification

The blood sample was inoculated in Tryptic soya broth (TSB) in ratio of 1:10 proportions and incubated overnight at 37^{o}C followed by subculture of the incubated culture in 5% blood agar, chocolate agar and MacConkey agar plates and incubated at 37°C for 18–24. The culture isolates were identified preliminary using standard bacteriological techniques by daily inspection for the presence of bacterial growth like turbidity, presence of hemolysis, coagulation of broth, and gas

production and their characteristic appearance on their respective media. The isolates were then processed according to the laboratory's standard operating procedure (SOP) of APHRRL for complete identification (Annex 3). Pure cultures of bacterial isolates were subjected to species identification and confirmation. Gram staining techniques followed by biochemical tests were undertaken to classify bacteria at species level. For Gram-negative bacteria series of biochemical tests including Indole, Simon's citrate, Urease, Triple sugar iron (TSI), lysine iron, and motility tests were performed following the SOP of the center. Whereas, for Gram-positives, bacteria were identified based on their preference of growth on blood agar plate followed by catalase, coagulase tests (Annex 3) as described by Bhandari et al.(2015).

3.6. Antimicrobial susceptibility test

After bacterial isolation, antimicrobial susceptibility testing (AST)was performed according to Kirby-Bauer (KB) disk diffusion method on Mueller Hinton agar (MHA) plates method to study the pattern of resistance among the isolates as described by the National Committee for Clinical Laboratory Standards (Wayne, 2002).

Mueller–Hinton agar plates (100 mm diameter) was inoculated with a standardized inoculums of the test bacteria prepared in a TSB (corresponding to 0·5 McFarland turbidity standard). Briefly, first, the inoculum was prepared by transferring 3-5 well-isolated colony of the same morphologic type from an agar plate culture to TSB and mixed well to adjust the turbidity of the broth to match 0.5 McFarland. Then, test organisms were uniformly swabbed over the Mueller–Hinton agar and exposed to a concentration gradient of antibiotic diffusion disks. During this procedure, antibiotic disks were applied to agar surface using sterile forceps and gentle pressure was used to ensure complete contact of disk with agar. More than 5 disks on 100mm plate were not placed on the agar (APHRRL, 2021).The plates were inverted and incubated within 15 minutes of disk application under suitable conditions, typically for 24 hours at 37°C as described by Benkova et al.(2020). The following commonly prescribed and commercially available antimicrobial discs for disc diffusion test were used. The antimicrobial discs and their concentrations were as follows: Amikacin (AMK) (30µg), Amoxicillin (AMC) (30 µg),

Cefepime (FEP) (30µg), Cefotaxime (CTX) (30µg), Ceftazidime (CAZ) (30µg), Chloramphenicol (CHL) (30µg),Ciprofloxacin (CIP) (5µg), Gentamycin (GEN) (10µg), Imipenem (IPM) (10µg), Oxacillin (OXA) (30µg), Penicillin (PEN) (10 IU), and (Ceftriaxone (CRO) (30µg).

3.6.1. Antimicrobial susceptibility test result interpretation

In this study, the result of the test was interpreted according to standard operating procedures of the (APHRRL 2021) and National Committee for Clinical Laboratory Standards (NCCLS) (Wayne, 2016). The inverted plates were held a few inches above a black non-reflecting surface to illuminate plate with reflected light. The diameters of the zone of inhibition was measured to the nearest millimeter by caliper (Figure1) and isolates were classified as either Susceptible (S), Intermediate (I), or Resistant (R) to the antimicrobial agents that have been tested based on the zone of inhibition (Iqbal *et al.*, 2020).

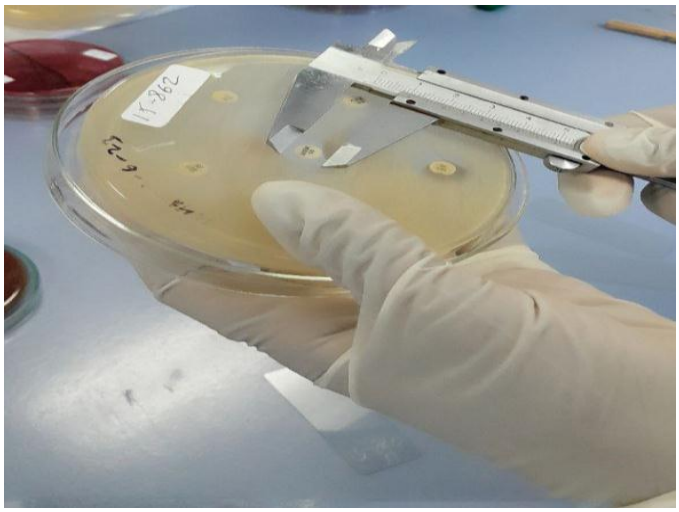


Figure 1: Measurement of zone of inhibition using caliper after 24 hours incubation at 37°C

The diameter of zone of inhibition used to interpretation isolates as sensitive, Intermediate and Resistant was used as described in the Annex 4 according to clinical and laboratory standards institute (CLSI, 2016).

3.7. Quality control

In this study reference strains of *Staphylococcus aureus* (ATCC 25923), and *Escherichia coli* (ATCC 25922) available at APHRRL were used as a control reference strains for identifications and drug susceptibility testing. However, negative control was prepared by randomly selecting prepared culture media and incubating it overnight to see if any growth occurs.

3.8. Data management and analysis

All collected data were entered to MS excel spread sheet program to create data base. SPSS version 22.00 (IBM Corp. Released 2013), was used for statistical analysis and the chi-square test was used to determine the relationship between variables. During analysis a statistical $p<0.05$ was considered as significant. Results were presented in the form of tables, graphs and others as appropriate.

3.9. Ethical Clearance

Official permission to conduct this study at APHRRL was obtained by the cooperative letter written by ASTU (started from department and then school). Ethical clearance of this study was obtained from Review board of Adama Public Health Research and Referral Laboratory Center (APHRRL) (Annex 6).

4. RESULTS

4.1. Socio-demographic Characteristics

Of the total 252 blood samples referred to APHRRL from different hospitals of the city, included in this study,173 (68.7%) samples were received from Adama Hospital Medical College (AHMC), 56 (22.2%) from Adama Public Health Research Institute(APHRI), 15 (5.9%) from Adama Comprehensive Specialized Hospital medical College (ACSHMC) and 8 (3.2%) were from Aklesia Memorial General Hospital (AMGH).Of these samples, referred to the center from August 2022 to May 2023, 150 (59.52%) were males and 102 (40.48%) were females (Figure 2).

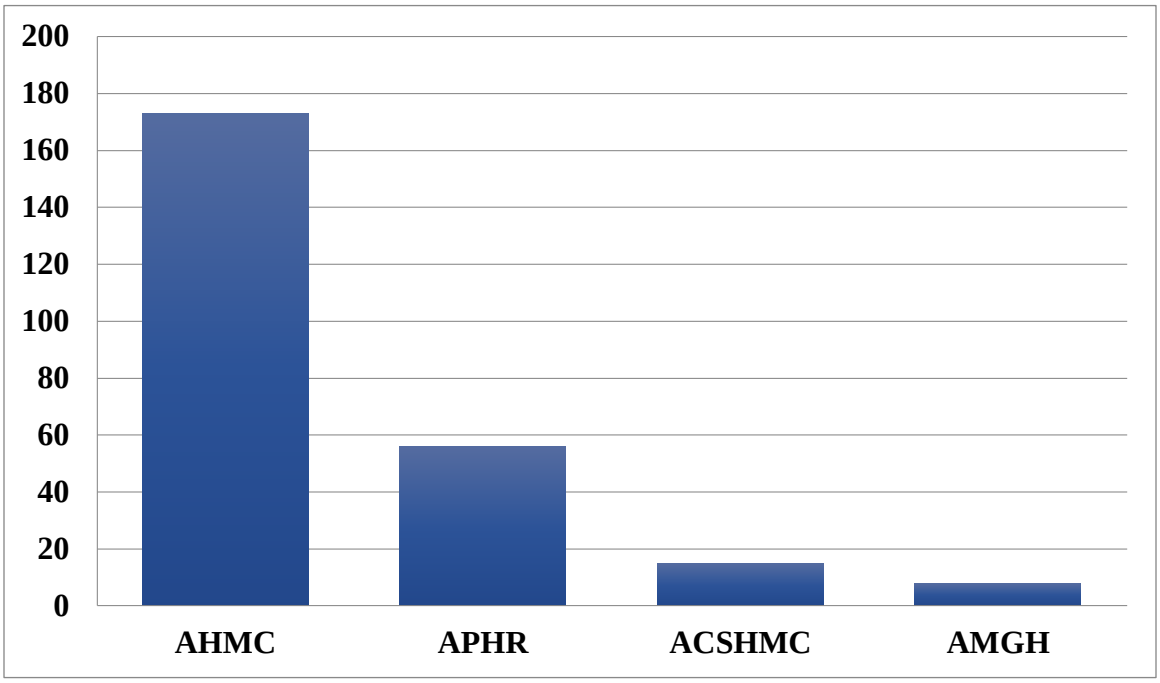


Figure 2: Graph which shows sex distribution and Hospitals involved in the study from August 2022 to May 2023

The ages of the participants were ranges from two days to 84 years old. Majority of the samples 31.7% (n=80) were in the age of 1 to 15 years followed by 23.4% (n=59) of the age group 16 to 31 years, 21.4% (n=54) of the age less than 1 year, 12.3% (n=31) of the age ranged from 32 to 50 and 8.3% (n=21) of the 51 to 70 years. Whereas, about 2.8% (n=7) were >70 years (Figure3).

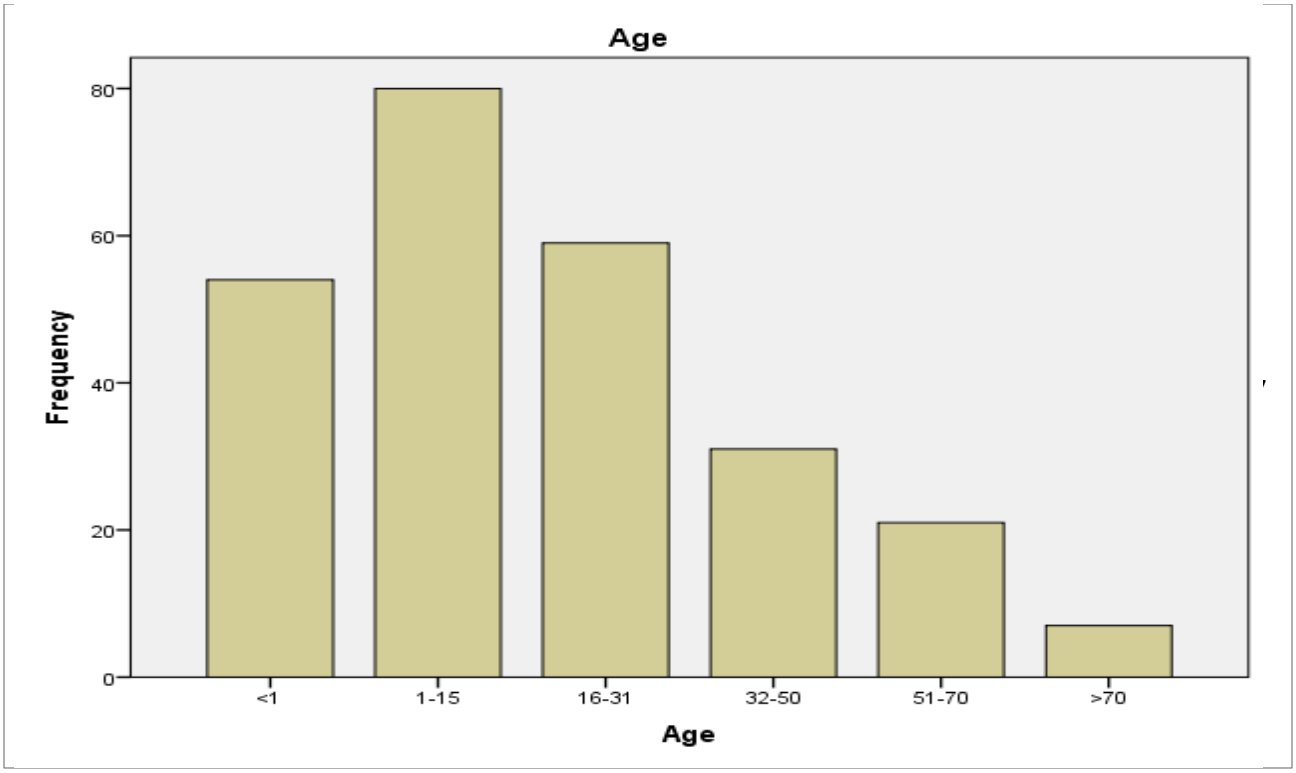


Figure 3: Graph which shows age distribution of BSI suspected blood samples referred to APHRRL from August 2022 to May 2023

4.2. Biochemical test results of Gram negative and positive bacterial isolates

The biochemical tests of both gram positive and negative bacteria were helped to identify the species of bacteria on the basis of biochemical activities as described in table 2 and 3.

Table 2: Biochemical test result of Gram positive bacterial isolates

Biochemical test type				
Growth on blood agar	**Catalase**	**Coagulase**	**Isolates**	**Bacteria name**
Positive	Positive	Negative	Isolate 1	*CONS*
Positive	Negative	Positive	Isolate 2	*Strep spp.*
Positive	Positive	Positive	Isolate 3	*Staph. aureus*

Table 3: Biochemical test results of Gram negative bacterial isolates

Biochemical test type							Isolates	Bacteria name
TSI		H_2S	Indole	Citrate	Urease	Motility		
Slant	Butt							
yellow	yellow	-ve	variable	+ve	+ve	+ve	Isolate 1	*Klebsiella*
yellow	yellow	-ve	-ve	+ve	-ve	+ve	Isolate 2	*Enterobacter*
yellow	yellow	-ve	+ve	-ve	-ve	+ve	Isolate 3	*E.coli*
red	red	-ve	-ve	+ve	-ve	-ve	Isolate 4	*Acinobacter*
red	yellow	-ve	-ve	+ve	+ve	+ve	Isolate 5	*Serratia*
red	red	-ve	-ve	+ve	-ve	+ve	Isolate 6	*Pseudomonas*
red	yellow	-ve	variable	-ve	-ve	-ve	Isolate 7	*Shigella*
red	neutral	+ve	-ve	+ve	+ve	+ve	Isolate 8	*Proteus*

TSI=Triple Sugar Iron, -ve=Negative, +ve=Positive

4.3. Bacterial isolates from blood culture results

In the present study, the predominant bacteria isolated from blood culture were *Coagulase Negative Staphylococcus (CONS)* 21 (23.9 %), followed by *Staphylococcus aureus* 17 (19.32 %) and *Klebiesella species* 13 (14.8 %). Of the culture positive, 48.7% isolates were Gram-negative bacteria species that constitutes *Klebiesella species* (14.8%), *Enterobacter species* (11.4%), *Escherichia coli* (8%), *Acinobacter species* (4.5%), *Serratia marcescens* (4.5%), *Pseudomonas aeruginosa* (3.4%), *Shigella* (1.1%) and *Proteus mirabilis* (1.1%) whereas, 51.3 % were Gram-positive species of bacteria including *CONS* (23.9 %), *Staphlococcus aureus*(19.32%) and *Streptococcus species* (8%) (Figure 4 and Table 4).

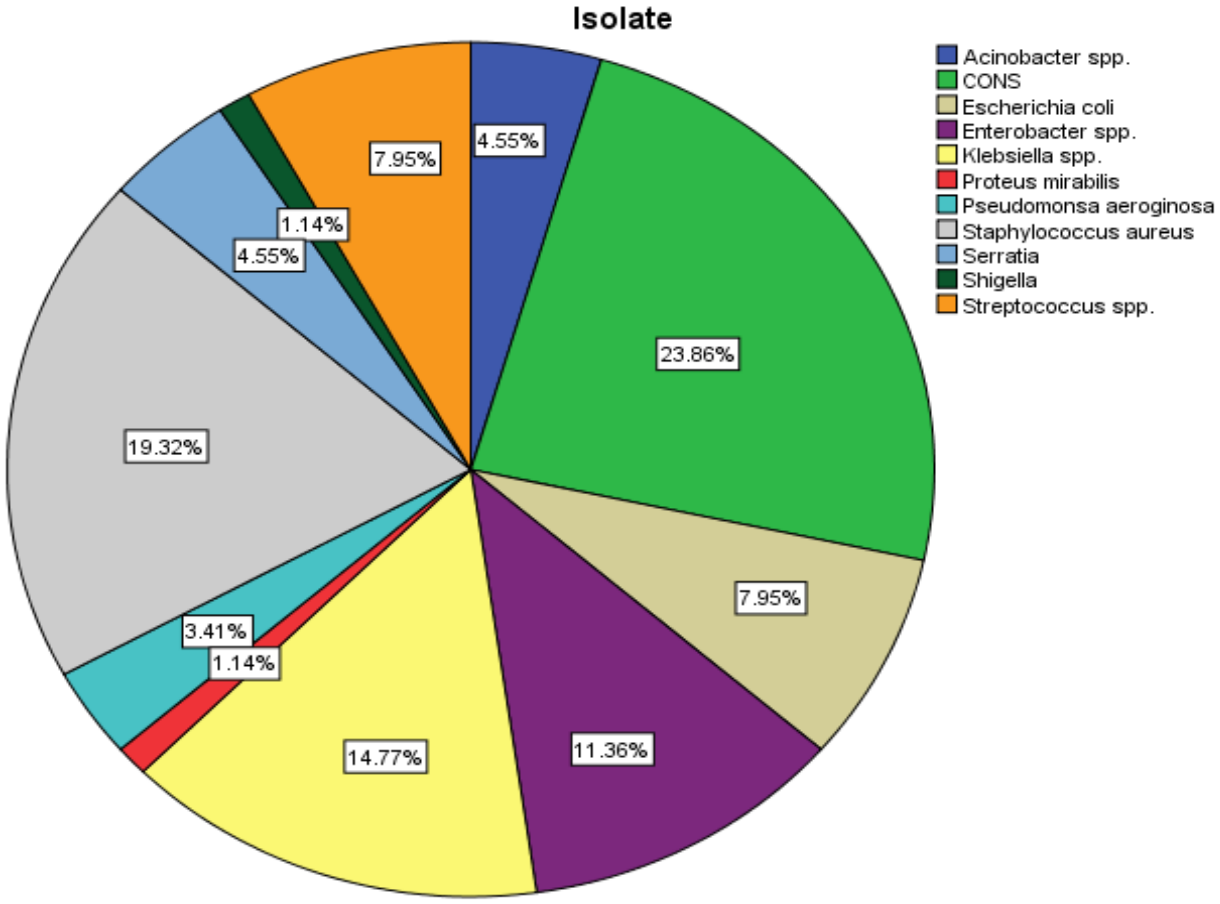


Figure 4: Percentage and types of bacterial isolates from BSI suspected blood samples referred To APHRRL from August 2022 to May 2023.

The overall prevalence of bacteria isolated from blood culture of samples referred to the center was 34.92 % (88/252). Among these isolates, 28 (31.8%) of the culture positive were from females and 60(68.2%) were males (Table 5). More bacteria were isolated form male than females, however the difference was not statistically significant (P=0.511, $\chi 2$=9.225, df=10). Additionally, the range of isolated bacteria varies with the age groups, where 1-15 years had the highest number of positive cases,34.1% (n=30), followed by age group of <1 year 23.9% (n=21),16-31 age 17% (n=15), 32-50 age 12.5%(n=11) and 51-70 age 11.4%(n=10). The age group >70 had the lowest isolate of bacteria when compared to the others 1.1% (n=1), but not statistically significant association was observed in the age category (P=0.071, $\chi 2$= 65.34, df=50) (Table 4). The distribution of isolated bacteria in different hospital varied, in which the highest

number of bacteria,58% (51/88), have been isolated from AHMC followed by APHRI (30.7%), ACSHMC (8%) and from AMGH (3.4%)(Table 4). However, the prevalence of these BSI causing bacteria in the hospitals was not statistically significant ($p>0.05$) ($P=0.648$, $\chi 2=26.5$, $df=30$).

Table 4: Frequency of bacterial isolates from different hospitals and by age class from BSI suspected blood samples referred to APHRRL from August 2022 to May 2023

Name of Hospital	Age category	Bacterial isolates											Total
		CONS n(%)	*S.aureu sn(%)*	*Kleb.spp n(%)*	*E.spp n(%)*	*Strep.spp n(%)*	*E. coli n(%)*	*Acinob. spp n(%)*	*Serratia m n(%).*	*Pseud. aeroginosa n(%)*	*shigella n(%)*	*P. mirabillis n(%)*	
`	<1	3(14.3)	2(11.8)	1(7.7)	3(30)	2(28.6)	0	2(50)	0	0	0	0	**13**
	1-15	6(28.8)	3(17.6)	3(23.1)	1(10)	0	2(28.6)	2(50)	1(25)	0	1(100)	0	**19**
AHMC	16-31	0	0	1(7.7)	2(20)	1(14.3)	4(57.1)	0	1(25)	0	0	0	**9**
	32-50	1(4.8)	2(11.8)	1(7.7)	0	0	1(14.3)	0	0	0	0	0	**5**
	51-70	1(4.8)	2(11.8)	1(7.7)	0	0	0	0	0	1(33.3)	0	0	**5**
	>70	0	0	0	0	0	0	0	0	0	0	0	**0**
	<1	3(14.3)	1(5.9)	1(7.7)	1(10)	0	0	0	0	0	0	0	**6**
	1-15	2(9.5)	1(5.9)	1(7.7)	0	2(28.8)	0	0	0	1(33.3)	0	0	**7**
	16-31	1(4.8)	1(5.9)	0	1(10)	1(14.3)	0	0	0	0	0	0	**4**
APHR	32-50	4(19)	0	0	1(10)	0	0	0	0	0	0	0	**5**
	51-70	0	1(5.9)	2(15.4)	0	0	0	0	0	0	0	1(100)	**4**
	>70	0	0	0	0	0	0	0	1(25)	0	0	0	**1**
	<1	0	1(5.9)	0	0	0	0	0	0	0	0	0	**1**
	1-15	0	1(5.9)	0	0	1(14.3)	0	0	1(25)	0	0	0	**3**
	16-31	0	0	1(7.7)	0	0	0	0	0	1(33.3)	0	0	**2**
ACSHM	32-50	0	1(5.9)	0	0	0	0	0	0	0	0	0	**1**
C	51-70	0	0	0	0	0	0	0	0	0	0	0	**0**
	>70	0	0	0	0	0	0	0	0	0	0	0	**0**
	<1	0	0	1(7.7)	0	0	0	0	0	0	0	0	**1**
	1-15	0	1(5.9)	0	0	0	0	0	0	0	0	0	**1**
	16-31	0	0	0	0	0	0	0	0	0	0	0	**0**
AMGH	32-50	0	0	0	0	0	0	0	0	0	0	0	**0**
	51-70	0	0	0	1(10)	0	0	0	0	0	0	0	**1**
	>70	0	0	0	0	0	0	0	0	0	0	0	**0**
	Total	**21**	**17**	**13**	**10**	**7**	**7**	**4**	**4**	**3**	**1**	**1**	**88**

AHMC =Adama Hospital Medical College, APHR=Adama Public Health and Research, ACSHMC=Adama Comprehensive Specialized Hospital Medical College, AMGH=Aklesia Memorial General Hospital Hospital

Table 5*: Distribution of bacterial isolates in sex and hospitals from BSI suspected blood samples referred to APHRRL from August 2022 to May 2023*

Sex	Name of Hospital	Bacterial Isolates											Total(%)
		CONS n(%)	*S.aureus n(%)*	*Kleb.spp n(%)*	*E.spp n(%)*	*Strep.spp n(%)*	*E. coli n(%)*	*Acinob. sppn(%)*	*Serratiam n(%).*	*Pseud. aerogino sa n(%)*	*shigella n(%)*	*P. mirabillis n(%)*	
Male	AHMC	8(21.6)	6(16.2)	5(13.5)	4(10.8)	2(5.4)	5(13.5)	3(8)	2(5.4)	1(2.7)	1(2.7)	0	**37(42)**
	APHR	8(44.4)	2(11.1)	2(11.1)	1(5.6)	3(16.7)	0	0	1(5.6)	0	0	1(5.6)	**18(20.5)**
	ACSHMC	0	3(60)	0	0	0	0	0	1(20)	1(20)	0	0	**5(5.7)**
	AMGH	0	1(33.3)	1(33.3)	1(33.3)	0	0	0	0	0	0	0	**3(3.4)**
Female	AHMC	3(21.4)	3(21.4)	2(14.3)	2(14.3)	1(7.1)	2(14.3)	1(7.1)	0	0	0	0	**14(15.9)**
	APHR	2(22.2)	2(22.2)	2(22.2)	2(22.2)	0	0	0	0	1(11.1)	0	0	**9(10.2)**
	ACSHMC	0	0	1(50)	0	1(50)	0	0	0	0	0	0	**2(2.3)**
	AMGH	0	0	0	0	0	0	0	0	0	0	0	**0**
Total		**21(23.9)**	**17(19.3)**	**13(14.8)**	**10(11.4)**	**7(8)**	**7(8)**	**4(4.5)**	**4(4.5)**	**3(3.4)**	**1(1.1)**	**1(1.1)**	**88(100)**

CONS=Cougulase Negative Staphlococcus, S. aureus=Staphlococcusaureus, Kleb.spp=klebisella species, E.spp= Enterobacter species, Strep.spp=streptococcus species, E. coli=Escherichia coli, Acinob.spp= Acinobacter species, Serratia m=Serratiamarcescens Pseud. aeroginosa=Pseudomonas aeruginosa, P.mirabillis= Proteus mirabilis

4.4. Antimicrobial Susceptibility/ Resistance pattern

In vitro antimicrobial susceptibility patterns of the isolated bacteria (n=88) from the blood cultures of BSI suspected samples referred to APHRRL against twelve (12) antimicrobial were presented in Table 6. The result of the test showed variations in their resistance/susceptibility patterns that range from 41.2% to 82.4%, and25% to 100% resistance for gram positive and negative bacteria, respectively.

4.4.1. Antimicrobial Susceptibility/Resistance pattern of Gram positive bacterial isolates

In Gram positive bacteria isolates the extent of resistance patterns ranged from 41.2% to 82.4% resistance. For instance, *Coagulase negative Staphylococcus isolates (CONS)* showed resistance in11/21 (52.4%) to Gentamycin, 15/21(71.4%) to Oxacillin and 14/21 (66.7%) toPenicillin. *Staphylococcus aureus* isolates were, 7/17 (41.2%), resistant to Gentamycin, 11/17 (64.7%) to Oxacillin and 14/17(82.4%) to Penicillin. The *Streptococcus species* isolates exhibited resistance,5/7 (71.4%), for Gentamycin, 4/7 (57.1%) for Oxacillin and 5/7 (71.4%) of Penicillin.

4.4.2. Antimicrobial Susceptibility/Resistance pattern of Gram negative bacterial isolates

In the present study, antimicrobial resistance levels for the Gram-negative bacteria isolates, causing BSI ranged from 25 to 100%. For instance, *Klebsiella species* were 76.9% resistant to Amoxicillin and Chloramphenicol, 69.2% to Cefepime, Imipenem and Ceftriaxone. The least level of resistance observed to Gentamycin and Ceftazidime, (46.2%) followed by Cefotaxime (53.8%), Amikacin (61.5%) and Ciprofloxacin (61.5%). *Enterobacter species* were 90% resistant to Amikacin, 80% to Amoxicillin, CefepimeandImipenem, 70% to Ceftazidimeand Chloramphenicol, 60% to Cefotaxime, Ciprofloxacin and Ceftriaxone (Table 6). Six of *Escherichia coli* isolates showed the highest resistant (85.7%) to Ciprofloxacin and Gentamycin followed by Amikacin, Cefotaxime, and Ceftazidime (71.4%). *Acinobacter species* isolate were 100% resistant to Amoxicillin,Ceftazidime and Chloramphenicol, 75% to Cefotaxime and Cefepime.*Pseudomonas aeruginosa* showed 100% resistant to Chloramphenicol and Ciprofloxacin, and 66.7% to each of Cefepime, Cefotaxime, Ceftazidime and Imipenem. Whereas, *Shigella* was 100% susceptible to Amikacin, Amoxicillin, Ceftazidime, Ciprofloxacin, Chloramphenicol and Ceftriaxone (Table 6).

Table 6: Antibiotic susceptibility patterns of Gram negative and positive isolates from BSI suspected blood samples referred to APHRRL from August 2022 to May 2023.

Isolates (N)		AMK-30 n (%)	AMC-20 n (%)	FEP-30 n (%)	CTX-30 n (%)	CAZ-30 n (%)	CHL-30 n (%)	CIP-5 n (%)	GEN-10 n (%)	IPM-10 n (%)	OXA-1 n (%)	PEN-10 n (%)	CRO-30 n (%)
CONS (21)	**S**	NA	NA	NA	NA	NA	NA	NA	2 (9.5)	NA	6(28.6)	7(33.3)	NA
	I	NA	NA	NA	NA	NA	NA	NA	8 (38.1)	NA	NA	NA	NA
	R	NA	NA	NA	NA	NA	NA	NA	11(52.4)	NA	15(71.4)	14(66.7)	NA
S. aureus (17)	**S**	NA	NA	NA	NA	NA	NA	NA	5(29.4)	NA	6(35.3)	3(17.6)	NA
	I	NA	NA	NA	NA	NA	NA	NA	5(29.4)	NA	NA	NA	NA
	R	NA	NA	NA	NA	NA	NA	NA	7(41.2)	NA	11(64.7)	14(82.4)	NA
Klebsiella spp. (13)	**S**	5(38.5)	2(15.4)	1(7.7)	2(15.4)	7(53.8)	3(23.1)	5(38.5)	1(7.7)	4(30.8)	NA	NA	3(23.1)
	I	0	1(7.7)	3(23.1)	4(30.8)	0	0	0	6(46.2)	0	NA	NA	1(7.7)
	R	8(61.5)	10(76.9)	9(69.2)	7(53.8)	6(46.2)	10(76.9)	8(61.5)	6(46.2)	9(69.2)	NA	NA	9(69.2)
Enterobacter spp. (10)	**S**	1 (10)	2(20)	2(20)	4(40)	3(30)	3(30)	2(20)	0	2(20)	NA	NA	3(30)
	I	0	0	0	0	0	0	2(20)	6 (60)	0	NA	NA	1(10)
	R	9(90)	8(80)	8(80)	6(60)	7(70)	7(70)	6(60)	4 (40)	8(80)	NA	NA	6(60)
Streptococcus spp. (7)	**S**	NA	NA	NA	NA	NA	NA	NA	0	NA	3(42.9)	2(28.6)	NA
	I	NA	NA	NA	NA	NA	NA	NA	2(28.6)	NA	NA	NA	NA
	R	NA	NA	NA	NA	NA	NA	NA	5(71.4)	NA	4(57.1)	5(71.4)	NA
E. coli (7)	**S**	2(28.6)	3(42.9)	2(28.6)	2(28.6)	1(14.3)	2(28.6)	0	0	3(42.9)	NA	NA	4(57.1)
	I	0	0	1(14.3)	0	1(14.3)	1(14.3%)	1(14.3)	1 (14.3)	0	NA	NA	0
	R	5(71.4)	4(57.1)	4(57.2)	5(71.4)	5(71.4)	4(57.1)	6(85.7)	6(85.7)	4(57.1)	NA	NA	3(42.9)
Acinobacter spp. (4)	**S**	2(50)	0	0	1(25)	0	0	1(25)	1 (25)	2(50)	NA	NA	1(25)

	I	0	0	1(25)	0	0	0	1(25)	1 (25)	1(25)	NA	NA	1(25)
	R	2(50)	4(100)	3(75)	3(75)	4(100)	4(100)	2(50)	2 (50)	1(25)	NA	NA	2(50)
Serratiamarcescens (4)	**S**	0	1(25)	0	0	1(25)	1(25)	0	1 (25)	0	NA	NA	2(50)
	I	0	0	0	1(25)	0	0	2(50)	2 (50)	0	NA	NA	1(25)
	R	4(100)	3(75)	4(100)	3(75)	3(75)	3(75)	2(50)	1 (25)	4(100)	NA	NA	1(25)
P. aeroginosa (3)	**S**	2(66.7)	3(100)	1(33.3)	1(33.3)	1(33.3)	0	0	0	1(33.3)	NA	NA	2(66.7)
	I	0	0	0	0	0	0	0	2 (66.7)	0	NA	NA	0
	R	1(33.3)	0	2(66.7)	2(66.7)	2(66.7)	3(100)	3(100)	1 (33.3)	2(66.7)	NA	NA	1(33.3)
Shigella (1)	**S**	0	0	1(1000)	1(100)	0	0	0	1 (100)	1(100)	NA	NA	0
	I	0	0		0	0	0	0	0	0	NA	NA	0
	R	1(100)	1(100)	0	0	1(100)	1(100)	1(100)	0	0	NA	NA	1(100)
Proteus mirabilis (1)	**S**	0	0	0	0	0	0	0	0	0	NA	NA	1(100)
	I	1(100)	0	0	0	0	0	0	0	0	NA	NA	0
	R	0	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1 (100)	1(100)	NA	NA	0
Total (88)	**R**	**30(69.8)**	**31(72.1)**	**31(72.1)**	**27(62.8)**	**29(67.4)**	**33(76.7)**	**29(67.4)**	**44 (50)**	**29(67.4)**	**30(66.7)**	**33(73.3)**	**23(53.5)**

AMK=Amikacin, AMC=Amoxicillin, FEP=Cefepime, CTX=Cefotaxime, CAZ=Ceftazidime, CHL=Chloramphenicol, CIP=Ciprofloxacin, GEN=Gentamycin, IPM=Imipenem, OXA=Oxacillin, PEN=Penicillin, CRO=Ceftriaxone, NA=Not applicable

5. DISCUSSIONS

Globally, BSIis an important cause of morbidity and mortality that can have serious immediate consequences that are shock, multiple organ failure, disseminated intravascular coagulation and death. Additionally, antimicrobial drug resistance (AMR) is a global problem(Kajumbula*et al*., 2018). Because of a lack of routine surveillance systems in sub-Saharan Africa, limited data are available that describes the local AMR patterns.There are few reports of these data from developing countries, especially from sub-Saharan Africa (Blondeau and Vaughan, 2000;Maina *et al*., 2016). As a result, accurate local prevalence of BSIs and microbial resistance data are vital for optimal treatment of patients. Therefore, the current study aimed to determine bacteriological profile and antimicrobial susceptibility pattern of blood stream infection suspected blood samples referred to APHRRL, Adama, Oromia regional State, Ethiopia.

The rate of BSI-causing bacteria from various hospitals has shown that the highest number of bacteria were isolated from Adama Hospital Medical College (AHMC) (58%), followed by Adama Public Health Research Institute (APHRI) (30.7%) and Adama Comprehensive Specialized Hospital Medical College (ACSHMC) (8%). Aklesia Memorial General Hospital (AMGH) has the lowest microorganisms (3.4%)(Table 4). However, the difference is not statistically significant ($p>0.05$). This finding is consistent with studies conducted in Ethiopia and other countries that discovered a variety of bacterial isolates with varying rates of prevalence (Dagnew *et al*., 2013; Maina *et al*., 2016; Hailu *et al*., 2016; Ahmed *et al*., 2017; Kajumbula *et al*., 2018; Makanjuola *et al*., 2018; Terfa *et al*., 2018; Beshah *et al*., 2022). This variation could be due to the difference in infections received from healthcare providers during treatment and operative factors such as duration of the procedure, skin antisepsis, surgical technique, antimicrobial prophylaxis practiced in these hospitals, and co-existing infections. Also, it might be due to the differences among the patients who came to the hospitals in their immune status, chronic disease, and others.

The results of this study showed that BSI was more prevalent in males than females. There was no significant association ($p>0.05$) between sex and isolates causing BSI. This finding is in line with other studies in Arba Minch General Hospital of Ethiopia (Birru *et al*., 2021) and Khan University Hospital of Kenya (Kohli *et al*., 2010), where the highest percentage of prevalence in males than females. But, it is not in agreement with the findings of Dagnew et al.(2013) and

Wasihun et al.(2015), who reported more BSI in females than males even if the sex and BSI were not signifcantly associated.Moreover, the rate of BSI in different age classes varied, which was highest in the age class of 1-15 (34.1%) years followed by <1 (23.9%)years. The least prevalence of BSI was observed in age class of >70 (1.1%) yearwhen compared to the others. However, the variation was not significant ($p>0.05$). Similar results obtained by Wasihun et al.(2015), suggested no significant association between age and BSI. The present finding is not consistent with the findings of other researchers (Zenebe *et al*., 2011;Dagnew *et al*., 2013;Gill and Sharma, 2016; Terfa Kitila *et al*., 2018), who revealed statistically significant association between age of patients and BSI,indicating that high BSI was seen in <1 year age group particularly in neonates. This may be due to the reason that the difference in number of study population and location of the study area and variation of individuals immune status.

In the present study, *CONS* (23.9%) was the most abundant isolated gram positive bacteria followed by *Staphylococcus aureus* (19.3%) and these findings are consistent with those of other studies conducted in Jimma (26.1%) (Zenebe *et al*., 2011), Gondar (42.3%)(Dagnew *et al*., 2013),Hawassa (24.8%) (Hailemariam *et al*., 2021)and other nations like Tanzania (67.4%) (Muleta *et al*., 2022). However, this was different from other studies in which *Staphylococcus aureus* was their predominant blood isolates such as in Jimma university medical center (26.7%) (Muleta *et al*., 2022), Addis Ababa (50.7%) (Terfa *et al*., 2018), Arba Minch (Birru *et al*., 2021)(31.8%), Mekelle (37.5%) (Wasihun *et al*., 2015), two studies in Nigeria (48.7% and 37.3%) (Meremikwu *et al*., 2005; Taiwo *et al*., 2008).The most likely explanation for the highest prevalence of these two bacteria is that they are frequently found in the environments than others. Moreover, they are also the most prevalent skin commensals, which can easily access blood stream during medical procedures and increase their prevalence rate.

The result of this study demonstrated that the overall prevalence of bacteria causing BSI isolated from blood culture was 31.8%. This finding was higher than the studies reported from different area of Ethiopia such as, 9.8% in Arba Minch(Birru *et al*., 2021), 18.2% in Gondar (Dagnew *et al*., 2013),8.8% (Zenebe *et al*., 2011)and 22.14% (Muleta *et al*., 2022)in Jimma and in other country like Kenya 5.8% (Kohli *et al*., 2010), Uganda 14% (Kajumbula *et al*., 2018) and Bangladesh 13.6% (Ahmed *et al*., 2017).However, it is comparably less than findings of the studies from Hawassa (35.9%) (Hailemariam *et al*., 2021), India (44%) (Prabhu *et al*., 2010) and

Nigeria (48.9%) (Meremikwu *et al*., 2005). Whereas, the result in current study is relatively in line with studies reported from Mekelle (28%) (Wasihun *et al*., 2015),Addis Ababa TikurAnbessa Specialized Hospital (28.06%) (Beshah *et al*., 2022), and Addis Ababa regional laboratory (32.8%) (Terfa *et al*., 2018). The variation of the findings from country to country or region, could be due to the difference in blood culture protocols, study population, the study design, geographical location, etiological agents, and infection control policies in the countries or regions as described by Mehl et al.(2017).

In the present study we found that 45/88(51.1%) gram positive bacteria and43/88 (48.9%) of gram negative was responsible for blood stream infection. The predominance of gram positive bacteria wasalso observed by several studies in Ethioipa such as 59.1 % of Gram positive and 40.9% of gram negative (Birru *et al*., 2021), 69% and 31.9% (Dagnew *et al*., 2013), in Kenya 53% and 39.3 % (Gill and Sharma, 2016), which makes the current study in line with the above studies.In contarst to this study, gram negative were frequently isolated from blood as reported by Hailu etal. (2016)(52.3 and 47.7 %) from Bahirdar, Beshah et al. (2022) (54.5 and 45.43%) from Addis Ababa, Ahmed et al. (2017) (72.1 and 27.9%) from Bangladesh, Taiwo et al. (2008) (52.7 and 47.3%) from Nigeria and Tohamy et al. (2018) (52.7 and 47.7%) from Egypt which represent gram negative bacteria and gram positive bacteria, respectively. This variation of the prevalence might be due to the factors of epidemiological changes of the bacteria that cause BSI and the incidence and etiology of BSIs have altered over time (Guimaraes *et al*., 2017).

Among gram negative bacterial isolates, *Klebsiella species* (14.8%) were predominantly isolated from the blood culture (Figure 5).This is in agreement with Hailemariam et al., (2021)(21.4%) (Beshah *et al*., 2022) (17.6%), (Maina *et al*., 2016) (12%), (Birru *et al.*, 2021) (18.2%) . But, not in agreement with the results of Muleta *et al*. (2022) from Jimma, Arya and Agarwal(2011) from Tanzania (Kajumbula*et al*., 2018)and from Uganda, who found *Klebsiella species* as the second most abundant gram negative bacteria next to *Escherichia coli.* Furthermore, the other studies from Bangladesh (Ahmed *et al*., 2017) reported *Salmonella typhi* as the most likely BSI causing bacteria among gram negative bacteria.

CONS, Staphylococcus aureus, Klebsiella species, Enterobacter species, Escherichia coli, Acinobacter species, Serratia marcescens, Pseudomonas aeruginosa, Shigella, Proteus mirabilis, and *Streptococcus species* were the isolated bacteria in this study, despite the fact that

the isolated organisms varied between studies due to various factors. Different study designs, study locations, epidemiologically distinct etiological agents, and seasonal variation are a few explanations that could account for the variation as suggested by Guimaraes et al. (2017).

Analysis of the *in-vitro* antimicrobial resistance test on all the bacterial isolates revealed that the highest levels of antimicrobial resistance to different antimicrobial agents ranging from 41.2% to 82.4% (gram positive) and 25% to 100% (gram negative bacteria). From the total of 12 antimicrobial used in this study, 11 of them were more than 50% resisted by all the bacterial isolates. High resistant pattern of the isolates causing BSI were observed in the present study. This results are partially comparable with the reports of Terfa et al. (2018) from Addis Ababa Regional Laboratory, Ethiopia and Birru et al.(2021) from Arba Minch General Hospital, Ethiopia. Besides, almost similar resistance findings were reported from the previous studies conducted in Ethiopia and elsewhere in another countries (Zenebe *et al*., 2011;Ebrahim-Saraie *et al*., 2016; Ishimwe and Rogo, 2018;Beshah *et al.*, 2022). In general, more or less similar observations have been made in cases of antimicrobial resistance pattern within the country and in other nations. This variation of the result could be due to possible terrible mishandling and incorrect application of the antibacterial medicines, this circumstance raises serious concerns about a very high resistance gene pool.

This study showed that, *CONS,* the predominant gram positive bacterial isolates, revealed the highest resistance to Oxacillin (71.4%) and the highest susceptibility to Penicillin (33.3%), and followed by Oxacillin, (28.6%). Similarly, *Streptococcus species* were also 71.4% resistant to Gentamycin and Penicillin but 49.2% susceptible to Oxacillin. *Staphylococcus aureus*, the second common bacteria of all isolates was 82.4% resistant to Penicillin and 35.3% susceptible to Oxacillin. In general, in this study, gram-positive bacteria isolates of BSI had a high level of resistance, >50%, to all tested antimicrobials. However, antimicrobials like Oxacillin showed a relatively high level of susceptibility compared to other antimicrobials. Almost similar findings were reported by Beshah et al.(2022) from Tinkur Anbessa, Specialized Hospital, Addis Ababa, Ethiopia, Prabhu et al.(2010) from India and Maina et al.(2016) from Kenya. Another study by Muleta et al.(2022) reported low level of resistance to gentamycin (2%), and high level of resistance to Penicillin (81%). This variability may be due to the frequency of use of

antimicrobials, self-medication practice, and control strategies of drug resistant bacteria that vary from for country to country (Widmer *et al*., 2015).

The antimicrobial resistance pattern of gram negative bacterial isolates exhibited a resistance (>50%) to the majority of antimicrobial agents being tested. *Kliebsiella species*, the first predominant isolates among gram negative bacteria showed that, the high level of resistance (> 70%) to Amoxicillin and Chloramphenicol, >60% to most of the tested antimicrobials. But in these isolates high level of susceptibility were observed to Ceftazidime (53.8%), Amikacin and Ciprofloxacin (38.5%) relative to the others. Moreover, *Enterobacter species were* ≥70% resistant to more than half of the tested antimicrobials. In general current study showed that gram negative isolates had high level of resistance than gram positive bacteria to the tested antimicrobial in which the current study is more or less in agreement with the result of Hailemariam et al. (2021).Even though, comparing individual resistance pattern of the present findings to other reports is difficult due to the fact that antimicrobial resistance varies by countries and laboratories, and sometimes even within the same country as a result a lack of consistency in the measurement and reporting the data (Tadesse *et al*., 2017).

Overall, in the present cross-sectional study, conventional blood culture techniques were employed to isolate causative agents of BSI only from suspected cases which most likely excluded cases of intermittent bacteria. Additionally, due to the short comings of some reagents, media, duration of time and the limited supply of funds, some tests were not carried out, for instance isolation of *Candida albicans*.

6. CONCLUSION AND RECOMMENDATIONS

Overall, the present study identified the most prevalent bacteria, their drug susceptibility profile, and the prevalence of BSI from blood samples referred to APHRRL. This study revealed that both grams positive and negative were responsible to cause BSI. Among the bacterial isolates, *CONS* was the predominantly isolated bacteria followed by *Staphylococcus aureus* and *Klebsiella species*. The bacterial isolates were found to be highly resistant to the commonly prescribed antimicrobial and their rate of resistance is alarming in the study area. Moreover, this study expanded our understanding of the epidemiology of BSI and their bacterial isolates with high rates of resistance to the most widely prescribed antibiotics.

Therefore based on the above conclusions, the following recommendations are forwarded;

- Further study and timely investigation of BSI, bacterial resistance pattern are highly recommended in this study in order to reduce the challenge of resistant bacteria.
- We also recommend periodic surveillance of etiologic agent and antibiotic susceptibility to prevent further emergence and spread of resistant bacteria pathogens in the study area.
- Instead of following general principles, the treatment of BSI should be dependent on culture and sensitivity test.
- Given that most isolated bacteria now exhibit high rates of resistance to the most widely used antimicrobials, selective alternative antibiotic treatment should be considered

7. REFERENCES

Ahmed, D., Nahid, M. A., Sami, A. B., Halim, F., Akter, N., Sadique, T., Rana, M. S., Elahi, M. S. Bin, and Rahman, M. M. (2017). Bacterial etiology of bloodstream infections and antimicrobial resistance in Dhaka, Bangladesh, 2005-2014. *Antimicrobial Resistance and Infection Control*, **6**(1); 1–11.

Aiken, A. M., Mturi, N., Njuguna, P., Mohammed, S., Berkley, J. A., Mwangi, I., Mwarumba, S., Kitsao, B. S., Lowe, B. S., Morpeth, S. C., Hall, A. J., Khandawalla, I., and Scott, J. A. G. (2011). Risk and causes of paediatric hospital-acquired bacteraemia in Kilifi District Hospital, Kenya: A prospective cohort study. *The Lancet*, **378**(9808); 2021–2027.

Arya, S. C., and Agarwal, N. (2011). Bacteria isolated from bloodstream infections at a tertiary care hospital in Dar es Salaam, Tanzania - antimicrobial resistance of isolates. *South African Medical Journal*, **101**(2); 85.

Benkova, M., Soukup, O., and Marek, J. (2020). Antimicrobial susceptibility testing: currently used methods and devices and the near future in clinical practice. *Journal of Applied Microbiology*, **129**(4); 806–822.

Beshah, D., Desta, A., Belay, G., Abebe, T., Gebreselasie, S., and Tessema, T. S. (2022). Antimicrobial Resistance and Associated Risk Factors of Gram-Negative Bacterial Bloodstream Infections in Tikur Anbessa Specialized Hospital, Addis Ababa. *Infection and Drug Resistance*, **15**;5043–5059.

Bhandari, P., Manandhar, S., Shrestha, B., and Dulal, N. (2015). Etiology of bloodstream infection and antibiotic susceptibility pattern of the isolates. *Asian Journal of Medical Sciences*, **7**(2); 71–75.

Birru, M., Woldemariam, M., Manilal, A., Aklilu, A., Tsalla, T., Mitiku, A., and Gezmu, T. (2021). Bacterial profile, antimicrobial susceptibility patterns, and associated factors among bloodstream infection suspected patients attending Arba Minch General Hospital, Ethiopia. *Scientific Reports*, **11**(1); 1–13.

Blondeau, J. M., and Vaughan, D. (2000). A review of antimicrobial resistance in Canada. *Canadian Journal of Microbiology*, **46**(10); 867–877.

Dagnew, M., Yismaw, G., Gizachew, M., Gadisa, A., Abebe, T., Tadesse, T., Alemu, A., and Mathewos, B. (2013). Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients attending Gondar University Hospital, Northwest Ethiopia. *BMC Research Notes*, **6**(1); 1–7.

Deen, J., von Seidlein, L., Andersen, F., Elle, N., White, N. J., and Lubell, Y. (2012). Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: A systematic review. *The Lancet Infectious Diseases*, **12**(6); 480–487.

Deku, J. G., Dakorah, M. P., Lokpo, S. Y., Orish, V. N., Ussher, F. A., Kpene, G. E., Angmorkie Eshun, V., Agyei, E., Attivor, W., and Osei-Yeboah, J. (2019). The Epidemiology of Bloodstream Infections and Antimicrobial Susceptibility Patterns: A Nine-Year Retrospective Study at St. Dominic Hospital, Akwatia, Ghana. *Journal of Tropical Medicine*, **2019**.

Diekema, D. J., Hsueh, P. R., Mendes, R. E., Pfaller, M. A., Rolston, K. V., Sader, H. S., and Jones, R. N. (2019). The microbiology of bloodstream infection: 20-year trends from the SENTRY antimicrobial surveillance program. *Antimicrobial Agents and Chemotherapy*, **63**(7).

Dunbar, S. A., Gardner, C., and Das, S. (2022). Diagnosis and Management of Bloodstream Infections With Rapid, Multiplexed Molecular Assays. *Frontiers in Cellular and Infection Microbiology*, **12**(March), 1–13.

Ebrahim-Saraie, H. S., Motamedifar, M., Mansury, D., Halaji, M., Hashemizadeh, Z., and Ali-Mohammadi, Y. (2016). Bacterial Etiology and Antibacterial Susceptibility Patterns of Pediatric Bloodstream infections: A two year study from nemazee hospital, shiraz, Iran. *Journal of Comprehensive Pediatrics*, **7**(1).

Garg, A., Anupurba, S., Garg, J., Goyal, R. K., and Sen, M. R. (2007). Bacteriological profile and antimicrobial resistance of blood culture isolates from a university hospital. *Journal, Indian Academy of Clinical Medicine*,**8**(2); 139–143.

Gill, M. K., and Sharma, S. (2016). Bacteriological profile and antibiotic resistance pattern in blood stream infection in critical care units of a tertiary care hospital in North India. *Indian*

Journal of Microbiology Research, **3**(3); 270.

Habyarimana, T., Murenzi, D., Musoni, E., Yadufashije, C., and Niyonzima, F. N. (2021). Bacteriological profile and antimicrobial susceptibility patterns of bloodstream infection at Kigali University Teaching Hospital. *Infection and Drug Resistance*,**14;** 699–707.

Hailemariam, M., Alemayehu, T., Agegnehu, A., Ali, M., and Azerefegne, E. (2021). Bacterial Blood Stream Infection in Southern Ethiopia. *Journal of Bacteriology and Parasitology*,**6**.

Hailu, D., Abera, B., Yitayew, G., Mekonnen, D., and Derbie, A. (2016). Bacterial blood stream infections and antibiogram among febrile patients at Bahir Dar Regional Health Research Laboratory Center, Ethiopia. *Ethiopian Journal of Science and Technology*, **9**(2), 103.

Hattori, H., Maeda, M., Nagatomo, Y., Takuma, T., Niki, Y., Naito, Y., Sasaki, T., and Ishino, K. (2018). Epidemiology and risk factors for mortality in bloodstream infections: A single-center retrospective study in Japan. *American Journal of Infection Control*, **46**(12); e75–e79.

Hurley, J. C., Cosgrove, S. E., and Carmeli, Y. (2003). Comparison of mortality associated with methicillin-susceptible and methicillin-resistant Staphylococcus aureus bacteremia: An ecological analysis (multiple letters). *Clinical Infectious Diseases*, 37(6); 866–869.

Iqbal, H., Mahboob, N., Ahmed, M., Mamun, K. Z., Rahim, A., and Azad, K. A. K. (2020). Bacteriological profile and antimicrobial susceptibility patterns of blood culture isolates among febrile patients in a Tertiary care hospital, Bangladesh. *Journal of Dhaka Medical College*, **27**(2); 114–122.

Ishimwe, E., and Rogo, T. (2018). Antibiotic resistance in children with bacteremia admitted in the largest tertiary hospital in rwanda. *Rwanda Medical Journal*, **75**(2); 5–8.

Kajumbula, H., Fujita, A. W., Mbabazi, O., Najjuka, C., Izale, C., Akampurira, A., Aisu, S., Lamorde, M., Walwema, R., Bahr, N. C., and Meya, D. B., Boulware, D. R., and Manabe, Y. C. (2018). Antimicrobial Drug Resistance in Blood Culture Isolates at a Tertiary Hospital, Uganda. *Emerging Infectious Diseases*, **24**(1); 174–175.

Kohli, R., Omuse, G., and Revathi, G. (2010). Antibacterial susceptibility patterns of blood stream isolates in patients investigated at the Aga Khan university Hospital, Nairobi. *East*

African Medical Journal, **87**(2); 74–80.

Laupland, K. B. (2013). Incidence of bloodstream infection: A review of population-based studies. *Clinical Microbiology and Infection*, **19**(6); 492–500.

Lee, H., Yoon, E. J., Kim, D., Jeong, S. H., Won, E. J., Shin, J. H., Si, H. K., Shin, J. H., Shin, K. S., Kim, Y. A., Uh, Y., Yang, J. W., Il, H. K., Park, C., and Lee, K. J. (2018). Antimicrobial resistance of major clinical pathogens in South Korea, May 2016 to April 2017: First one-year report from kor-GLASS. *Eurosurveillance*, **23**(42); 0–10.

Lewis, J. M., Feasey, N. A., and Rylance, J. (2019). Aetiology and outcomes of sepsis in adults in sub-Saharan Africa: A systematic review and meta-analysis. *Critical Care*, 23(1), 1–11.

Lochan, H., Pillay, V., Bamford, C., Nuttall, J., and Eley, B. (2017). Bloodstream infections at a tertiary level paediatric hospital in South Africa. *BMC Infectious Diseases*, 17(1), 1–9.

Lwanga, S. K., Lemeshow, S., and World Health Organization. (1991). Sample size determination in health studies: a practical manual. *World Health Organization.*

Maharath, A., and Ahmed, M. S. (2021). Bacterial Etiology of Bloodstream Infections and Antimicrobial Resistance Patterns from a Tertiary Care Hospital in Malé, Maldives. *International Journal of Microbiology*, **2021**.

Maina, D., Omuse, G., Revathi, G., and Adam, R. D. (2016). Spectrum of microbial diseases and resistance patterns at a private teaching hospital in Kenya: Implications for clinical practice. *PLoS ONE*, **11**(1); 1–12.

Makanjuola, O. B., Fayemiwo, S. A., Okesola, A. O., Gbaja, A., Ogunleye, V. A., Kehinde, A. O., and Bakare, R. A. (2018). Pattern of Multidrug Resistant Bacteria Associated With Intensive Care Unit Infections in Ibadan, Nigeria. *Annals of Ibadan Postgraduate Medicine*, **16**(2); 162–169.

http://www.ncbi.nlm.nih.gov/pubmed/31217775%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6580401

Marchello, C. S., Dale, A. P., Pisharody, S., Rubach, M. P., and Crump, J. A. (2020). A Systematic Review and Meta-analysis of the Prevalence of Community-Onset Bloodstream

Infections among Hospitalized Patients in Africa and Asia. *Antimicrobial Agents and Chemotherapy*, **64**(1).

McNamara, J. F., Righi, E., Wright, H., Hartel, G. F., Harris, P. N. A., and Paterson, D. L. (2018). Long-term morbidity and mortality following bloodstream infection: A systematic literature review. *Journal of Infection*, 77(1), 1–8.

Mehl, A., Åsvold, B. O., Lydersen, S., Paulsen, J., Solligård, E., Damås, J. K., Harthug, S., and Edna, T. H. (2017). Burden of bloodstream infection in an area of Mid-Norway 2002-2013: A prospective population-based observational study. *BMC Infectious Diseases*, 17(1), 1–14.

Meremikwu, M. M., Nwachukwu, C. E., Asuquo, A. E., Okebe, J. U., and Utsalo, S. J. (2005). Bacterial isolates from blood cultures of children with suspected septicaemia in Calabar, Nigeria. *BMC Infectious Diseases*, **5**;1–4.

Muleta, D., Simieneh, A., Sahile, S., Mesfin, G., Abdissa, D., and Workineh, L. (2022). Bacterial Profile and Their Antimicrobial Resistance Pattern among Adult Patients with Suspected Bloodstream Infection at Jimma University *Medical*. **32**(September).

Nazir, A., Sana, I., Peerzada, B. Y., and Farooq, T. (2018). Study of prevalence and antimicrobial susceptibility pattern of blood culture isolates from a tertiary care hospital of North India. I*nternational Journal of Research In Medical Sciences*. **6**(12); 4046-4052.

Negussie, A., Mulugeta, G., Bedru, A., Ali, I., Shimeles, D., Lema, T., and Aseffa, A. (2015). Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Culture Isolates among Septicemia Suspected Children in Selected Hospitals Addis Ababa, Ethiopia. *International Journal of Biological and Medical Research*, **6**(1); 4709–4717.

Oli, K., Haimanot, R. T., Gedebou, M., and Tsega, E. (1990). Ceftriazone in the treatment of septicaemia due to multipledrug resistant bacteria. *Central African Journal of Medicine*, **36**(3); 68–71.

Prabhu, K., Bhat, S., and Rao, S. (2010). Bacteriologic Profile and Antibiogram of Blood Culture Isolates in a Pediatric Care Unit. *Journal of Laboratory Physicians*, **2**(02); 085–088.

Reddy, E. A., Shaw, A. V, and Crump, J. A. (2011). *National Institute of Health Public access*, **10**(6); 417–432.

Santos Guimaraes, R. de O., Marquez Cunha, T., Souza Oliveira, A. C., de Araujo, L. B., Santos Pedroso, R. dos, and de Brito Roder, D. V. D. (2017). Bloodstream Infection: The Influence of Risk Factors, Etiology and Antimicrobial Therapy on Mortality Rates. *Journal of Nursing and Care*, **06**(02); 4–9.

Stewardson, A. J., Allignol, A., Beyersmann, J., Graves, N., Schumacher, M., Meyer, R., Tacconelli, E., De Angelis, G., Farina, C., Pezzoli, F., Bertrand, X., Gbaguidi-Haore, H., Edgeworth, J., Tosas, O., Martinez, J. A., Ayala-Blanco, M. P., Pan, A., Zoncada, A., Marwick, C. A., Falcone, C. (2016). The health and economic burden of bloodstream infections caused by antimicrobial-susceptible and non-susceptible Enterobacteriaceae and Staphylococcus aureus in European hospitals, 2010 and 2011: A multicentre retrospective cohort study. *Eurosurveillance*, **21**(33).

Tadesse, B. T., Ashley, E. A., Ongarello, S., Havumaki, J., Wijegoonewardena, M., González, I.J., andamp; Dittrich, S. (2017). Antimicrobial resistance in Africa: A systematic review. *BMC Infectious Diseases*,**17**(1); 1–17.

Taiwo, S. S., Fadiora, S. O., and Fayemiwo, S. A. (2008). High antimicrobial resistance among bacterial isolates of blood stream infections (BSI) in a Nigerian University Teaching Hospital. *World Journal of Microbiology and Biotechnology*, **24**(2); 231–236.

Tekleab, A. M., Amaru, G. M., and Tefera, Y. A. (2016). Reasons for admission and neonatal outcome in the neonatal care unit of a tertiary care hospital in Addis Ababa: a prospective study. *Research and Reports in Neonatology*, **17**.

Terfa Kitila, K., Taddese, B. D., Hailu, T. K. mariam, Sori, L. M., K mariam, Geleto, S. E., Mengistu, G. Z., Tesfaw, D. D., K mariam Hailu, T., Gebeyehu, C. sisay, Balcha, H. M., Desalegn, D. M., and Bika, A. T. (2018). Assessment of Bacterial Profile and Antimicrobial Resistance Pattern of Bacterial Isolates from Blood Culture in Addis Ababa Regional Laboratory, Addis Ababa, Ethiopia. *Clinical Microbiology: Open Access*, **07**(02).

Tian, L., Zhang, Z., and Sun, Z. (2019). Antimicrobial resistance trends in bloodstream infections at a large teaching hospital in China: A 20-year surveillance study (1998-2017). *Antimicrobial Resistance and Infection Control*, **8**(1); 1–8.

Timsit, J. F., Soubirou, J. F., Voiriot, G., Chemam, S., Neuville, M., Mourvillier, B., Sonneville, R., Mariotte, E., Bouadma, L., and Wolff, M. (2014). Treatment of bloodstream infections in ICUs. *BMC Infectious Diseases*, **14**(1); 1–11.

Tohamy, S. T., Aboshanab, K. M., El-Mahallawy, H. A., El-Ansary, M. R., and Afifi, S. S. (2018). Prevalence of multidrug-resistant Gram-negative pathogens isolated from febrile neutropenic cancer patients with bloodstream infections in Egypt and new synergistic antibiotic combinations. *Infection and Drug Resistance*, **11**;791–803.

Vasudeva, N., Nirwan, P. S., and Shrivastava, P. (2016). Bloodstream infections and antimicrobial sensitivity patterns in a tertiary care hospital of India. *Therapeutic Advances in Infectious Disease*,**3**(5); 119–127.

Viscoli, C. (2016). Bloodstream Infections: The peak of the iceberg. *Virulence*, **7**(3); 248–251.

Walker, T., Dumadag, S., Lee, C. J., Lee, S. H., Bender, J. M., Abbott, J. C., and She, R. C. (2016). Clinical impact of laboratory implementation of verigene BC-GN microarray-based assay for detection of gram-negative bacteria in positive blood cultures. *Journal of Clinical Microbiology*, **54**(7); 1789–1796.

Wasihun, A. G., Wlekidan, L. N., Gebremariam, S. A., Dejene, T. A., Welderufael, A. L., Haile, T. D., and Muthupandian, S. (2015). Bacteriological profile and antimicrobial susceptibility patterns of blood culture isolates among febrile patients in mekelle hospital, Northern Ethiopia. *SpringerPlus*, 4(1).

Widmer, A. F., Lakatos, B., andamp; Frei, R. (2015). Strict Infection Control Leads to Low Incidenceof Methicillin-Resistant Staphylococcus aureus Bloodstream Infection over 20 Years.I*nfection Control and Hospital Epidemiology*, **36**(6); 702–709.

Willmann, M., Kuebart, I., Marschal, M., Schröppel, K., Vogel, W., Flesch, I., Markert, U., Autenrieth, I. B., Hölzl, F., and Peter, S. (2013). Effect of metallo-β-lactamase production and multidrug resistance on clinical outcomes in patients with Pseudomonas aeruginosa bloodstream infection: A retrospective cohort study. *BMC Infectious Diseases*, **13**(1); 1–9.

Zenebe, T., Kannan, S., Yilma, D., and Beyene, G. (2011). Invasive bacterial pathogens and their antibiotic susceptibility patterns in Jimma University specialized hospital, Jimma, Southwest Ethiopia. *Ethiopian journal of health sciences*, ***21***(1); 1-8.

8. ANNEXES

Annex 1: Data record format of samples referred to the laboratory

Serial no	Sample type	Sample sender	Sample Id	Sex	Age	Isolates
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Annex 2:Data record format of antimicrobial susceptibility test results

Isolates	Susceptibility Pattern	Antimicrobials											
		AMK	AMC	FEP	CTX	CAZ	CHL	CIP	GEN	IPM	OXA	PEN	CRO
	S												
	I												
	R												
	S												
	I												
	R												

Annex 3:Standard operating procedure for each and every sample collection and processing procedures

3.1. Specimen collection, Transport and Processing

a. Disinfect the vein puncture site with 70% alcohol

b. Withdraw about 5-10 ml of blood sampleaseptic allyfrom two separate peripheral veins maintaining a time gap by using vacutainertube.

c. Transfer the sample into the blood culture bottle containing Tryptic Soy Broth sterile culture medium.

d. Transport Blood culture broths to the microbiology laboratory and incubate at 37°C.

e. Inspect routinely for a week for the presence of bacterial growth, like uniform/subsurface turbidity, coagulation of broth, surface floccular deposit, pellicle formation, and gas production

f. Blood culture broth with no bacterial growth after 7 days is sub-cultured before being reported as a negative result.

g. If the result is positive, identify further the isolates with colony morphology, gram staining reaction and biochemical tests.

h. Perform antimicrobial susceptibility testing for the isolated organisms according to Clinical Laboratory Standard

3.2. Gram Staining Procedure/Protocol

Fix material on a slide with methanol or heat. If the slide is heat fixed, allow it to cool to the touch before applying the stain.

a. Flood heat-fixed smear of cells for 1 minute with crystal violet staining reagent.
b. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
c. Flood slide with the mordant: Gram's iodine. Wait 1 minute.
d. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
e. Flood slide with a decolorizing agent (Acetone-alcohol decolorize). Wait 10-15 seconds
f. Flood slide with a counterstain, safranin. Wait 30 seconds to 1 minute.
g. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.

h. Observe the results of the staining procedure under oil immersion (100x) using a Bright field microscope.

Gram-negative bacteria will stain pink/red and Gram-positive bacteria will stain blue/purple.

3.3. Sub-Culture

Prior to sub-culturing, put the vial in an upright position, and place an alcohol wipe over the septum. To release pressure in the vial, use an appropriate venting unit[81]. The needle should be removed after the pressure is released and before sampling the vial for subculture. The insertion and withdrawal of the needle should be done in a straight-line motion, avoiding any twisting motions.

Subcultures are performed by streaking a loopful on appropriate media:

- For gram-negative rods: MacConkey agar
- For small gram-negative rods: blood agar; chocolate agar
- For staphylococci: blood agar, mannitol salt agar;
- For streptococci: blood agar with optochin, bacitracin, and bile easculin

3.4. Biochemical tests, materials/reagents and procedures

The staining is followed by use of various biochemical reagents and tests to get closer to the identification of bacteria. There are many biochemical tests available for bacterial identification. Few of them are required to be carried out depending upon the bacteria. The commonly used biochemical tests are as mentioned below.

3.4.1.Indole Test

Purpose: The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. It is used as part of the IMViC (indole, MR-Vp Citrate) procedures, a battery of tests designed to distinguish among members of the family *Enterobacteriaceae.*

Procedure: Inoculate the tube of tryptone broth with a small amount of a pure culture. Incubate at 37°C for 24 to 48 hours. To test for indole production, add 5 drops of Kovác's reagent directly to the tube. A positive indole test is indicated by the formation of a pink to red color

("cherryredring") in the reagent layer on top of the medium within seconds of adding the reagent. If a culture is indole negative, the reagent layer will remain yellow or be slightly cloudy.

Indole positive bacteria: E. coli, Vibrio cholera
Indole negative bacteria: Klebsiella, Salmonella, Shigella spp.

3.4.2.Citrate Test

Purpose: The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator. The citrate test is often part of a battery of tests used to identify gram-negative pathogens and environmental isolates. Detection of citrate utilization for the differentiation of Enterobacteriaceae

Procedure: Inoculum is streaked over the slant of Simmon's citrate agar in a tube and incubated for 24- 48 hrs.

Interpretation: Citrate positive bacteria: Klebsiella spp.

Citrate negative bacteria: E. coli.

3.4.3.Catalase test

Purpose: The catalase test facilitates the detection of the enzyme catalase in bacteria. It is essential for differentiating catalase-positive Micrococcaceae from catalase-negative Streptococcaceae.

Procedure: Place a microscope slide inside a petri dish. Keep the petri dish cover available. Using a sterile inoculating loop or wooden applicator stick, collect a small amount of organism from a well-isolated colony and place it onto the microscope slide. Be careful not to pick up any agar. This is particularly important if the colony isolate was grown on agar containing red blood cells. Carryover of red blood cells into the test may result in a false-positive reaction. Using a dropper or Pasteur pipette, place 1 drop of 3% H_2O_2 onto the organism on the microscope slide. Do not mix. Immediately cover the petri dish with a lid to limit aerosols and observe for immediate bubble formation (O_2 + water = bubbles). Observing for the formation of bubbles against a dark background enhances readability.

Catalase positive bacteria: Staphylococcus spp. and Catalase negative bacteria: Streptococcus spp.

3.4.4.Coagulase Test

Purpose: The coagulase test differentiates strains of Staphylococcus aureus from other coagulase-negative species. S. aureus strains are capable of coagulating plasma in the tube test and will produce clumps of cells in the slide test. The coagulase test can be performed using two different procedures - Slide test and tube test. The slide test is simple, giving results within 10 seconds, but it can give false negatives. The tube test is the definitive test, however, it can take up to 24 hours to complete. For both tests, clumping or clots of any size indicate a positive response.

Procedure: The slide test is performed by preparing a suspension of bacterial cells mixed into a drop of rabbit plasma on a microscope slide. If bound coagulase is present on the bacterial cells, then the presence of plasma will cause the bacterial cells to clump. The clumping will occur because the clumping factor is an adhesin, which causes the cells to bind to fibrinogen in the plasma. This will result in visible clumping of bacterial cells on the microscope slide. Figure given below illustrates the visible clumping of cells on the microscope slide.

The tube coagulase test is performed by mixing bacterial cells into a larger volume of plasma in a small test tube. As the bacteria multiply in the plasma, they secrete staphylocoagulase. Staphylocoagulase initiates blood coagulation by activating prothrombin. Formation of a clot will be noted within 24 hours for a positive response.

Coagulase positive bacteria: Staphylococcus aureus
Coagulase negative bacteria: Staphylococcus epidermis, Staphylococcus saprophyticus

3.4.5.Oxidase test

Purpose: The oxidase test is a biochemical reaction that assays for the presence of cytochrome oxidase, an enzyme sometimes called indophenol oxidase. In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product.

Procedure: There are many method variations to the oxidase test. These include, but are not limited to, the filter paper test, filter paper spot test, direct plate method, and test tube method.

Filter Paper Test Method: Soak a small piece of filter paper in 1% Kovács oxidase reagent and let dry. Use a loop and pick a well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate and rub onto treated filter paper.

Observe for color changes: Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes.

Oxidase positive bacteria: Pseudomonas, Vibrio cholera.
Oxidase negative bacteria: E. coli, Klebsiella, Salmonella.

3.4.6.Bile Solubility

Principle / Intended use: The bile solubility test is a confirmatory test used in the identification of *Streptococcus pneumoniae*.

Procedure: Two tubes are required for bile solubility testing of each suspect strain of *S. pneumoniae*. Take a loop of the suspect strain from fresh growth on a blood agar plate and prepare a bacterial cell suspension in 0.5 ml of sterile saline. The suspension of bacterial cells should be cloudy, similar to that of a 0.5 or 1.0 McFarland turbidity standard.Divide the suspension into two equal amounts (*i.e.* 0.25 ml per tube). Add 0.25 ml of saline to one tube and 0.25 ml of 10% sodium deoxycholate (bile salts) to the other. Shake the tubes gently and incubate them at 35°– 37°C for up to 2 hours. Examine the tubes periodically for lysis of cells in the tube containing the bile salts. A clearing of the tube, or a loss in turbidity, is a positive result.

Interpretation: Positive test: Clearing of turbidity (tube) or dissolution of a colony (plate) - *bile soluble.* Negative test: No clearing or colony dissolution - *bile insoluble*

3.4.7.Triple Sugar Iron

Principle / Intended use: Triple Sugar Iron test determines the ability of an organism to ferment sugars and to produce hydrogen sulfide in anaerobic and aerobic conditions. A Triple Sugar Iron

slope contains three sugars (lactose, sucrose, and glucose), phenol red (a pH indicator), sodium thiosulphate and an iron source (ferrous sulfate). If an organism is able to ferment any of the sugars, the acid will be produced which will change the pH of the media and result in a color change from red to yellow. The location of this color change indicates if the organism is able to ferment the sugar under aerobic (slope) or anaerobic (butt) conditions.

Method: Pick a single colony for the test and stab inoculate the butt of the Triple Sugar Iron slope and on withdrawal "squiggle" the loop up the slope. Loosely cap the slope. Incubate the slope at 35-37°C in air for 18-24 hours.

Interpretation:

- Red Slant/ Yellow Butt: Ferments glucose
- Yellow Slant/ Yellow Butt: Ferments/Lactose/Sucrose/glucose
- Red Slant (no change)/ Red Butt(no change): No fermentation
- Red Slant/ Yellow Butt, Blackening: Ferments glucose and H_2S produced
- Yellow Slant/ Yellow Butt, Blackening: Ferments/Lactose/Sucrose/glucose, H_2S produced
- Red Slant (no change)/ Red Butt(no change), Blackening: No fermentation, H_2S produced

Note:Blackening of the whole butt indicates large amounts of H_2S. Blackening of the stab inoculation line indicates small amounts of H_2S.

3.4.8.Lysine Iron Agar

Purpose/Principle: To to differentiate gram negative bacilli based on decarbolation or deamination of lysine. Lysine deamination is an aerobic process which occurs on the slant of the media. Lysine decarboxylation is an anaerobic process which occurs in the butt of the media.Lysine iron agar contains lysine, peptones, a small amount of glucose, ferric ammonium citrate, and sodium thiosulfate. When glucose is fermented, the butt of the medium becomes acidic (yellow). If the organism produces lysine decarboxylase, cadaverine is formed. Cadaverine neutralizes the organic acids formed by glucose fermentation, and the butt of the medium reverts to the alkaline state (purple). If the decarboxylase is not produced, the butt remains acidic (yellow). If oxidative deamination of lysine occurs, a compound is formed that, in

the presence of ferric ammonium citrate and a coenzyme, flavin mononucleotide, forms a burgundy color on the slant. If deamination does not occur, the Lysine iron agar slant remains purple. Bromocresol purple, the pH indicator, is yellow at or below pH 5.2 and purple at or above pH 6.8.

Method

1. The medium is tubed, sterilised and slanted so that a short slant and deep butt are formed.
2. With a straight inoculating needle, inoculate LIA by twice stabbing through the center of the medium to the bottom of the tube and then streaking the slant.
3. Cap the tube tightly and incubate at 35°-37°C in ambient air for 18 to 24 hours.
4. Examine at 18 – 24 and 40 – 48 hours for growth and color changes in tube butt and slant, and for blackening at the apex of slant

Expected Results:Alkaline slant/alkaline butt**,** Alkaline slant/alkaline butt, H_2S positive**,** Alkaline slant/acid butt**,** Red slant/acid butt.

Lysine Decarboxylation (detected in butt):

- **Positive Test:** Purple slant/purple butt (alkaline), the butt reaction may be masked by H_2S production
- **Negative Test:**Purple slant/yellow butt (acid), fermentation of glucose only

Lysine Deamination (detected on slant):

- **Positive Test:**Red slant
- **Negative Test:**Slant remains purple

H_2S production

- Positive test: Black precipitate
- Negative test: No black color development
- Gas production: demonstrated by the presence of bubbles or cracks in the medium

3.4.9.Urease test

Purpose: The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive bacteria from other Enterobacteriaceae.

Procedure: Christensen's Urea Agar - Use a heavy inoculum from an 18- to 24-hour pure culture to streak the entire slant surface. Do not stab the butt as it will serve as a color control. Incubate tubes with loosened caps at 35^{o}C. Observe the slant for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production is indicated by a bright pink (fuchsia) color on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. The culture medium will remain a yellowish color if the organism is urease negative.

Urease positive bacteria: Proteus spp., Morganellamorganii.
Urease negative bacteria: E. coli.

3.4.10. Bacitracin Test

Purpose/principle: This test is used for presumptive identification and differentiation of beta-hemolytic group A streptococci (*Streptococcus pyogenes*– susceptible) from other beta-hemolytic streptococci. It is also used to distinguish staphylococci species (resistant) from micrococci (susceptible). The antibiotic bacitracin inhibits the synthesis of bacterial cell walls. A disk impregnated with a small amount of bacitracin (0.04 units) is placed on an agar plate, allowing the antibiotic to diffuse into the medium and inhibit the growth of susceptible organisms. After incubation, the inoculated plates are examined for zones of inhibition surrounding the disks.

Procedure:Using an inoculating loop, streak two or three suspect colonies of a pure culture onto a blood agar plate.Using heated forceps, place a bacitracin disk in the first quadrant (area of heaviest growth). Gently tap the disk to ensure adequate contact with the agar surface.Incubate the plate for 18 to 24 hours at 35°-37°C in ambient air for staphylococci and in 5% to 10% carbon dioxide (CO2) for streptococci differentiation.Look for a zone of inhibition around the disk.

Interpretation;Positive: Any zone of inhibition greater than 10 mm; susceptible **Negative:** No zone of inhibition; resistant

3.4.11. Optochin sensitivity test

Principle: Optochin (ethyl hydrocupreine hydrochloride) sensitivity test is used for the presumptive identification of alpha-hemolytic streptococci as *Streptococcus pneumoniae*. Optochin is used to differentiate *Streptococcus pneumoniae* from other alpha-hemolytic streptococci. *Streptococcus pneumoniae* strains are sensitive to Optochin although some strains are optochin-resistant and other alpha-hemolytic streptococcal species are Optochin-resistant. The Optochin test is performed on a blood-agar medium using a disk diffusion principle. Optochin sensitive *Streptococcus pneumoniae* surrounding the disk impregnated with optochin are lysed, due to changes in surface tension, creating a clear zone of inhibition.

Procedure: Take a few isolated colonies of the alpha- hemolytic organism and streak onto a blood-agar plate.Place an Optochin disk, in the streaked area and incubate it at 35-37^{o}C.Examine the plate after 18/24 hs of incubation and measure the zone of inhibition if applicable.

Interpretation: If the zone of inhibition is 14 mm or more, around a 6-mm disk, then identify the organism as *Streptococcus pneumoniae*. If the inhibition zone is less than 14 mm, further testing (bile solubility or serology) should be done for the identification of *Streptococcus pneumoniae.*

3.4.12.Motility

Principle: The motility test is used to aid identification of certain bacteria. Both negative and positive tests can be useful particularly in the identification of non-fermenting gram negative bacilli when coupled with the OF test or when characteristic motility of any organism is examined (e.g. "tumbling" motility of *Listeria* spp.).

Method: Pick a suspect colony into a tube of peptone water and leave standing at room temperature for four hours and examine using the method below. If negative keep the culture at room temperature overnight and examine again the following morning. Place one drop of suspension using a sterile pastette onto a coverslip and invert this onto a slide containing a ring of plasticine / blutak. Examine as a wet preparation using the x40 objective and focussing on the edge of the drop.

Interpretation: Motile: Active movement from one side of the field to another.

Non-Motile: No active movements as above including the "shimmering" appearance due to Brownian movement.

3.5. Antimicrobial Sensitivity Test

Purpose/principle: This procedure provides instructions to determine the drug sensitivity pattern of bacteria using the Kirby-Bauer disk diffusion method. The antimicrobial will diffuse in a radial manner from the disc and will inhibit bacterial growth around it.

Materials: 0.5 McFarland standards, MHA, Normal saline, Test tube, sterile wooden applicator sticks with cotton, Antimicrobial disks, Measuring caliper/ruler and Candle jar.

Sample: 2ml pure colony equivalent to 0.5 McFarland.

Procedure: Pure colony suspension will be prepared by adding it into normal saline equivalent to 0.5 McFarland standards. The suspension will be streaked on the entire surface of MHA. Then selected antimicrobial agents according to the CLSI guideline will be placed on the plate aseptically (by disc dispenser or manually by sterilized forceps). Then after incubation at 35°C - 37°C for 16 – 24 hrs, zone of inhibition is measured in mm and the result is interpreted based on CLSI breakpoint.

Result interpretation

Sensitive: the susceptible category implies that isolates are inhibited by the usually achievable concentration of antimicrobial agent when the recommended dosage is used for the site of infection.

Intermediate: the intermediate category include isolates with antimicrobial agent zone diameters that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug can be used. This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretation, especially for drugs with narrow pharmacotoxicity margin.

Resistant: the resistant category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate zone diameters that fall in the range where specific microbial resistant mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

Procedural Notes: make sure the turbidity is equivalent to 0.5 McFarland and the thickness of the Mueller Hinton agar is 4 mm.

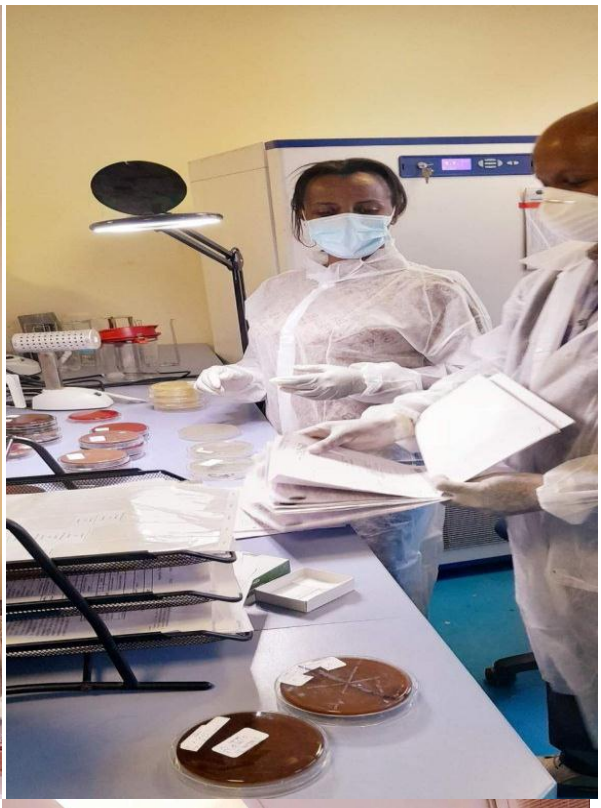
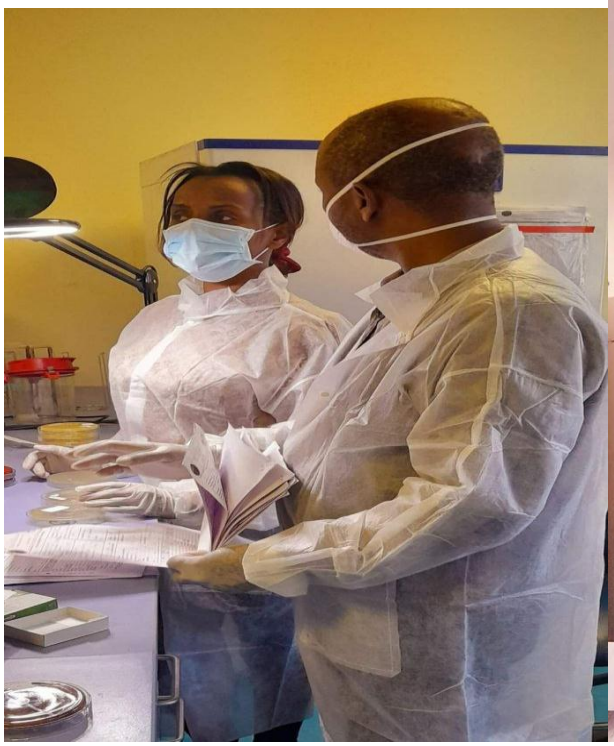
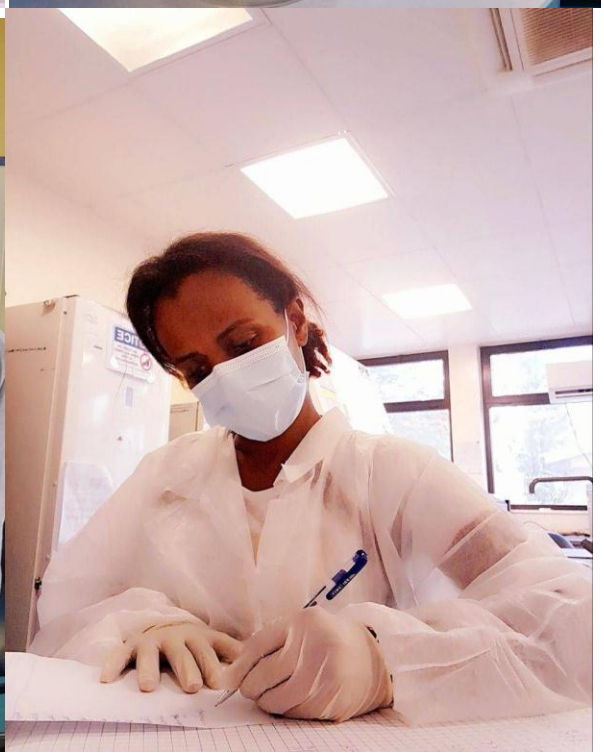
Utility: To detect the *in vitro* relationship between an organism and an antibiotic and predict antibiotic resistance pattern in the community. Zone sizes will be interpreted using manufacturer inserted leaflet and CLSI recommendation as shown in table below**.**

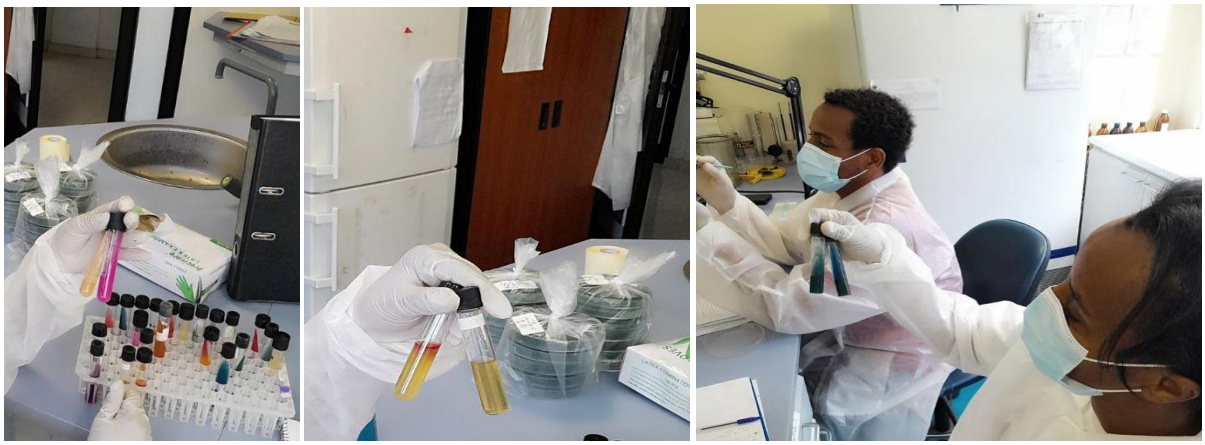
Annex 4: Antimicrobial agents with zone diameter interpretive standards for bacterial isolates

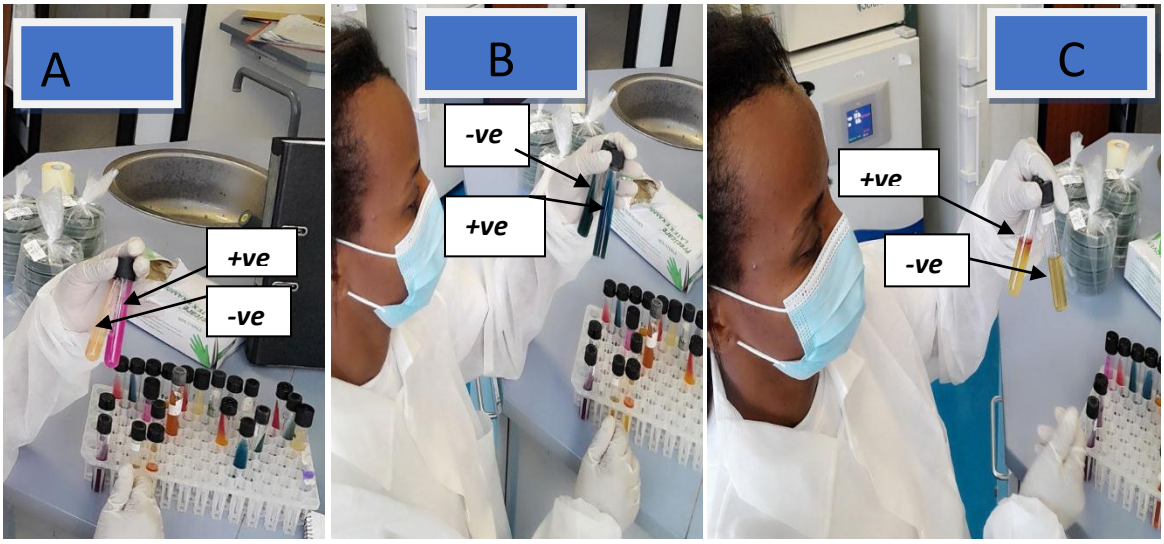
Staphylococcus aureus and Coagulase-negative Staphylococcus spp				
Drug	Conc. (µg)	Sensitive	Intermediate	Resistant
Penicillin	10 units	≥ 29	-	≤ 28
Cefoxitin	30	≥ 22	-	≤ 21
Clindamycin	2	≥ 21	15-20	≤ 14
Trimethoprim/Sulfamethoxazole	1.25/23.75	≥ 16	11-15	≤ 10
Chloramphenicol	30	≥ 18	13-17	≤ 12
Beta-hemolytic Streptococci (large colony-forming groups A, C, G and S. agalactiae)				
Penicillin	10 units	≥ 24		
Erythromycin	15	≥ 21	16-20	≤ 15
Clindamycin	2	≥ 19	16-18	≤ 15
Cefotaxime	30	≥ 24		
Chloramphenicol	30	≥ 21	18-20	≤ 17
Enterobacteriaceae				
Drug	Conc. (µg)	Sensitive	Intermediate	Resistant
Ampicillin	10	≥ 17	14-16	≤ 13
Cefazolin	30	≥ 23	20-22	≤ 19
Gentamicin	10	≥ 15	13-14	≤ 12
Amoxicillin/clavulanate	20/10	≥ 18	14-17	≤ 13
Cefotaxime	30	≥ 26	23-25	≤ 22
Ciprofloxacin	5	≥ 21	16-20	≤ 15
Trimethoprim/Sulfamethoxazole	1.25/23.75	≥ 16	11-15	≤ 14
Chloramphenicol	30	≥ 18	13-17	≤ 12

Pseudomonas aeroginosa				
Ceftacidime	30	≥18	15-17	≤14
Gentamycin	10	≥15	13-14	≤12
Tobramycin	10	≥15	13-14	≤12
Piperacillin	100	≥21	15-20	≤14
Ciprofloxacin	5	≥21	16-20	≤15
Meropenem	10	≥19	16-18	≤15
Piperacilin-Tazobactum	100/10	≥21	15-20	≤14
Enterococcus spp.				
Ampicillin	10	≥17	-	≤16
Chloramphenicl	30	≥18	13-17	≤12
Vancomycin	30	≥17	15-16	≤14

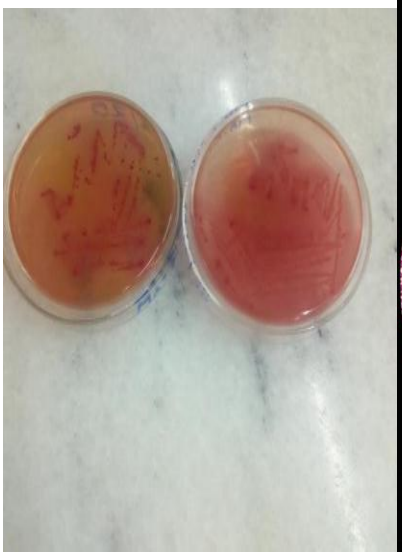
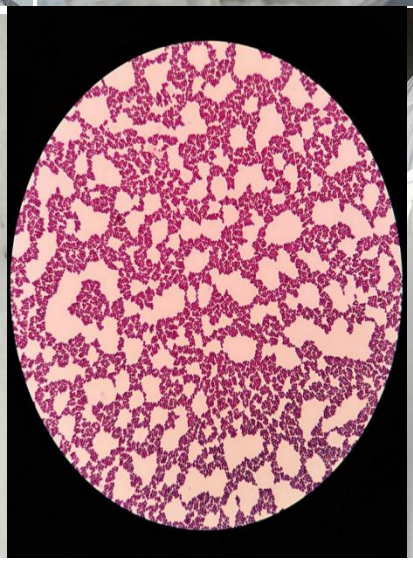
Annex 5: Miscellaneous images taken during the laboratory work



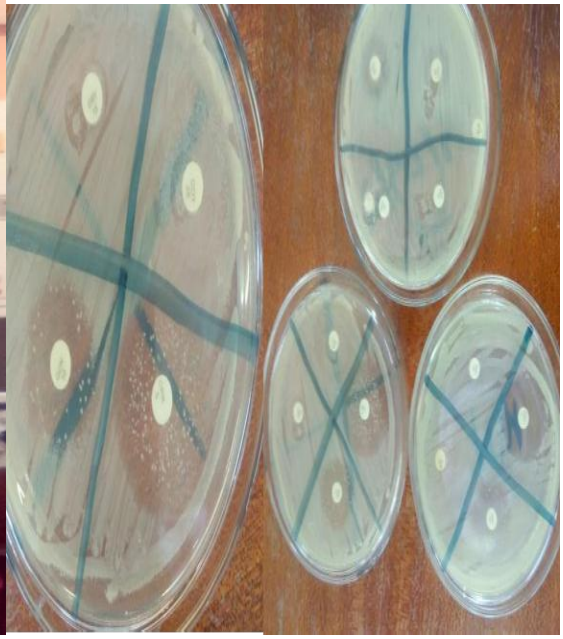

A
+ve
-ve
B
-ve
+ve
C
+ve
-ve





MICRO BIO.



Annex 6: Ethical clearance letter

BIIROO FAYYAA OROMIYYAATTI GIDDUGALA
LAABORATORII RIIFARAALAA, QO`ANNOO QORAANNOO
FI FAYYAA HAWAASA ADAAMAA

OROMIA HEALTH BUREAU ADAAMA PUBLIC
HEALTH RESEARCH & REFERRAL LABORATORY CENTER

Number GGLRAQAPHTA/D854/16

Date 14/01/16

To Roman Abaje

Subject: Letter of Research Completion

Adama Public Health Research and Referral Laboratory center PHEM & Research sub process section was received ethical clearance letter and proposal of Roman Abaje (PI) sent from Adama science and Technology university on the research project entitled '' ***Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Stream Infection Suspected Blood Samples Referred to Adama Public Health Research and Referral Laboratory Center*** '' on December, 2022 reviewed and discussed. Therefore, from the perspectives of ethics, relevance, originality, and technical competence, the project proposal review is deemed acceptable.

Based on this Adama public Health Research and referral laboratory center accepted Mr. Roman Abaie research. So she was conducted here research at Microbiology section on the title Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Stream Infection Suspected Blood Samples of 252 patient samples from August 2022 to May 2023.

Sincerely yours

Masfin Baqqalaa Tolasaa
መስፍን በቀለ ቶሎሳ
Mesfin Bekele Tolosa
Hojii Gaggeessa Ol'anaa
ዋና ስራ አስኪያጅ
Chief Executive Officer (CEO)

Tel:-+251221127962/+251221113505/+251221110718 Fax: +25122-111-35-22 Email:- aphrrlc@gmail.com adamaistc@gmail.com Box 688