ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCE
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Multi-Drug Resistant Bacterial Isolates Among Septicemia Suspected Under Five Children In Tikur Anbesa Specialized Hospital, Addis Ababa Ethiopia

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

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ABBREVIATIONS

AMR	Antimicrobial Resistance
AST	Antimicrobial susceptibility testing
ATCC	American Type Culture Collection
BC	Blood Culture
BSI	Bloodstream Infection
CoNS	Coagulase Negative Staphylococci
CRE	Carbapenem resistant enterobacteriaceae
CTL	Catheter lock therapy
ESBL	Extended Spectrum B-Lactamase
GNB	Gram negative bacteria
ICU	Intensive Care Unit
MIC	Minimum inhibitory concentration
MRSA	Methicillin resistant *Staphylococcus aureus*
PYR	PyrrolidonylArylamidase
SPSS	Statistical Package for Social Science
TASH	Tikur Anbesa Specialized Hospital
VISA	vancomycin intimidate *Staphylococcus aureus*
ZOI	Zone of inhibition

Abstract

Background: Bloodstream infections due to bacterial pathogens are a major cause of morbidity and mortality among pediatric patients. Emergence of drug resistance in high classes of antibiotics among the bacterial pathogens is another issue of the public health concern.

Objective: To determine Multi-Drug Resistant Bacterial Isolates among Septicemia Suspected under Five Children in Tikur Anbesa Specialized Hospital, Addis Ababa Ethiopia.

Methods: Across-sectional study was conducted from September 2017 to June 2018 among pediatric patients with febrile illness aged under five in Tikur Anbesa Specialized Hospital. Three hundred forty blood samples were collected and processed following standard microbiological techniques and culture was performed using BacT/Alert machine in combination with conventional method. Antimicrobial susceptibility testing of the isolates was performed by Kirby-Bauer disc diffusion method and MIC technique

Result: A total of 137(40.2%) bacterial pathogens were isolated from 340 pediatric patients suspected of BSI with febrile illness. Of these isolates, about 46% of them were Gram positive and 54% were Gram negative bacteria. Of the isolates 43 (31.4%) Klebsiella *pneumoniae, 29*(21.2%) *S. aureus*, 15(10.9%) *CoNS and* 12 (8.7%) *Acinitobactor* species were the most frequently isolated pathogens. The overall prevalence of MDR 51.1%, CRE 30.5% and ESBL 25.4% were alarmingly high in bacterial isolates. *Klebsiella pneumoniae* isolates *were 95.6*% MDR, 23.7% ESBL, and 27.1% CRE dominated followed by *Staphylococcus aureus* 55.2% MRSA in children.

Conclusion and Recommendation:

In this study, *Klebsiella pneumoniae* and *S. aureus* are common pathogens associated with BSI in pediatrics with high antimicrobial resistance. High frequency of staphylococcus species and MRSA were a treat of children. Emergency of an intermediate vancomycin susceptibility of an isolate among MRSA calls an attention on treatment options. ESBL producing organisms were common in *Klebsiella species* and *Escherichia coli* isolates. Since most of isolates exhibit multidrug resistant, routine in-vitro susceptibility of antimicrobials and update antibiogram for treatment is essential.

Key words: Blood stream infection, BacT/Alert, multi-drug resistance, ESBL, MRSA, CRE, VRSA

1. INTRODUCTION

1.1 Background

Blood stream infection (BSI) remains one of the most important causes of morbidity and mortality throughout the world. Approximately 200,000 cases of bacteremia occur annually with mortality rates ranging from 20-50% worldwide [1]. Blood stream infection (BSI) accounts for 10-20% of all nosocomial infections and is the eighth leading cause of mortality, in the United States some 17% of result in death [2]. In sub Saharan countries including Ethiopia septicemia is an important cause of illness and death in children, the mortality rate approaches 53% which makes it a significant health problem in developing countries [3].

In many studies a wide range of bacteria has been described in febrile patients including gram negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella species, Neisseria meningitidis, Haemophilus influenzae*, and gram positive such as *Coagulase negative staphylococci (CONS*), *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae,* and *Enterococcus faecium* [4]. The diagnosis of these infections can be confirmed by blood culture, which is routinely available in few Hospitals in developing countries [5].

Bacterial pathogens isolated from BSI are a leading cause of significant patient morbidity and mortality. The impact of specific etiologic agents on BSI patient outcome are tremendous; BSI increases the mortality rate, prolongs patient stay in an intensive care unit and in the Hospital, and leads to increased health care costs [6, 7].

The timely and appropriate use of antibiotics is currently the only way to treat bacteremia. However, many bacterial pathogens have become resistant to antibiotic regimens and become a serious public health concern with economic and social implications throughout the world. Antibiotics resistance is a growing problem in developing countries such as Ethiopia. In Ethiopia the unregulated over-the-counter sale of these antimicrobials, mainly for self-treatment of suspected infection in humans, and to a lesser extent for use in animals without prescription, would inevitably lead to emergence and rapid dissemination of resistance [8]. Many studies have found that inadequate empirical therapy of bacteraemic infections is associated with adverse outcomes, including increased mortality and increased drug resistance emergence [9-10].

During the past few decades, antimicrobial resistance has increased worldwide, and the perspectives are alarming [11-12]. The nature, the magnitude and ways to cope with this problem

are studied and described in the western world, while this base of knowledge is lacking in developing African countries [13- 15]. We lack reports on mortality related to distribution of pathogens and their resistance patterns. Without such reports, guidelines for empiric treatment of severe bacterial diseases cannot be given. While updated studies on outcome in sepsis in Africa are almost non-existent, there are a few reports on bacterial culture results. The most alarming reports on antimicrobial resistance concern patients admitted to Hospitals [16], while community- acquired infections may have lower profiles of resistance [17]. In Ethiopia, the resource situation has not allowed antimicrobial resistance to be prioritized as major public health concern despite the obvious needs [18]. The aim of this study was to identify and determine multi- drug resistance bacteria among blood culture samples from under five patients attending to Tikur Anbesa Specialized Hospital by BacT/Alert and biochemical test.

1.2 Statement of the Problem

Globally blood stream infection is one of the most significant causes of morbidity and mortality among neonates and children. Out of 5.9 million child deaths in 2015, almost 1 million occur in the first day of life and close to 2 million occurs in the first week of life. The main causes of neonatal deaths were preterm birth complications (35 %), intrapartum related complications (24 %), and sepsis (15 %) globally [18].

In the last 30 years, the frequency, etiology, and epidemiology of nosocomial BSI have changed with the evolution of medical care, particularly among the increasing number of Hospitalized patients who require intensive care. Nearly 75% of primary bloodstream infections have been caused by Gram-negative bacilli. This is due to the development of potent anti-staphylococcal β-lactam agents. *Staphylococcus aureus* gave way to Gram-negative bacilli, however, by the early 1980s; Gram-positive cocci began to re-emerge as predominant nosocomial pathogens [19].

Mortality and morbidity from infections are greater when caused by antimicrobial-resistant bacteria. Antimicrobial resistance (AMR) within a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors. Increasingly, governments around the world are beginning to pay attention to a problem so serious that it threatens the achievements of modern medicine. *Enterobacteriaceae* and non-fermentative Gram-negative bacilli are of great concern because antimicrobial therapy for infections due to these resistant pathogens remains a clinical dilemma in Hospitalized patients [20].It is also noted that there is an increase in the resistance among Gram-negative bacilli to third generation cephalosporin which is caused by expression of Extended-Spectrum B-lactamase (ESBL) enzymes. Therefore, infections due to ESBL isolates continue to pose a challenge to infection management worldwide ‘[21].

The impact of specific etiologic agents on BSI patient outcome is tremendous; BSI increases the mortality rate, prolongs patient stay in an intensive care unit and in the Hospital, and increased health care costs [22-23]. Furthermore, inadequate empirical therapy of bacteremia infections is associated with adverse outcomes, including mortality [24- 25].

The timely and appropriate use of antibiotics is currently the only way to treat bacteremia. However, many bacterial pathogens have become resistant to antibiotic regimens and become a serious public health concern with economic and social implications throughout the world. Antibiotics resistance is a growing problem in developing countries such as Ethiopia. In Ethiopia the unregulated and over-the-counter sale of these antimicrobials, mainly for self-treatment of suspected infection in humans, and to a lesser extent for use in animals without prescription, would inevitably lead to emergence and rapid dissemination of resistance. Many studies have found that inadequate empirical therapy of bacteraemic infections is associated with adverse outcomes, including increased mortality and increased drug resistance emergence.

However, there are only a few studies from Ethiopia, which have studied the organisms involved in blood stream infection (BSI) and their susceptibility pattern. We therefore conducted this study to determine the multi-drug resistance of bacterial isolates associated with BSI among under five children in Addis Ababa, Ethiopia. So that we provide current appropriate information about the alternative of drug of choice and an insight into the present situation regarding the etiology and antimicrobial susceptibility of major bacteria isolated from under five children In Tikur Anbesa Specialized Hospital (TASH). Moreover, it is very important to update the available scarce information with regard to the increment of multidrug resistance and widespread of carbapenem resistance entrobacteriaceae (CRE) and extended spectrum β-lactamase producing entrobacteriaceae in our Hospital.

1.3. Significance of the Study

Blood stream infections (BSI) due to bacterial pathogens are a major cause of morbidity and mortality in Ethiopia and other developing countries. In these countries, most patients are treated empirically based on their clinical symptoms. Therefore, up to date etiological data for major pathogens causing bloodstream infections may play a positive role in better healthcare management. Researchers have observed significant changing trends in the microbiology, epidemiology and clinical as well as prognostic significance of positive blood cultures over a period of time. For these reasons, surveillance of bloodstream infections from blood cultures and their antibiotic resistance patterns are vital to the care of patients and prevention of BSI and this information will be generated in this perspective.

The data that can be obtained from this study will be part of the solution for efforts made to understand the management of BSI in the Hospital. There is paucity of objective data on the causative agents and their susceptibility in these Hospital populations particularly in our study area. It may help physicians to be specific for the choice of appropriate antibiotics for empiric treatment and superiorly this study provides information on the prevalence of extended spectrum beta-lactamase, vancomycin resistant Mithcillin resistant *Staphylococcus aureus* and carbapenem resistant producing bacteria. This will help for policy makers to amend or develop new infection control programs in local situations.

2. Literature Review

2.1 Epidemiology of BSI

The epidemiology of BSI varies depending on the geographic location, age and co-morbid illnesses. The epidemiology of pediatric BSI is also influenced by numerous factors, including age, geographical location, nutritional status and vaccine coverage [26]. Outcomes reflect the capacity of the host to combat infection, pathogen virulence and access to medical treatment, including effective antimicrobials [27].

As an example, *Salmonella enterica* is a frequently isolated pathogen from blood samples in both African and Asian regions, however their serotypes differ substantially. *S. Paratyphi* is the predominant organism in the Salmonella group in Africa whereas *S. Typhi* is the most frequently isolated organism in Asia. Besides their isolation rate, their antibiotic susceptibility pattern varies substantially [28]. So, understanding of local epidemiology may play an important role in making proper empirical treatment choices before laboratory test results are available. This is especially true for Bangladesh and other developing countries where healthcare systems operate on poor hygiene system and lack proper facilities to contain infections. In these countries, early treatment is usually based on the patient's clinical symptoms rather than diagnostic results. Therefore, patient's early prognosis to final outcome might be much improved by available epidemiologic data for the most frequently isolated pathogenic organisms. There was a report of multi-resistant Klebsiella spp. In the pediatric department of Tikur Anbesa Specialized Hospital, Addis Ababa (TASH), in 1988 with resistance rates for chloramphenicol, gentamicin and co-trimoxazole of 96%, 89% and 86%, respectively [18]. However, complete data on BSI causing organisms from Addis Ababa, Ethiopia is scarce [29, 30].

2.2 Transmission and Risk Factors for BSI

Several predisposing factors are associated with BSI (Hospitalized patients, age, Intensive care unit, underlined conditions). An increasing age is associated with a higher risk of BSI. The risk for bacteremia in Hospitalized patients that are 85 years or more of age is more than twice than that of patients in the age group of 65-84 years. The increased risk of BSI associated with higher age may reflect the increasing prevalence of underlying co-morbidity that predisposes to acquiring BSI, such as diabetes mellitus, renal and liver disease. Intravascular catheters provide a

port d’entrée for microorganisms to enter the bloodstream and thus pose a risk factor for BSI. The same applies for patients on hemodialysis, intravenous drug abusers, and patients who have undergone invasive procedures in the ICU [31].

2.3 Pathogenesis

The spectrum of bloodstream infections (BSIs) ranging from mild infection to severe sepsis and septic shock causes high mortality, longer Hospitalizations, and excessive cost to the patients and healthcare system. (BSIs) caused by different pathogenic microorganisms and their toxin in blood flow. If there is only transient and a few numbers of blood bacteria without obvious toxemia, it is called bacteremia. Now sepsis and bacteremia are both referred to as bloodstream infection (BSI) [32].

Most episodes of occult bacteremia spontaneously resolve, particularly those caused by *Streptococcus pneumoniae* and *Salmonella*, and serious sequelae are increasingly uncommon. However, serious bacterial infections occur, including pneumonia, septic arthritis, brain abscesses, osteomyelitis, cellulitis, meningitis, and sepsis, possibly resulting in death. The significance of the history in a febrile child varies according to the patient’s age. Elements of the history includes: duration of fever, a specific illness, history of an underlying medical condition and prematurity, history of another reason for an increased temperature, history of gastroenteritis, epidemiology and other risk factors for invasive pneumococcal disease [33].

2.4 Diagnosis of BSI

Sepsis is associated with mortality rates ranging from 20% to 50% in children [18].Therefore, identification of bloodstream infections is among the most critical tasks performed by the clinical microbiology laboratory. While the criteria for achieving an adequate blood culture specimen in adults have been well described, there is much more ambiguity in pediatric populations [34]. Early diagnosis followed by prompt appropriate treatment improves the prognosis of septic patients. One important therapeutically aspect is early initiation of calculated antibiotic therapy. Each hour of delay in administration of antibiotics is associated with an average 8% decrease in survival rate of septic shock. Blood cultures (BC) must be obtained before antibiotic therapy [35] which should be reassessed on the basis of culture results and clinical data. Timely and

continuous reassessment is important, since inappropriate antibiotic therapy deteriorates outcome, whereas adequate therapy is associated with favorable outcome [36].
Typically, BC specimens become positive within 24–36 hours after sampling and therapy can be optimized based on presumptive bacterial identification. A complete microbial identification and susceptibility profile, however, is usually not available before 24–72 hours later. Despite advances in BC techniques, BC positivity rates remain low and may vary significantly, depending on severity of sepsis and ongoing antibiotic treatment [36]. It has been suggested that nucleic-acid-based technology such as PCR is more sensitive and can also shorten the time to result when compared with conventional BC technique [37].

2.5 Treatment and Prevention

Despite appropriate antibiotic therapy, treatment failure (persistent bacteremia, premature device removal due to infection or relapse of infection) occurs in at least 25% of BSI episodes in which salvage is attempted. Relapse can occur many months after infection [38].

Failure is highest in infections caused by certain organisms (such as *S. aureus*, *Candida* spp., *Bacillus* spp. and Mycobacteria), tunnel and pocket infection, and in ports [39]. This is attributable to biofilm, organized communities of microorganisms in a sessile state, predominantly on the luminal surface of the catheter [40]. In addition to the risk of treatment failure, prior BSI increases the risk of future episodes [41].This might be associated with line-care technique, biofilm or catheter-associated thrombus acting as a nidus for reinfection, or the presence of multispecies biofilm.

Empiric antibiotic therapy for suspected BSI should take into account the clinical condition of the patient, documented past colonization or infection with resistant organisms, known allergies and local resistance patterns. Initial therapy should usually be with vancomycin combined with a broad-spectrum agent active against Gram-negative bacteria including *Pseudomonas aeruginosa*, such as an aminoglycoside (e.g., gentamicin), and antipseudomonal penicillin (e.g., Piperacillin-tazobactam), or a cephalosporin (e.g. ceftazidime or cefepime) [42].

Once the causative organism is identified, targeted therapy should be selected based on susceptibility testing. Ideally, a single narrow spectrum agent should be used. Catheter lock therapy (CLT) may be used in addition to systemic therapy with the aim of reducing treatment

failure by targeting biofilm bacteria. A small dose of antimicrobial agent is instilled to fill the catheter lumen and is allowed to dwell for an extended period of time. This permits high concentrations of antimicrobial agent at the site of infection and can be repeated in each lumen throughout the course of antibiotic treatment. For example, local vancomycin concentrations of up to 5 mg/mL, 1000 times higher than the usual minimum inhibitory concentration, are achievable. This targets intraluminal colonization and would not be expected to improve outcomes for extra luminal colonization or infected catheter-associated thrombus [43].

Various interventions to reduce bloodstream infections in infants have been studied, with their focuses on healthcare professionals' hands (e.g., the improvement of compliance with hand hygiene protocols, the use of gloves, the introduction of hand alcohol), the usage of intravenous (IV) devices (e.g., closed IV administration devices, the introduction of IV teams, IV care bundles) or 'other aspects' (e.g., multimodal interventions, neonatal intensive care unit design, and feeding the infant with human milk). However, the results of these studies have not been unanimous, and a recent systematic overview of the effectiveness of various interventions is lacking [44].

A study done by Tabah et al., in Europe documented that carbapenem-resistance rates have progressively increased, with *Acinetobacter* spp., *Klebsiella* spp and *Pseudomonas* spp. showing carbapenem resistance in 69%, 37% and 5.7% of cases, respectively, in patients with HA-BSIs managed in ICU in Europe[45].

ESBL producing Enterobacteriaceae have spread quickly worldwide and became well reported in many publications. Among these, the study conducted in Afghanistan, from January 2010 to June 2012 in pediatric patients, out of all gram-negative isolates, 51.9% strains were ESBL producers and among gram positive isolates 51% were MRSA [46].

In Ethiopia, the study done by Legese et al the overall prevalence of ESBL- and carbapenemase-producing *Enterobacteriaceae* among children under fifteen years were 78.57% (n=22/28) and 12.12%, respectively. Among the *Enterobacteriaceae* tested, *Klebsiella pneumoniae* (84.2%, n=16/19), *Escherichia coli* (100%, n=5/5), and *Klebsiella oxytoca* (100%, n=1/1) were positive for ESBL [47].

3 Objectives

3 .1 General objective

To determine multi-drug resistant bacterial isolates among septicemia suspected under five children in Tikur Anbesa Specialized Hospital, Addis Ababa Ethiopia.

3.2 Specific objectives

- To assess the bacterial pathogens and potential risk factors causing BSI in under five children.
- To determine the prevalence of Methicillin Resistant Staphylococcus aureus (MRSA) and Vancomycin resistant Staphylococcus aureus (VRSA) among septicemic children
- To determine the prevalence of Carbapenem resistant enterobacteriaceae (CRE)
- To determine the prevalence of Extended Spectrum beta-lactamase (ESBL) producing enterobacteriaceae by comparing single disk and double disk synergy methods

3.3. Hypothesis

What are the most common bacterial pathogens and potential risk factors for blood stream infection and circulating multi -drug resistance bacteria among children under five years of age admitted to Tikur Anbesa Specialized Hospital?

4. Material and Methods

4.1. Study area

The study was conducted in Tikur Anbesa Specialized Hospital (TASH), the teaching Hospital of health Science College, Addis Ababa University. TASH is the largest specialized Hospital in Ethiopia, with over 700 beds, and serves as a training center for undergraduate and postgraduate medical students, dentists, nurses, midwives, pharmacists, medical laboratory technologists, radiology technologists, and others who shoulder the health problems of the community and the country at large. With more than 70 percent of childhood deaths attributable to communicable diseases and malnutrition, Ethiopia's healthcare resources have been directed primarily to treat and prevent diseases such as malaria and diarrhea [48].

4.2 Study design and period

A cross-sectional study was conducted from September 2017 to June 2018 to identify the bacterial profiles and antimicrobial susceptibility pattern among septicemia under five patients with acute febrile illness in Tikur Anbesa Specialized Hospital in Addis Ababa.

4.3 Population

4.3.1 Source population

All under 5 years pediatric patients who were suspected for septicemia and seek medical care at the study site during the study period

4.3.2 Study population

All under 5 years pediatric patients who were requested for blood culture in the study site during the study period.

4.4 Inclusion and Exclusion criteria

4.4 .1 Inclusion criteria

- Children aged under five years including neonates with fever
- Patients who are diagnosed with sepsis, Sever sepsis and septic shock
- All children who gave blood sample and their parents volunteer to give permission to participate on the study

4.4.2 Exclusion criteria

- None febrile patients under five years
- Patients who took antibiotics currently within the last 7 days

4.5 Study variables

4.5.1 Dependent variable

Prevalence of bacterial isolates and antimicrobial resistance

4.5.2 Independent variable

- Socio-demographic characteristics
- Frequency of antimicrobial prescription
- Hospitalization
- Care in an intensive care unit,
- Antibiotic use within the preceding 07 days

4.6. Measurement and data collection

4.6.1. Sample size calculation

The sample size for the study that infers the total population was determined using a single population proportion formula. The study considered the previous study of prevalence and antibiotic resistance of bacterial pathogens isolated from children under five in septicemia patients at Tikur Anbesa Specialized Hospital 27.9% bacterial isolation (49), at 95% level of confidence and 5% margin of error.

n = (Zα)2 (pq)/ d2 Where: n = sample size Zα/2 = level of confidence P = diarrhea prevalence q = 1-p d2 = margin of error (0.05)

$n = \frac{z^2 * p * q}{d^2}$, p=0.279, q=0.721, d=0.05, Zα/2=1.96

$\frac{1.96^2 * 0.279 * 0.721}{0.05^2} = 309.$

Considering 10% non- response rate, the totals of 340 children patients were enrolled in the study.

4.6.2. Sampling technique

The study subjects were selected using convenient sampling technique from all patients attending Tikur Anbesa specialized Hospital among under five children with febrile illness clinically diagnosed at pediatric OPD, ICU and impatient pediatric ward admitted during the study period. Sampling technique was employed for those children fulfill the inclusion criteria.

4.6.3. Data collection procedure

Well standardized questionnaire was used to collect socio-demographic characteristics (sex, age, clinical presentation (fever, vomiting and household income). Patients visiting outpatient departments (pediatric and general medicine) and those admitted in the inpatient units were investigated for bloodstream infections by respective unit physicians. At the onset of fever (>37∘C) or in the presence of any clinical symptoms compatible with infection.

4.6.4. Laboratory analysis

4.6.4.1 Blood sample collection

A venous blood culture specimen was taken with aseptic technique by cleansing of the collection site with 70% alcohol and subsequently followed by 10% povidone-iodine solution by trained laboratory personnel. About 2.5-5ml of blood specimen was collected and inoculated into aerobic 30ml BacT/ALERT PF Plus pediatric bottles at the blood to broth ratio of 1: 10-1:30. At least 2 sets of blood cultures were collected from a patient with suspected bacteremia prior to the initiation of antimicrobial therapy.

4.6.4.2 Culture Isolation and Identification

Venous blood to BacT/ALERT culture bottles were incubated in automatic BacT/ALERT® 3D at 37°C of 5% CO_2 for 5 days for the primary isolation of the microorganism. Two aerobic blood culture bottles were used for each patient and growth in both bottles were considered positive. The microbial growth that could be detected by flag and audible sound of the instrument will subsequently be sub culture on 5% sheep blood Agar, chocolate, and MacConkey Agar plate (Oxoid Ltd, UK) and incubate at 37°C for 18-24 for bacterial isolation. The MacConkey agar plate was incubated aerobically while chocolate and blood agar were incubated in microaerophilic atmosphere (5-10% CO_2) candle Jar. A negative result was checked by

examining the flag and doing gram stain and a final subculture at the end of 5th day (why five days) prior to discarding as negative. The significant growth colonies were examined morphologically for size, consistency, shape, hemolytic and ability to ferment lactose [50].

Blood agar, chocolate and MacConkey agar (Oxoid Ltd, UK) were used for subcultures and gram stain was performed for preliminary result. All positive cultures from blood samples were characterized by colony characteristics, Gram stain and biochemical tests. In case of gram negative bacteria convectional biochemical test and serological identification was performed for *Salmonella* and *Shigella* spp [50- 51].

4.6.4.3 Antibiotic Susceptibility Test

Kirby-Bauer disc diffusion method

Pure Colony of isolated bacterial organism was mixed with 0.85% normal saline and measured at 0.5 McFarland standards for susceptibility testing. The bacterial isolates were tested against the following drugs commonly used; for gram positive bacteria Clindamycin (2µg), Cefoxitin (30µg),Penicillin(10µg),Trimethoprim-Sulphamethoxazole (1.25/23.75µg), Erythromycin(15µg), Oxacilin(1µg), Ampicillin(10µg), Vancomycin (30µg), for gram negative bacteria Tobromycin (10µg),Amoxicilin-Clavulanate(20/10µg),Amikacin(30µg),Gentamycin(10µg), Ampicilin(10µg), Piperacillin-Tazobactam(100/10µg), Cefotaxim (30µg), Cefepime (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Impenem/ Meropinem (10µg), Trimethoprim- Sulfamethoxazole (1.25/23.75µg), Nalidixic Acid (30µg) were tested. Kirby-Bauer's Disk Diffusion method was used for susceptibility of the isolates on Muller Hinton agar and 5% sheep blood with Muller Hinton agar was referred to the standard interpretative chart reporting the zone sizes of each antimicrobial in the book of Cheesbrough, 2009 and CLIS guidelines 2017 [52].

4.6.4.4 Methicillin resistant Staphylococcus aureus (MRSA)

Phenotypic test for Methicillin resistant *Staphylococcus aureus* (MRSA) isolates were detected by cefoxitin disk (30 μg) method of CLSI 2017. *S. aureus* isolates are deemed methicillin resistant when the zone of inhibition (ZOI) for cefoxitin was ≤21mm. Similarly, inducible macrolide-lincosamide-streptogramin-B (iMLSB) resistance was detected in *S. aureus* by disk approximation using clindamycin (2 μg) and erythromycin (15 μg) on Mueller–Hinton agar plates. After overnight incubation, isolates with flattened zone of inhibition adjacent to the

erythromycin disk (referredto as a "D" zone) were considered to exhibit inducible clindamycin resistance.

4.6.4.5 Vancomycin resistant Staphylococcus aureus (VRSA)

The MIC of vancomycin was determined by a broth micro-dilution method using Mueller–Hinton broth as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). VRSA was considered to be resistant at ≥ 16 µg/mL and VISA are resistant to vancomycin at concentrations from 4-8 µg/ml and VSSA with MIC ≤2 µg/mL CLSI guideline 2017 [52].

4.6.4.6 Detection of Carbapenem Resistance

All the Carbapenem (imipenem or Meropenem) resistant or intermediate isolates were checked for the presence of carbapenemase using modified Hodges test (MHT), also known as the clover leaf test as per CLSI 2017. Presence of indentation indicates a positive test and the isolate was a carbapenemase producing strain. No growth of the ATCC *E. coli* 25922 along the organism growth streak indicates a negative test and the isolate is not a carbapenemase producer.

4.6.4.7 Detection of Extended spectrum beta-lactamase

Initial screening for ESBL was done by the diameters of zones of inhibition produced by Ceftazidime (30µg), Ceftriaxone (30µg) and Cefotaxime (30µg) found to be within the CLSI screening criteria. These breakpoints indicative of thought for ESBL production are: for CAZ≤22mm, CRO, ≤ 25 mm and for CTX≤ 27mm. Phenotypic detection of ESBL production was confirmed by double disk synergy test and combined disk test according CLSI(2017) guidelines.

4.6.4.4 .1 Combined disk (double disk potentiate) Test (CDT)

A Ceftazidime (30 µg) disk and Cefotaxime (30µg) dick were used alone and their combination with Clavulanic acid (30 µg/10 µg) for phenotypic confirmation of the presence of ESBLs. A ≥5 mm increase in zone diameter for either of the Cephalosporin disks and their respective Cephalosporin/Clavulanate disk were interpreted as ESBL producer. This method (according to CLSI) is used as reference phenotypic method for comparing double disk synergy method.

4.6.4.4 .2 Double Disk Synergy Test (DDST)

The organism to be tested was spread onto a Mueller–Hinton agar plate. The antibiotic disks used are Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30µg), Aztreonam (30µg) and Amoxicillin/ Clavulanic acid (20/10 µg). The four antibiotics were placed at distances of 20 mm (edge to edge) from the Amoxicillin/Clavulanic acid disk placed in the middle of the plate. After 24-h incubation, if an enhanced zone of inhibition between either of the cephalosporin antibiotics and the Amoxicillin/Clavulanic acid disk occurred, the test was considered positive.

4.7 Data Quality assurances

Media preparation as per manufacturer instructions and laboratory Standard Operating Procedures (SOP) was strictly followed. Verify that media meet expiration date and quality control parameters per CLSI. Labeling container, media, filling the forms were carried out.
Visual inspections of cracks in media or plastic petri-dishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed. Use *ATCC* control strain for each isolated bacterium including *E. coli* 25922, *S. aureus* 25923, *Pseudomonas areuginosa* 27853, *H.influnzae* 10479. Report the results on log sheet and stored for further data. Samples were stored at -80 ^{0}c in skim milk.

4.8 Data analysis and interpretation

SPSS versions 20.0 was employed to analyze the work and to make inferences on the frequency of occurrence of the bacterial pathogens associated with febrile illness and to show the resistance pattern to antibiotic substances. Descriptive statistics to analysis by using frequency, proportions graphs, crosstabs and odds ratio. Bivariate analysis was performed for each factors associated with bacteria pathogens in pediatrics with BSI. Regression analysis was conducted to identify associated factors and how they are associated with dependent variables .The strength of association was presented by odds ratio and 95% confidence interval and p-value of <0.05 was considered as statistical significant association between risk factors and positive blood cultures causing BSI

4.9 Ethical Considerations

The study was conducted after ethical clearance was obtained from the research ethical committee of Department of Medical Laboratory sciences. An informed consent was obtained before collection of blood specimens and results were used in the management of patients. Those

patients who clinically diagnosed as BSI in pediatric OPD and admitted willing to participate in the study and able to give blood sample during the study period were informed about the purpose of the study and written consent was sought for the study. Any information related with the patient result and clinical history was kept confidential.

4.10 Dissemination and Utilization of Results

After the completion of the study the research were disseminated to Department of Medical Laboratory Sciences, School of Allied Health Science, College of Health Science, and Addis Ababa University. It will also be submitted for scientific publication.

4.11 Operational Definitions

Antimicrobial resistance: occurs when microorganisms change in ways that render the medications used to cure the infections they cause ineffective.

Extended spectrum β-lactamase (ESBL): Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam.

Multidrug resistance (MDR): is antimicrobial resistance shown by a species of microorganism at least to one drug in three different classes of antibiotics.

5 Results

5.1 Clinical and socio-demographic Characteristics of Study population

A total of 340 pediatric patients under five years of age suspected of bacteremia were enrolled from September 2017 to June 2018 in Tikur Anbesa Specialized Hospital.

Table 1: clinical and sociodeographic characteristic of study participants for blood culture in TASH, 2018**.**

Variables	*Frequency (%)*
Sex	
Male	122(35.9)
Female	218(64.1)
Age group	
Neonates (<28days)	111(32.6)
Infants (<1years)	115(33.8)
Children(<5years)	114(33.5)
Clinical condition	
CHF	29(8.5)
Hospital acquired infection	43(12.6)
Early onset of neonatal sepsis	71(20.8)
Late onset of neonatal sepsis	48(14.1)
Sepsis	102(30.0)
Mengitidis	3(0.8)
Neuroblastoma	5(1.5)
Community acquired pneumonia	7(2.0)
Endocarditis	11(3.2)
Neutrophenic fever	9(2.6)
Othrs	12(3.5)
Unit of diagnosis	
ICU	83(24.4)
Impatient	181(53.2)
Pediatric OPD	76(22.4)
Duration of admission	
1-2 days	61(17.9)
3-4 days	76(22.4)
5-6 days	41(12.0)
=>7 days	84(24.7
Cause of high fever	
Suspected bacteremia	340(100)
Malaria	0(0)
Others (viral, fungal...)	0(0)
Symptom of BSI	
High grade Fever only	267(78.5)
Mild Fever and others	73(21.5)
Antibiotics taken before 07 days	
Yes	148(43.5)
No	192(56.5)
History Hospital acquired infection	
yes	52
No	298
Complication of BSI	
Yes	92
No	248

Among the study participants 122(35.9%) were males and 218 (64.1%) were females resulting in an overall female to male ratio of 1.7:1. The mean age of pediatrics participated in this study was 1.04±1.0 (SD) years [Table 1]

From the study patients 76(22.4%) were from pediatric OPD and 181(53.2%), 83(24.4%) from inpatient ward and ICU ward respectively. The proportion of culture positive patients in the ICU 59/83(71.1%), impatient 66/181(36.5%) and pediatric OPD 10/76 (13.2%) patients were identified as shown in table 1

Patients showed different clinical diagnosis before confirmed their BSI in blood culture, most of were sepsis 102(30.0%) followed by Early onset of neonatal sepsis /EoNS/ 71 (20.9%),Late onset neonatal Sepsis/LoNS 48(14.1%) and Hospital acquired infection 43(12.6%). However, the distribution of positive blood culture among patients with

different clinical diagnosed disease type suspected of having BSI showed that among clinical disease in endocarditis 7/11(63.6%), Hospital acquired infection 26/43(60.5%), sepsis 49/102 (48%) and late neonatal sepsis 21/48 (44%) high positive blood culture were identified as shown in Figure

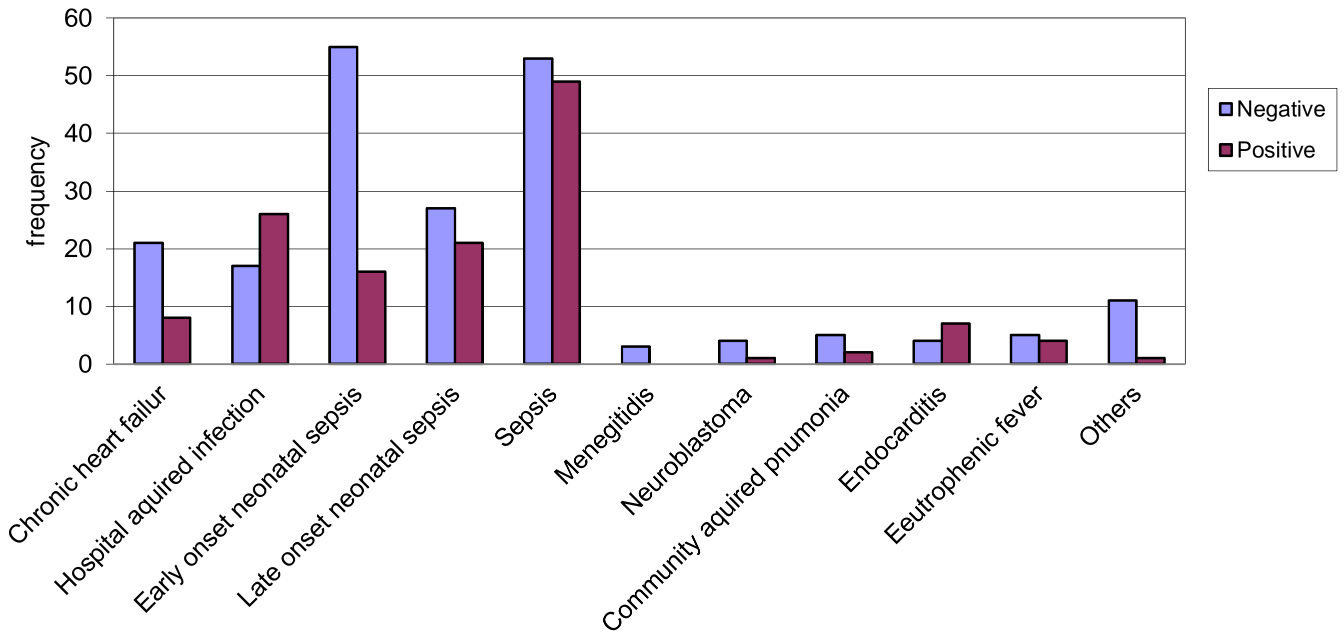


Figure 1: Distribution of positive blood culture among clinically diagnosed disease suspected septicemia in TASH, 2018

5.2 Blood stream infection and associated factors for positive blood culture

Analysis of data using logistic regression model showed that among the clinical condition, high proportion of positive blood culture was isolated in Hospital acquired infection and Endocarditis however no significant association between clinical condition and blood culture ($p>0.05$). The duration of admission in the Hospital for those admitted longer duration (≥7 days) showed high proportion of positive blood culture compared to short duration (1-2 days) but no significant association for positive BSI with p value>0.05 and AOR =0.28[0.06, 1.21]).

With regard to patients with high grade fever only in clinical symptoms showed significantly associated ($p<0.05$& AOR=3.2 [1.4, 7.3]) positive blood culture and was about three times more likely infected with bacteria compared to patients with mild fever and other clinical symptoms (chills, fast heartbeat, shivering and vomiting). Pervious exposure status of the patient for Hospital acquired infection was significantly associated ($P<0.05$& AOR=25.0 (5.0, 111.1).

Having complication of BSI for development of sepsis/septic shock/ septicemia was also significantly associated to positive blood culture ($p<0.05$& AOR=20.0 (6.25, 50.0) in our study as shown in Table 2.

Table 2 **:**Associated factors for positive blood culture in TASH, 2018

	Blood culture result		**Bivariate**	**multivariate**
Variables	**Positive n (%)**	**Negative n (%)**	**COR (95%CI)**	**AOR (95%CI)**
Sex				
Male	55(45.1)	67(54.9)	1	
Female	80(36.7)	138(63.3)	1.4(0.9,2.2)	
Age group				
Neonates (<28days)	39(35.1)	72(64.9)	1.5(0.9,2.6)	
Infants (<1years)	44(38.3)	71(61.7)	1.3(0.7,2.2)	
Children(<5years)	52(45.6)	62(54.4)	1	
Clinical condition				
CHF	8(27.6)	21(72.4)	2.1(0.4,9.8)	
Hospital acquired infection	26(60.5)	17(39.5)	0.5(0.1,2.2)	
Early onset of neonatal sepsis	16(22.5)	55(77.5)	2.7(0.6,11.4)	
Late onset of neonatal sepsis	21(43.8)	27(56.2)	1.0(0.2,4.3)	
Sepsis	49(48.0)	53(52.0)	0.8(0.2,3.4)	
Mengitidis	0(0)	3(100)	-	
Neuroblastoma	1(20.0)	4(80.0)	3.2(0.2,41.2)	
Community acquired pneumonia	2(28.6)	5(71.4)	2.0(0.2,16.3)	
Endocarditis	7(63.6)	4(36.4)	0.4(0.0,2.7)	
Neutrophenic fever	4(44.4)	5(55.6)	8.8(0.7,100.2)	
Othrs	1(8.3)	11(91.7)	1	
Unit of diagnosis				
ICU	59(71.1)	24(28.9)	0.06(0.02,0.14)	
Impatient	66(36.5)	115(63.5)	0.25(0.12,0.53)	
Pediatric OPD	10(13.2)	66(86.8)	1	
Duration of admission				
1-2 days	10(16.4)	51(83.6)	1	
3-4 days	30(39.5)	46(60.5)	**0.30(0.13,0.68)***	0.76(0.25,2.29)
5-6 days	21(51.2)	20(48.8)	**0.18(0.07,0.46)***	0.45(0.13,1.58)
=>7 days	63(75.0)	21(25.0)	**0.06(0.02,0.15)***	0.28(0.06,1.21)
Cause of high fever				
Suspected bacteremia	135(39.7)	205(60.3)	-	
Malaria	0(0)	0(0)		
Others (viral, fungal...)	0(0)	0(0)		
Symptom of BSI				
High grade Fever only	82(30.7)	185(69.3)	**5.9(3.3,10.6)***	**3.2(1.4,7.3)****
Mild Fever and others	53(72.6)	20(27.4)	**1**	1
Antibiotics taken before 07 days				
Yes	49(33.1)	99(66.9)	1	
No	86(44.8)	106(55.2)	0.6(0.3,0.9)	
History of Hospital acquired infection				
yes	49(94.2)	3(5.8)	**50.0(12.5,125.0)***	**25.0(5.0,111.1)****
No	86(29.9)	212(70.1)	**1**	**1**
Complication of BSI				
Yes	87(94.6)	5(5.4)	**100.0(33.3,200.0)***	**20.0(6.25,50.0)****
No	48(19.4)	200(80.6)	**1**	**1**

COR -crude odd ratio, AOR-adjusted odd ratio, 1 reference category, **significance $p<0.05$

Age boundary according to WHO age classification, 2007

5.3 Bacteria pathogens causing BSI

Of 340 paired blood sample bottles, a total of 137(40.2%) bacterial pathogens were isolated from pediatric patients suspected of BSI with febrile illness. Among positive blood culture results about 46% of them were Gram positive bacteria while 54% were Gram negative bacteria.

Klebsiella pneumoniae was the highest incidence 31.4%, *S. aureus* accounted for 21.2% of the total isolates followed by *coagulase negative Staphylococcus (CoNS) 10.9% and Acinitobactor* species (8.7%). Double infection from species *pseudomonas+ Klebsiella oxytoca* were identified in one patient as shown in figure 2

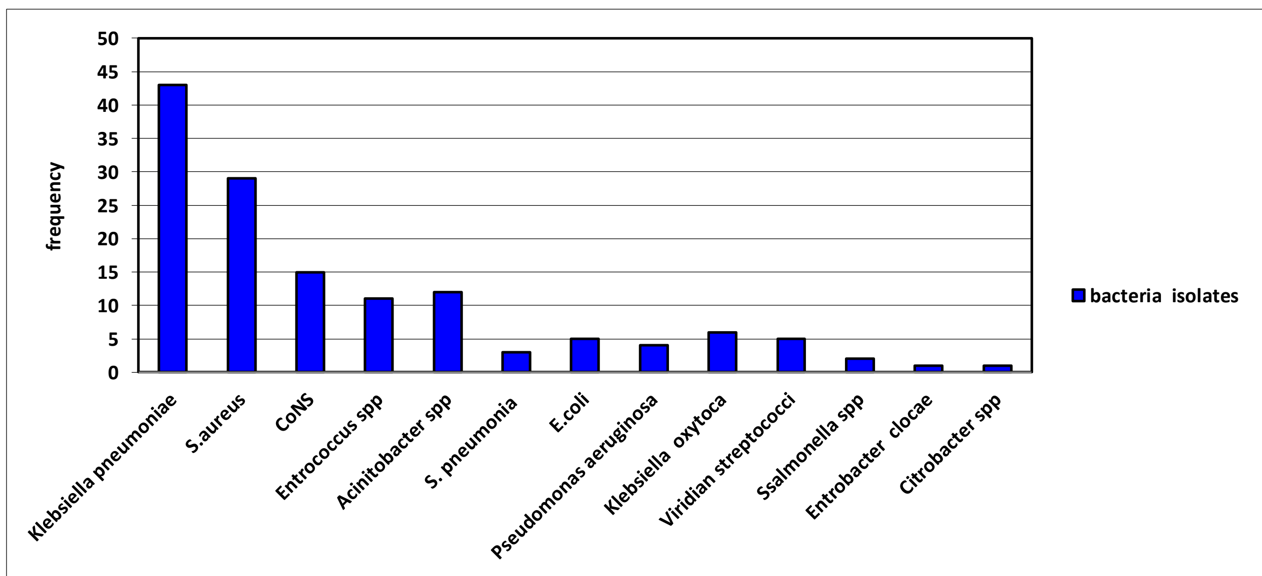


Figure 2: Distribution of bacteria pathogens isolated from blood stream infection among suspected septicemia patients in TASH, 2018

5.4 Antimicrobial susceptibility Testing

Trends of antibiotics prescribed were assessed prior to blood sample collection before 7days and about 148(43.5%) participants have taken antibiotics empirically, of these 49(33.1%) were culture positive during the study. Ampicillin and Gentamicin were among the most common empirical prescribed antibiotics. After collection of positive blood culture results about 20 antibiotics were applied in 137(40.2%) isolates and it revealed that the most prescribed antibiotics cotrimosazole, gentamycin, and ciprofloxacillin showed high resistance.

5.4.1Antimicrobial susceptibility pattern of Gram positive bacterial isolates

Isolates from pediatric patients showed high level of resistance among tested antimicrobials. The predominant gram positive bacteria *Staphylococcus aureus* isolates were resistant to oxacillin (55.2%), penicillin (55.2%), Trimethoprim- Sulphamethoxazole (37.9%) and were sensitive to erythromycin (72.4%), clindamycin (65.5%), Trimethoprim- Sulphamethoxazole (62.1%). Another gram positive isolates of *Coagulase negative Staphylococcus /CoNS* were resistance to Trimethoprim- Sulphamethoxazole (13.3%), oxacillin (6.7%), penicillin (6.7%) and were sensitive oxacillin and penicillin with the same rate 93.3%. *Enterococcus spp* were resistance to ampicillin (18.2%) but sensitive to vancomycin (100%) and ampicillin (81.8%). on the other hand, *Viridian Streptococci* were completely sensitive to tested antimicrobials clindamycin, ampicillin, penicillin and erythromycin. Furthermore, isolates of streptococcus pneumonia were resistance to Trimethoprim- Sulphamethoxazole (33.3%) and sensitive completely to penicillin, clindamycin, ampicillin, erythromycin and Augmentin (Table 3).

Table 3: Antimicrobial susceptibility of gram positive bacterial isolates associated with bloodstream infections among pediatric patients in Tikur Anbesa Specialized Hospital, 2018

Gram positive bacterial isolates		**Antimicrobial susceptibility pattern**							
		SXT	**CN**	**AMP**	**OXA**	**P**	**E**	**VAN**	**AGU**
S .auresus	R%	11(37.9)	10(34.5)	NA	16(55.2)	16(55.2)	8(27.6)	NA	NA
(n=29)	S%	18(62.1)	19(65.5)	NA	13(44.8)	13(44.8)	21(72.4)	NA	NA
Coagulase negative	R%	2(13.3)	0(0)	NA	1(6.7)	1(6.7)	0(0)	NA	NA
staph./CoNS(n=15)	S%	13(86.7)	15(100)	NA	14(93.3)	14(93.3)	15(100)	NA	NA
Enterococcus spp	R%	NA	NA	2(18.2)	NA	NA	NA	0(0)	NA
(n=11)	S%	NA	NA	9(81.8)	NA	NA	NA	11(100)	NA
Viridian streptococci	R%	NA	0(0)	0(0)	NA	0(0)	0(0)	NA	NA
(n=5)	S%	NA	5(100)	5(100)	NA	5(100)	5(100)	NA	NA
S. pneumonia	R%	1(33.3)	0(0)	0(0)	0(0)	0(0)	0(0)	NA	0(0)
(n=3)	**S%**	**2(66.7)**	**3(100)**	**3(100)**	3(100)	3(100)	**3(100)**	NA	**3(100)**

P =Penicillin, AMP = ampicillin, SXT = cotrimoxazole (trimethoprim + sulphamethoxazole), CN=clindamycin, ox=oxacillin, VAN=vancomycin, E=erythromycin, AGU=Augmentin

5.4.2 Antimicrobial susceptibility pattern of Gram negative bacterial isolates

In this study, susceptibilities of beta-lactam antibiotics, fluoroquinolones, aminoglycosides, and carbapenems towards 72 gram negative isolates (with exception of *Salmonella species*) were carried out from pediatric patients as recommended by CLSI 2017. The predominant gram negative isolates from BSI were *Klebsiella pneumoniae species* showed resistance to ampicillin (100%) and cotrimosazole (90.7%). On the other hand, the isolates susceptible to meropenem (62.8%) and Piperacillin-Tazobactam(58.1%). All *Acinitobactor species* were highly resistance to tested antimicrobials such as cefepime (100%), ceftazidime (90.9%), 72.7% for each meropenem and ciprofloxacillin. *Pseudomonas spp* also showed fifty percent (50%) resistance to anti-pseudomonal antibiotics gentamycin, ciprofloxacillin, cefepime, Amikacin and ceftazidime but it was susceptible 75% to meropenem and Piperacillin-Tazobactam. All *Salmonella species* completely susceptible to Ciprofloxacillin, ceftriaxone, and ampicillin and less susceptible to cotrimosazole (50%) as shown in Table 4.

Table 4: Antimicrobial susceptibility of gram negative bacterial isolates associated with bloodstream infections among pediatric patients in Tikur Anbesa Specialized Hospital, 2018

Gram negative Bacteria isolates		Antimicrobial susceptibility pattern											
		SXT	GEN	CIP	CRO	AMP	MEM	FEP	AMK	TORB	PZT	AGU	CAZ
E.coli(n=5)	R%	5(100)	3(60.0)	5(100)	4(80.0)	4(80.0)	0(0)	4(80.0)	0(0)	4(80.0)	3(60.0)	3(60.0)	5(100)
	S%	0(0)	2(40.0)	0(0)	1(20.0)	1(20.0)	5(100)	1(20.0)	5(100)	1(20.0)	2(40.0)	2(40.0)	0(0)
P.aeruginosa	R%	NA	2(50.0)	2(50.0)	NA	NA	1(25.0)	2(50.0)	2(50.0)	1(25.0)	1(25.0)	NA	2(50.0)
(n=4)	S%		2(50.0)	2(50.0)			3(75.0)	2(50.0)	2(50.0)	3(75.0)	3(75.0)		2(50.0)
Klebsiella	R%	39(90.7)	38(88.4)	38(88.4)	37(86.0)	43(100)	16(37.2)	38(88.4)	22(51.2)	38(88.4)	18(41.9)	37(86.0)	37(86.0)
pneumoniae. (n=43)	S%	4(9.3)	5(11.6)	5(11.6)	6(14.0)	0(0)	27(62.8)	5(11.6)	20(46.5)	5(11.6)	25(58.1)	6(14.0)	6(14.0)
Klebsiella	R%	5(83.3)	3(50.0)	4(66.7)	6(100)	6(100)	2(33.3)	6(100)	3(50.0)	4(66.7)	3(50.0)	6(100)	6(100)
oxatica(n=6)	S%	1(16.7)	3(50.0)	2(33.3)	0(0)	0(0)	4(66.7)	0(0)	3(50.0)	2(33.3)	3(50.0)	0(0)	0(0)
Acinitobactor	R%	NA	9(81.8)	11(72.7)	NA	NA	8(72.7)	10(90.9)	6(54.5)	9(81.8)	6(54.5)	NA	11(100)
(n=11)	S%		2(18.2)	3(27.3)			3(27.3)	1(9.1)	5(45.5)	2(18.2)	5(45.5)		0(0)
Entrobacter	R%	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)
clocae(n=1)	S%	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)
Citrobacter	R%	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)
(n=1)	S%	0(0)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)
Salmonella	R%	1(50.0)	NA	0(0)	0(0)	0(0)	NA	NA	NA	NA	NA	NA	NA
spp (n=2)	S%	1(50.0)		2(100)	2(100)	2(100)							

SXT—Sulphamethoxazol-trimethoperem/cotrimosazole, GN—Gentamycin, CIP-Ciprofloxacillin, CRO-ceftriaxone, AMP-ampicillin, MEM—Meropenem, FEP-Cefepime, AMK –Amikacin, CAZ- ceftazidime, Torbomycin, Piperacillin-Tazobactam, AGU—Augmentin/Amoxycillin-Clavulanic acid, NA- Not applicable

5.4.3 Multi-drug resistant isolates

Antibiogram pattern of the isolates in this study showed that multidrug resistance among gram positive isolates 2(6.9. %) and 7(24.1%) of S. *aureus* isolates were resistance to three and five antibiotics respectively. On the other hand, among gram negative isolates the prevalence of multidrug resistance (MDR) in *Pseudomonas aeruginosa* showed that two (50%) of the isolates exhibit resistance to three antibiotics. In *Klebsiella pneumoniae.* majority of isolates 35(81.4%) were resistance to eight and more tested antibiotics even though 2(4.6%), 1(2.3%), 2(4.6%) and 2(4.6%) isolates were resistance consecutively to three ,four ,six and seven antibiotics respectively . Among eleven *Acinitobacter* spp 7(63.6%) isolates were resistance to eight and

more antimicrobials and 1(9.1%) was resistance to seven antibiotics .the least isolate of gram negative bacteria *Entrobacter cloacae* 1(100%) was resistance to eight and more antibiotics. However there was no MDR in *Viridian streptococci, CoNS, S. pneumonia, Enterococcus species, and Citrobacter* and *Salmonella species.* In general, 51.1% of the isolates in our study developed multidrug resistance to at least to one drug in three different classes of antibiotics (≥3 antibiotics) Table 5.

Table 5: Resistance antibiogram of gram positive and gram negative isolates from BSI among pediatric patients in Tikur Anbesa Specialized Hospital, 2018

					Resistance antibiogram				
Bacterial isolates	**RO%**	**R1%**	**R2%**	**R3%**	**R4%**	**R5%**	**R6%**	**R7%**	**≥R8%**
Viridian streptococci(n=5)	5(100)	-	-	-	-	-	-	-	-
S .aureus(n=29)	11(37.9)	0(0)	7(24.1)	2(6.9)	0	7(24.1)	-	-	-
CoNS(n=15)	13(86.7)	2(13.3)	-	-	-	-	-	-	-
S. pneumonia (n=3)	1(33.3)	2(66.7)	-	-	-	-	-	-	-
Enterococcus spp (n=5)	9(81.8)	2(18.2)	-	-	-	-	-	-	-
E.coli(n=5)	1(20)	-	1(20)	1(20)	1(20)	-	1(20)	-	-
Pseudomonas (n=4)	2(50)	-	-	2(50)	-	-	-	-	-
Klebsiella pneumoniae. (n=43)	-	-	1(2.3)	2(4.6)	1(2.3)	-	2(4.6)	2(4.6)	35(81.4)
Klebsiella oxytoca (n=6)	-	-	1(16.7)	-	-	1(16.7)	1(16.7)	-	3(50)
Acinitobactor (n=11)	3(27.3)	-	-	-	-	-	-	1(9.1)	7(63.6
Entrobacter clocae(n=1)	-	-	-	-	-	-	-	-	1(100)
Citrobacter (n=1)	-	-	1(100)	-	-	-	-	-	-
Salmonella spp(n=3)	2(66.7)	1(33.3)	-	-	-	-	-	-	-

RO,R1,R2,R3,R4,R5,R6,R7,R8,R9,R10 sensitive, resistance to 1,2,3,4,5,6,7,8,9,10 antibiotics respectively

5.4.4 Methicillin resistant *Staphylococcus aureus* (MRSA)

Susceptibility test of cefoxitin disk from a total of 29 *Staphylococcus aureus* isolates, 16(55.2%) were resistance to cefoxitin. All resistance to cefoxitin (55.2%) predicted for oxacillin resistance and other β-lactamase. Therefore the prevalence of methicillin-resistance (oxacillin resistance) MRSA was 55.2% of the isolates. Minimum inhibitory concentration (MIC) test done among 29 *Staphylococcus aureus* isolates for Vancomycin susceptibility showed that one (3.4%) of *S. aureus* isolate recovered from the blood of a patient had an MIC of 4 μg/ml and was confirmed by the CLSI as VISA .For the remaining 28 isolates, a shift in vancomycin MICs from 0.5 to 1.0 μg/ml was observed .However no VRSA isolates were detected.

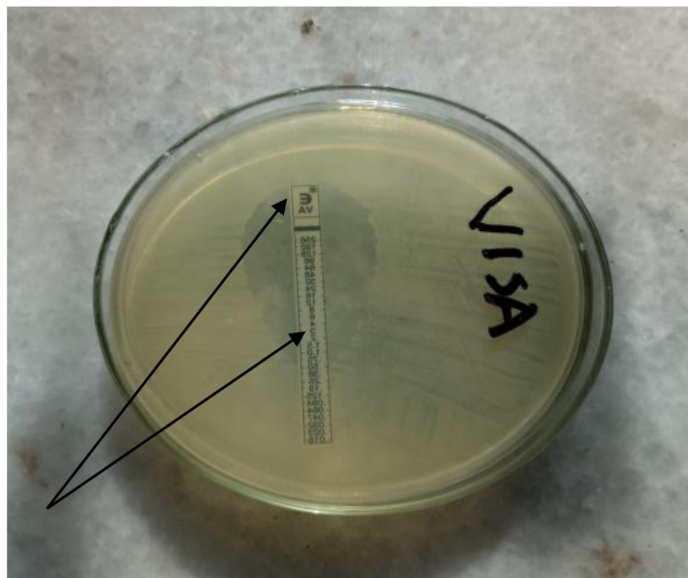


Figure 3: vancomicin intermidate *Staphylococcus aureus* using MIC test

5.4.5 Carbapenem resistant *Enterobacteriaceae* (CRE)

Out of 59 *enterobacteriaceae* isolates 18 (30.5%) of them were resistant to Carbapenem (Meropenem) by producing KPC and 41 (69.5%) were sensitive. The predominate carbapenem resistance *enterobacteriaceae* species in our study were *Klebsiella pneumonia* 27.1% *(n=16/59)* followed by *Klebsiella oxytoca* 3.4 %*(n=2/59)*. Moreover, other gram negative non-enterobacteriaceae isolates capable of developing carbapenem resistance were identified in *Acinitobactor species* 12.2% (n=9/74) and *Pseudomonas areuginosa* 1.3 %(n=1/74) of the total gram negative isolates. A Positive Modified Hodge test showed a clover leaf-like indentation of the *Escherichia coli* 25922 strain growing along the test organism growth streak within the disk

diffusion zone indicating production of carbapenemase as shown in figure 4

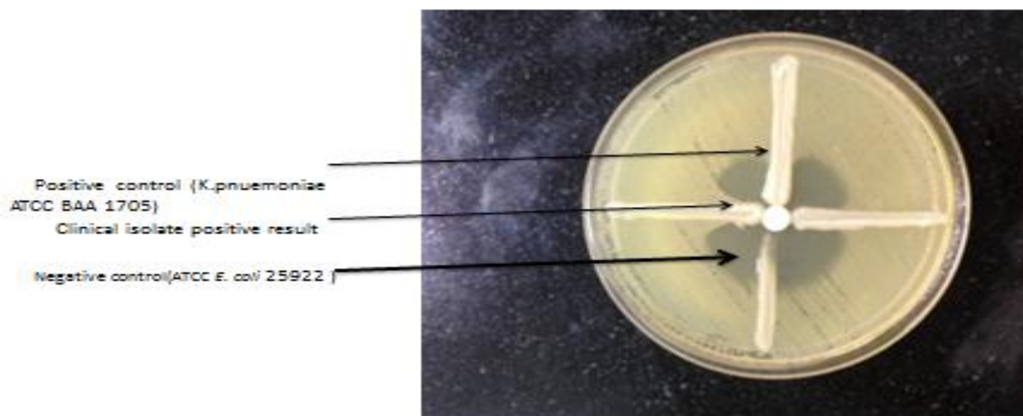


Figure 4: Modified Hodge test for detection of carbapenemase producing enterobacteriaceae

5.4.6 Extended spectrum beta-lactamase producing enterobacteriaceae

Screening a total of 74 gram negative bacteria 59(79.7%) enterobacteriaceae isolates were suspected of ESBL producing organisms. *Klebsiella pneumoniae* 27.1% (n=16/59) *and Escherichia coli* 1.7% (n=1/59) were among gram negative enterobacteriaceae isolates showing ESBL producers.

5.4.6.1 Combined disk (double disk potentiate) Test (CDT)

The overall prevalence of ESBL producing enterobacteriaceae was 28.8% (n=17/59). Among the suspected 17 isolates 100% (n=17/17) were phenotypically confirmed for ESBL using combination disk method, *K. pneumoniae* 100% (n=16/16) and *E. coli* 100% (n= 1/1) were positive for ESBL (figure). For result interpretation we use this result as the CLSI recommend this technique as reference for other phenotypic methods. We also use this test result to compare the findings of double disk method.

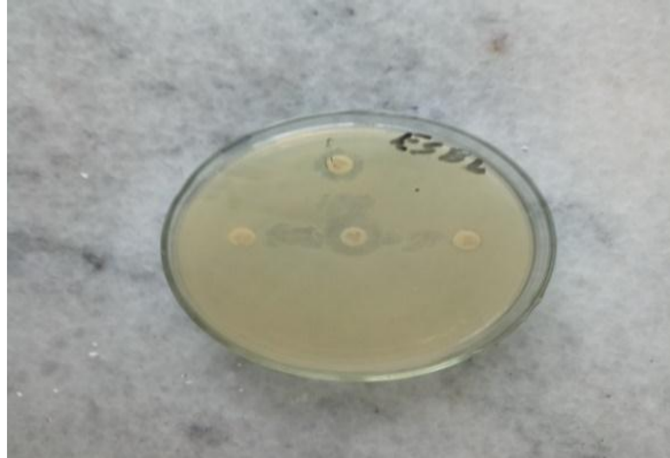
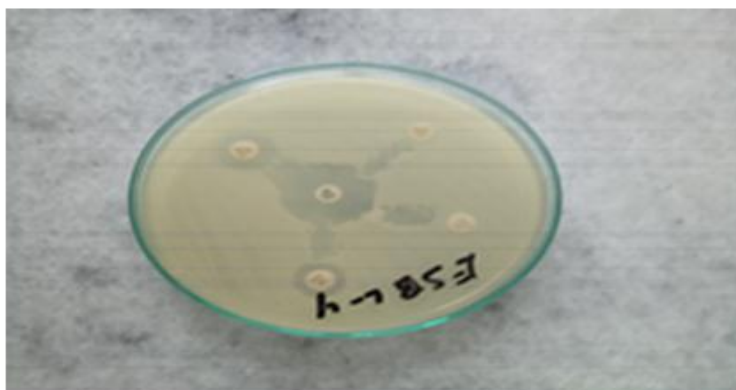


Figure 5: Identification of extended spectrum β-lactamase producing gram negative bacteria using Combined disk (double disk potentiate) Test method

5.5.6.2 Double Disk Synergy Test (DDST)

All isolates (n=17) were further tested for ESBL production by double disk synergy procedure, another phenotypic confirmatory method. The double disk Synergy method indicated 82.3% (n=14/17) were confirmed for ESBL producing enterobacteriaceae. Thus, *K. pneumoniae* showed from the 100% (n=17/17) which were positive by the reference (CDT) method, 82.3% (n=14/17) were positive by this method while 17.6% (n=3/17) were negative. However E. *coli* 100% (n=1/1) was ESBL positive concordant done by two methods (Figure 6).



The inhibition zone between Amoxacilin –clavulanic acid adisk at the center surounded by third generation cephalosporin disk confirmed ESBL

Figure 6: phenotypic confirmatory identification of extended spectrum β-lactamase producing gram negative bacteria using Double Disk Synergy Test (DDST) method.

6 Discussions

Blood stream infection (BSI) in pediatric patients associated with febrile illness is a major public health problem especially in developing countries where high child morbidity and mortality rate. So timely detection of bacteremia in blood culture set is a promising diagnostic tool established to rule out bacteremia and determination of its antimicrobial Susceptibility profile is necessary for clinicians to decide appropriate empirical therapy, which ultimately decreases the emergence of drug resistance [50]. The present study included 340 pediatric patients under five years of age clinically diagnosed with different disease suspected of bacteremia. Even though no statistical significant association for positive blood culture, high proportion of Endocarditis (63%), Hospital acquired infection (60%) and sepsis (48%) were clinically diagnosed in patients with BSI [Table1].

In this study, overall prevalence of bloodstream infection based on significant bacterial growth in the blood cultures obtained from suspected patients was 137 (40.2%) which was in agreement with studies prevalence range of 35%-45% , with the study done in Gondar, northern Ethiopia 39.5% [51] and other similar studies conducted in African countries such as in Egypt 40.7% [52] and Tanzania 38.9% [53] and also in India by Zakariya *e*t al.,41.6% [91] and Khanal et al., [54] has reported 44% of positive blood cultures. Meanwhile, the present study was higher than the studies conducted in Addis Ababa, Ethiopia 13.0% [55], 27.9% [46], and other African counties such as Tanzania 7.7% [56] and Ghana 19.9% [57]. The difference between studies might be due to differences patient condition in which our study includes more patients from ICU and impatient than outpatients in addition blood culture was performed by using more sensitive automated BACTEC/ALERT system. However we have isolated bacteria lower than the studies in Nigeria 47.6% [58], this could be the patient condition in which others only include impatient and isolate anaerobic bacteria.

Among the total isolates of bacteria, 46% gram positive and 54% gram negative bacteria were causing blood stream infection in children which is in agreement with the previous studies done in Addis Ababa, Ethiopia 46.4%, 51.8% [49] elsewhere in India 46.4%, 51.8% [59] Kabul, Afghanistan 44.8%, 51.7%[60], Nepal 44.8%, 55.2% and 44%, 56%[61,86] were gram positive and gram-negative respectively but inconsistent compared to the study done in USA by Larru., *e*t al., 72%, 22% [62] and in South Africa by *Crichton e*t al., 59.3%, 40.7% [63] Gram-positive

bacteria and Gram-negative respectively were documented. This was due to difference in socioeconomic, geographical and infection control mechanisms.

The predominant Gram-positive organisms causing BSI in our study were *staphylococcus aureus* 29/137(21.2%) followed by *coagulase negative staphylococcus (CoNS)* (10.9%) which was comparable to other previous studies in Gondar, Ethiopia in which the rate of isolation was highest in *Staphylococcus aureus* 42%, *coagulates negative staphylococci* 26% [64]. In addition other publication by Anjum *e*t al., [65] has isolated Staphylococcus aureus 19.7% followed by *coagulase negative Staphylococcus aureus* (CONS) 7.23% and in India *27.37*% *Staphylococcus aureus* and 20.1% *coagulase negative Staphylococcus* (CONS) were reported [66]. Our result was lower compared to the study done in Ghana [67] Staphylococcus aureus 66.7%, coagulase negative Staphylococci (CoNS) 17.6%.

In this study, the most common causes of bloodstream infections were gram-negative bacteria, in particular *Klebsiella pneumoniae* (31.4 %) followed by *Acinitobacter species* (8.7%), this was supported by the study done in Jimma Ethiopia 31.4% [68],in African countries in Kenya 13%[69], Ghana 26% [70] , Bouaké, central Côte d'Ivoire 22.5%[71] in Asian such as in India by 25.8% ,30.5%[72,73] in Brazil, Latin America by Berezina *et al* [74], veitnam 20% [75] the most common isolate was *Klebsiella pneumoniae.* However it was inconsistent that the predominant GNB isolation rate varies from country to country where in India by *Kante et al.,* [76], Indonesia by Murni *e*t al., [77] frequently isolated pathogen in BSI was Pseudomonas other than *Klebsiella pneumoniae in* the same age group. The possible difference might be due the difference prescription of antibiotics for empirical treatment of patients before blood culture and difference of management in pathogens causing nosocomial infection across the counties in addition in our Hospital setting, nosocomial infections were not proper patient isolation system in the ward which further increase the survival of high drug resistant bacteria including *Klebsiella pneumoniae.*

A polymicrobial infection in our study was isolated in a single patient and etiologies both were from gram negative bacteria that tends to increase the severity of the diseases which is in agreement with previous studies [78,79] even though some microbiologists consider polymicrobial growth as a contamination, but sepsis should be clinically correlated [79].

The trend of empirical treatment in our study (43.5%) and the most prescribed antibiotics were ampicillin, gentamicin, ciprofloxacillin and third –generation cephalosporin (most common ceftriaxone) in which ampicillin and gentamicin were the most common combined drugs used. This was supported by the previous study in Tamale, Ghana [80].

The rate of antimicrobial resistance in gram positive and gram negative isolates were ranging from 0%-55.2% and 0%-100% respectively. The susceptibility pattern of ampicillin in our gram positive isolates was effective against *S. pneumoniae 100%, viridian streptococcus* 100% and enterococcus spp 81.8% in addition other most affordable drug in our country cotrimosazole showed high sensitive against *S.auresus* 62.1%, *Coagulase negative staph. /CoNS* 86.7% *and S. pneumonia* 66.7%. However the predominant gram positive bacteria *Staphylococcus auresus* showed 55.2% resistance to oxacilin and penicillin. High prevalence of methicillin (oxacillin) resistance *Staphylococcus aureus* (MRSA) which is a surrogate of Cefoxitin test in Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia was 55.2% which was in agreement with the previous study in Addis Ababa 38.5% [49] and in Jimma, Ethiopia by Balta and Deribie 51.8% [81]. Vancomycin has been the treatment of choice for presumed resistant Gram-positive bacterial infections including MRSA in children even though Linezolid represents an important option to vancomycin for the treatment of serious infections caused by antibiotic-resistant Gram-positive cocci [82, 83]. In our study, among 16 MRSA isolates done by MIC test for Vancomycin susceptibility showed that one (3.4%) of S. *aureus* isolate recovered from the blood of a patient had an MIC of 4 µg/ml was confirmed by the CLIS as VISA. For the remaining 15 isolates, a shift in vancomycin MICs from 0.2 to 1.5 µg/ml were sensitive. However no VRSA isolates were detected. The result was concordance with the study by Wang *e*t al., in Los Angeles, California[84] but inconsistent to the report from Centers for Disease Control and Prevention done by Miller *e*t al.,[85] in which two isolates were VRSA.The reason behind might be due to variable nature of antibiotic susceptibility pattern both in time and location of different country.

The antimicrobial susceptibility of gram negative bacteria predominately *Klebsiella pneumoniae* isolates were high level of resistance to ampicillin(100%), cotrimosazole (90.7%) and gentamycin (88.4%),despite of sensitive to meropenem (62.8%), Piperacillin-Tazobactam (58.1%) was consistent with the studies by Zenebe *e*t al [86] reported 100%resistance to

ampicillin and Cotrimosazole, in Bahir dar ,Ethiopia by Hailu *et al*.,[87] ampicillin 91.4%,cotrimosazole 77.1% and gentamicin 71% while in India the resistance of ampicillin, cotrimosazole and gentamycin done by Kumar *e*t al.,[72] were 97%,88%,67% respectively. it was also comparable in Kaneti children Hospital, Nepal by kari *e*t al [88] reported 100% resistance to ampicillin and least sensitive to Cotrimosazole and Gentamycin. The highest potent drugs 3rd and 4th generation cephalosporin, quinoles and carbapenem antibiotics also showed resistance which is a concern for treatment of BSI in pediatrics with septicemia.

The second most predominant GNB isolates in our study were *Acinitobacter species* resistance to most tested antimicrobials ceftazidime100%, cefepime 90.9% gentamycin 81.8%, torbomycin 81.8% ciprofloxacillin 72.7%, meropenem 72.7% was comparable with other previous studies where high resistance of *Acinitobacter species* was published [89,90]. However our result was high rate of resistance compared to the study conducted in South India by Zakariya *e*t al., [91] in which meropenem 100% sensitive, while 67% were sensitive to gentamicin, ceftriaxone, ciprofloxacin, ceftazidime and Amikacin reported. This is the fact that we had relatively many isolates and might be due to inappropriate empirical use of meropenem as the first line treatment since most of isolates are from ICU patients in our Hospital.

The overall prevalence of multidrug resistance isolates MDR in our study was 51.1% of which most of them were Gram-negative bacteria with a very high resistance to beta-lactam antibiotics. This result is supported by the previous study in Ethiopia [49]. Among Gram negative bacterial isolates, *Klebsiella 95.9%* and *Acinetobacter72.2%* were dominant species. This was consistent with the study in north India [92].

The present study identified carbapenem resistance enterobacteriaceae (CRE) with the rate of 30.5% comparable with study conducted in Tanzania 35% [93]. The most carbapenem resistance was detected in 72.2% isolates of *Acinetobacter spp.* and in 62.8% of *Klebsiella pneumoniae.* This was inconsistent with the study in north India 64%, 92% [92] respectively.

The prevalence of ESBL-producing Enterobacteriaceae in our study is 25.4%. Among 43 *Klebsiella pneumoniae* isolates 14(32.5%) *and* 5 *E.coli* isolates 1(20.0%) ESBL-producers which is in line with the study conducted in south India by Zakariya *e*t al., 32.0% [91] and in Mali by Sangare *e*t al.,, 29.4% [94] ESBL producing *Klebsiella pneumoniae.*

7 Strength and limitation of the study

7.1 Strength of the study

- Identify multi drug resistance bacteria isolates in BSI patients under five years at different patient ward
- Show the prevalence of MRSA and VISA in children which is a critical concern in our TSAH needs indicate follows up.
- Point out last line drugs resistance antimicrobials including carbapenems that indicates resistance of β-lactam antibiotics by producing carbapanamase enzyme mostly observed in *Klebsiella pneumoniae* carbapanamase producing (KPC).
- The study also detected ESBL-producing entrobacteriacae among the gram negative isolate

7.2 Limitation of the study

Even though our study identifies numerous bacteria pathogens causing BSI in pediatrics under five years, we could not able to isolate other possible pathogens including anaerobic bacteria.

8 Conclusion and Recommendation

8.1 Conclusion

Blood stream infection in pediatric patients dominantly caused by Gram negative organisms even though high frequency of staphylococcus species and MRSA were a treat of children. An intermediate vancomycin susceptibility of an isolate among MRSA calls an attention on treatment options. Among the dominate gram negative isolates *Klebsiella pneumoniae and Acinitobactor species* were multidrug resistant including 3rd and 4th generation cephalosporin, quinoles, aminoglycosides, carbapenem. The prevalence of MDR 51.1%, CRE 30.5% and ESBL 25.4% were alarmingly high in bacterial isolates in this study.

The duration of Hospitalization, history of Hospital acquired infection and complication of clinical suspected septicemia with high grade fever were significantly associated with positive blood culture in pediatric patients.

8.2 Recommendation

Based on the findings of this study, we recommend that blood culture should be done for pediatric patients prior to antimicrobial therapy with most sensitive BacT/Alert machine. Clinicians should avoid prescribing last line antibiotics for pediatrics in ICU.

Since Majority of antibiotics even last line antibiotics alarmingly resistance, laboratory based treatment should be routinely done. Isolation of patients confirmed nosocomial infection is mandatory to minimize transmission of resistance gene to others including MRSA, CRE, MDR and ESBL producing organisms in Hospital admitted patients.

Strengthening of antimicrobial surveillance system and antimicrobial stewardship are necessary for better management of antibiotics in addition to infection prevention practice in the Hospital settings.

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10 ANNEXES

Annex I: Information Sheet

Study title Bacterial Isolates and Antimicrobial Resistance Pattern among Septicemia Suspected under Five Children in Tikur Anbesa Specialized Hospital, Addis Ababa Ethiopia

Principal investigator: Mequanint Mitiku

Dear participant

I am learning in Addis Ababa University College of health science Department of Medical Laboratory Sciences, School of Allied Health Science. I would like to interview you few questions about blood stream infection for the cause of febrile illness.

Advisor: Kassu Desta

Ethical approval and confidentiality: The ethical clearance was obtained from the research ethical committee of Department of Medical Laboratory sciences. I assure you all the information regarding to the result being confidential. Your participation is voluntary and you are not obligate to answer any questions and give any specimens that you are not volunteer to answer. I would like to appreciate if you give me a few minutes to answer my questions but if you are not comfortable feel free to stop it any time.

Are you volunteer to continue? If yes, continue to next page for interview. If no, continue next participant

Introduction: Bloodstream infections due to bacterial pathogens are a major cause of morbidity and mortality among pediatric patients. Most patients are treated empirically based on their clinical symptoms. Microorganisms present in circulating blood whether continuously, intermittently, or transiently are a threat to every organ in the body.The condition can be life threating in critically ill patients in intensive care unit (ICUs) of the Hospital. Emergence of resistance among the bacterial pathogens causing these infections is another issue of the public health concern. Therefore, up to date etiological data for major pathogens causing bloodstream infections may play a positive role in better healthcare management

Purpose of the study: this study is isolate bacteria and their antimicrobial resistance pattern in under five patients with septicemia. You are kindly requested to be included in the study which

will have importance for management of your child treatment and your cooperation and willingness for about 30 minute of interview and providing specimen will be very helpful in identifying the problems related to the issue.

The specimen that you will provide about 2.5-5ml blood specimen within short time. Giving to these specimens does not affect your health.

Role of principal investigator. Culture the specimen and isolate the bacteria .in addition, antimicrobial susceptibility testing will be carried out in microbiology laboratory

Thank you for your cooperation!!!

Annex II: Consent form

I have read the information sheet concerning this study (or have understood the verbal explanation) and I understand what will be required of me and what will happen to me if I take part in it. I also understand that any time I may withdraw from this study without giving a reason whenever discomfort me about my child.

May I continue the interview?

1. Yes ____________Continue the interview

2. No ______________Stop the interview and thank the respondent

Witness's signature certifying that the informed consent has been given

Witness's signature ______________ Date ______________

Introduction to the Interview

Thank you for deciding to participate in the interview and for coming to this session. Previously (on the statement of consent form), we have discussed briefly on the purpose of the research, how you were identified, and your part in the research study. Now I am going to have discussion with you on the relevant topic items. Before going to the discussion, would you tell me important backgrounds such as age, educational background etc.? There is no right or wrong answers. All answers /responses/ ideas you provide are equally important and you are requested to respond honestly from your experiences and beliefs. I may interrupt and probe your ideas. Once again I would like to tell you that what we are going to discuss is very confidential and it will be used only for the research.

Thank you

Annex III: Questionnaire

Demographic information

1. Pediatric age________ years

2. Medical registration number (MNR)_______

3. Sex: ☐ male ☐ female

Clinical information

1. Clinical condition of the patient
2. Current unit of diagnosis; ☐ ICU ☐ impatient ☐ pediatric OPD

3. If from inpatient for how long did the patient admitted? ☐ 1- 2 days ☐ 3-4days ☐ 5-6 days ☐ ≥7 days

4. Have you a history of incubation after delivery in incubator? ☐ yes ☐ no

5. The child body temperature? ☐ $37^{o}c$ ☐ $<36.5^{o}c$ ☐ $>37.5^{o}c$

6. What was the cause of high fever? ☐ suspected bacteremia ☐ malaria ☐ others specify
____

7. What was the Symptom of BSI? ☐ Fever ☐ chills ☐ shivering ☐ vomiting ☐ fast heart beat

8. Have taken any antibiotic treatment for BSI before 48hours case? ☐ yes ☐ no
9. Please state antibiotic If yes specify _________________________
10. Does the patient had history of the following conditions?
    a) Renal and/or liver disease ☐ Yes ☐ no
    b) Intravascular catheters ☐ yes ☐ no
    c) Hemodialysis ☐ yes ☐ no
    d) Intravenous drug abusers ☐ yes ☐ no
    e) Hospital acquired infection ☐ yes ☐ no
11. Does the BSI got complication and develop to sepsis/septic shock/ septicemia?
    ☐ Yes ☐ no

Thank you!

12. Laboratory data

Fill the following laboratory data according to the SOP and international guide line of reporting of results

Biochemical tests

lactose	Indole	urea	manitol	H_2S	TSI	gas/ glu	citrate	motility	lysine	LDC	malonate

Antimicrobial susceptibility testing

isolated microorganism	Antimicrobial susceptibility result																		
	Gentamicin	Ciprofloxacin	Ceftriaxone	Clindamycin	Amikacin	Cefoxitin	Ampicillin	Nalidixic Acid	Meropenem	Cefepime	Piperacillin	trimethoprimsulphame thoxazole	Oxacillin	Amoxicillin+ clavuninic acid	Penicillin	Erythromycin	Vancomycin	Tobromycin

R-resistance, S-Sensitive, I-Intermediate

ቅጽV:ስለ ጥናቱ ማስተዋወቂያ ና በጥናቱ ለመሳተፍ ፈቃደኝነት መጠየቂያ የአማርኛ ቅጽ ከአምስት አመት በታች ለሆኑ ህፃናት በደም ውስጥ ህመም የሚያስከትሉ ባክቴሪያወችን መለየት እና የመድህኒት መለማመድ ላይ የሚደረግ ጥናት

1. ስለ ጥናቱ ማስተዋወቂያ ቅፅ

ጤና ይስጥልኝ? እኔ ስሜ_______________________ይባላል፡፡የምማረው በአዲስ አበባ ዩኒቨርቲ ህክምና ትምህርት ቤት ሲሆን ጥናቴን የምሰራው ከአምስት አመት በታች ለሆኑ ህፃናት በደም ውስጥ ህመም በሚያስከትሉ ባክቴሪያወች መለየት እና የመድህኒት መለማመድ ላይ ሲሆን፤የጥናቱ አላማ በህፃናት ላይ ከአምስት አመት በታች ለሆኑ ህፃናት በደም ውስጥ ህመም የሚያስከትሉ ባክቴሪያወችን መለየት እና የመድህኒት መለማመድ፡፡ጥናቱ የሚካሄደው በጥቁር አንበሳ ሪፈራል ሆስፒታል በሚታከሙ ህፃናት ይሆናል፡፡እርሰዎንም ከዚህጋርተያያዥነት ያላቸውን ጥያቂዎች እንጠይቀዎታለን ፡፡ ጥናቱ ለእርሰዎ የህፃናት ትኩሳት መንስኤ፣ህክምና እና መከላከያው በሚታዘዙ ፀረተህዋሥያን የፈሽነት ደረጃ ግንዛቤ ለማገኘት ጥንቃቄ ለማድረግ ይረዳል፡፡የደም ናሙና በላብራቶሪ ሲመረመር ምንም አይነት ችግር የማያስከትል ሲሆን የመድሃኒት ትእዛዝና የባለሙያ ምክር ይሰጥዎታል፡፡እርሰዎንም በዚህ ጥናት እንዲሳተፉ በትህትና እንጠይቀዎታለን ፡፡በዚህ ጥናት በመሳተፈዎ የምናገኘው መረጃ ለጥናታችን ውጤታማነት እነዲሁም በጥናቱ ውጤት ላይ ከፍተኛ አስተዋፅዎ ይኖረዋል ፡፡ስለዚህም በዚህ ቃለ-መጠይቅ በመሳተፈዎ ምስጋናዬ የላቀ ነው፡፡በጥናቱ በመሳተፈዎ ምክንያት የሚመጣበዎት ምንም አይነት ችግር አይኖርም ፡፡ ነገር ግን 2-3 የሻይ ማንኪያ የደም ናሙና ለጥናቱ እንዲሰጡ እጠይቅዎታለሁ፡፡ በጥናቱ ውስጥ የሚሠጡት መረጃ ሙሉ በሙሉ ሚስጢራዊነቱ የተጠበቀ ነው፡፡ ስለዚህ በጥናቱ ለመሳተፍ የእርሠዎ ሙሉ ፈቃድ አስፈላጊ ነው፡፡በተጨማሪም ለመመለስ የማይፈልጓቸው ጥያቂዎች ካሉ ጥያቄዎችን ለመመለስ አይገደዱም፡፡እንዲሁም በጥናቱ ላለመሳተፍ ከፈለጉ በማንኛውም ጊዜ ማቋረጥ ይችላሉ ፡፡በጥናቱ ባለመሳተፈዎ በርሰዎ ላይ የሚያስከትለው ወይም የሚያመጣው ምንም አይነት ጉዳት የለውም ፡፡ቃለ መጠየቁን ለማካሄድ 30 ደቂቃ ይወስዳል፡፡ ቃለ መጠየቁን በተመለከተ ወይም አጠቃላይ ስለጥናቱ ማንኛውንም አይነት ጥያቄ ና አስተያየት ቢኖረዎት በሚከተሉ ትአድራሻዎች መጠቀም ይችላሉ፡፡

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ከመጠየቁ በፊት የተጠያቂውን ስምምነት ማረጋገጫ ቅፅ VI

ከላይ በመግቢያው ላይ የተጠቀሰውን መረጃ አንብቢያለሁ ወይም በቃል የተሰጠኝን ማብራሪያ ተረድቻለሁ፡፡ በዚህ መሰረት ከእኔ የሚጠበቅብኝን ድርሻ በሚገባ አውቄያለሁ እና በዚህ ጥናት ላይ በመሳተፌ ሊከሰቱ የሚችሉትን ሁኔታዎች ተገንዝቢያለሁ፡፡ጥናት በማንኛውም ሠዓት ያለምንም ቅድመ ሁኔታ ና ምክንያት እራሴን ከተሳታፊነት የማግለል ሙሉ መብት እንዳለኝ ተረድቻለሁ፡ይህን ውሳኔዬን ተከትሎ በእኔም ሆነ በቤተሰቦቼ ላይ በምንፈልገው የጤና አገልግሎት ላይ ምንም አይነት አዊ ተጽዕኖ እንደማይደርስብኝ ተረድቻለሁ፡፡

ፊርማ --------------- ቀን ----------------

ጥናቱን በተመለከተ የቃል ማብራሪያ የተሰጠ መሆኑን የሚያረጋግጥው የቃለ መጠይቁ አድራጊ ስምና ፊርማ

የጠያቂው ስም---

ፊርማ---------------

ቀን-----------------------

መጠይቁን እንድቀጥል ፈቃደኛነዎት;

1.አዎፈ ቃደኛ ናቸው ---------------------- ቃለ መጠይቁ ይቀጥላል

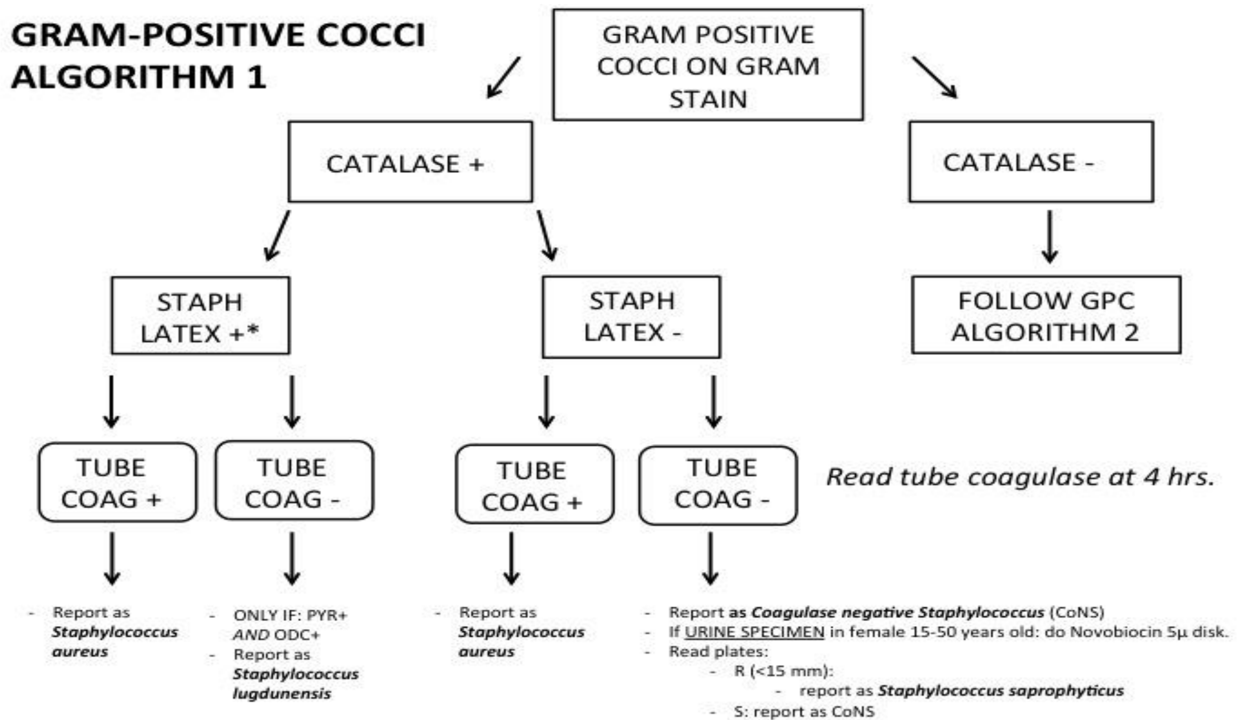
2. አይ ፈቃደኛ አደለሁም------------------ ቃለ መጠይቁን በማቆም አመስግነው ይለያዩ

የመጠየቁ ውጤት መግለጫ

ሀ.ሙሉ በሙሉ የተሞላ ለ. በከፊል የተሞላ ሐ.ተጠያቂው ፈቃደኛ አይደለም መ. ሌላ ካለ------

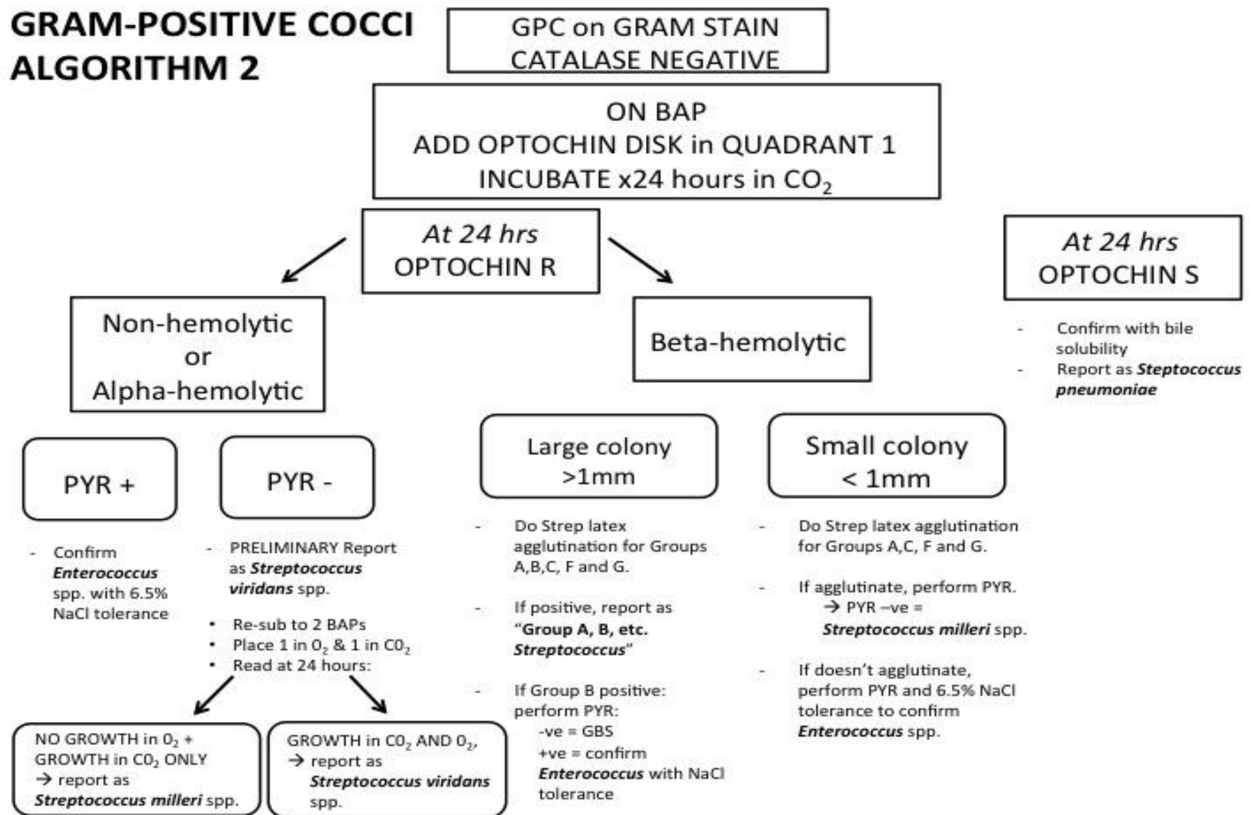
መጠየቁን የሞላው ሰው ስም---------------ፊርማ-------------------ቀን--------------------

Annex IV: Gram Positive Cocci 1



Staph Latex + isolates are for the most part S. aureus; isolates that appear creamy and hemolytic on BAP and which are Staph latex + can be called "presumptive S. aureus" and AST should be performed. If Latex test positive and Tube coagulase negative → perform API Staph to identify species.

Annex V: Gram Positive Cocci 2



Annex VI: Flow Chart For Gram Negative Rodes

I	Lactose	Indol	UrEa	Mannitol	H_2S	Gas glu	Citrate	Motility	Lysin	Organism	Additional
LACTOSE			+	+	+	+	+	+	-	Citrobactor	Urea +
OR		-		+			+	+	-	Entrobactor cloacae	
ONPG					-	+		-	+	Klebsiella pneumoniae.	Malonate +
(ortonitrophnile galactosidase)							-/+	-	-/+	Klebsiella ozenae	
POSITIVE							+/-	+	-	Ent. agglomerans (Erwinia)	
NB:-	+					-	-	-	-	Klebs.rihinoscleromitus1 Shigella sonnei (2)	
1. Ornithine (-)					-		+	+	+	Serratia (3)	
2. Ornithine (+)			-	+		+	+	+	+	Ent.arogens (4) or Hafnia (5)	
3.Gas variable week.									–	Ent.Cloacae (6) or Citrobactor (7)	
4.Additional inositol (+)					+	+	+	+	+	Citrobactor	
5.Additional inositol (-)									+	Arizona	Malonate +
			+	+	-	+	+	-	+/-	Klebsella oxytoca	
6.MR$^-$, VP$^+$		+					+	+	+	Citrobactor diversus	
7.MR$^+$, VP$^-$				+	-	+	-	+	+	E. Coli	
			-					-	+	E. Coli	
						-	-	-	-	E. Coli (A—D)	
				-	-	-	-	-	-	Sh. Dysente or E. Coli A-D	
II	Lact	Ind	Ur	Ma	H_2S	Gas	Cit rat	Mot	Lys		

	ose	ol	E a	ito l		glu	e	ilit y	in	Organism	Additional
LACTOSE				+/-	-	+	+	+	-	Providencia rettgeri	PAD (+)
AND			+	_	-	+	-	+	-	Morganella morganii	PAD (+) LDC(-)
ONPG					+	+	-/+	+	-	Proteus vulgaris	PAD (+)
NEGATIVE		+				+	+	+	-	Providenica alkalifaciens 4	PAD (+)
				-	-	-	+	+	-	Providinica Stuartii	PAD (+)
NB:-							-	-	-	Shegella dysentriae	
+ 90% or more positive	-		-		+	+	-	+	+	Edwardsiella	
-90%or more negative							-	-	+	E. coli (A—D)	
+/- majority negative				+	-	-			-	Shigella spp.	
-/+majority negative							+	+	-	Providenicia stuartii (B)	PAD (+)
				-	-	-	-	-	-	Shigella dysentriae	
		-	-		-	-	-	-	-	Shigella spp.	
				+		+	-	+	_	Salmonella Group A	
					+	+	+	+	+	Salmonella or Arizona	
						-	-	+	+	Salmonella thyphi	VC (+)
			+	-	+	+	+/-	+	-	Proteus mirabilis	PAD (+)
	d	-	D	+	-	+d	+	+	+	Seratia marcescers	Ox +/-
III	Lac t ose	In d ol	U r E a	M a ito l	H_2 S	Ga s glu	Cit rat e	Mo t ilit y	Lys in	Organism	Additional
NON FERMENTATIVE	-	-	D	+/-	-	-	+	+/-	+/-	Pseudomonas Arogenesa	Cat + Oxi +
	+/-	-	-s	-	-	-	+/-	-	-	Acitinobactor	Cat + Oxi -
	-	-	-	-	-	-	d	+	-	Alcaligens spp.	Cat + Oxi -

Annex VII: AST

CLSI 2017 breakpoints

Zone Diameter Interpretive Standards for *Enterobacteriaceae*, in mm

Testing conditions Media: Mueller-Hinton agar. Use maximum 12 disks on a 150 mm plate; Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center. Number of disks to test = 12 Inoculum: direct colony suspension equivalent to 0.5 McFarland standards Incubation: 35+/- 2 °c ,ambient air 16-18 hr						
When test? #	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Ampicilin	10 µg	≤ 13	14-16	≥ 17	Report amoxicillin with S/I/R result from ampicillin
A	Gentamicin	10 µg	≤ 12	13-14	≥ 15	Do not report for Salmonella and Shigella spp.
A	Tobramycin	10 µg	≤ 12	13-14	≥ 15	Do not report for Salmonella and Shigella spp.
A	Amikacin	30 µg	≤ 14	15-16	≥ 17	Do not report for Salmonella and Shigella spp.
A	Cefotaxime	30 µg	≤ 22	23-25	≥ 26	
A	Ceftriaxone	30 µg	≤ 19	20-22	≥ 23	
A	Ceftazidime	30 µg	≤ 17	18-20	≥ 21	
A	Trimethoprim+Sulfamethoxazole	1.25/23.75µg	≤ 10	11-15	≥ 16	
A	Ciprofloxacin (breakpoint for Salmonella only)	5 µg	≤ 20	21-30	≥ 31	
A	Ciprofloxacin (breakpoint for non-Salmonella)	5 µg	≤ 15	16-20	≥ 21	
A(Salm)	Nalidixic acid	30 µg	≤ 13	14-18	≥ 19	Only test for Salmonella or if requested (drug N/A)
AU	Nitrofurantoin (PO only)	300 µg	≤ 14	15-16	≥ 17	
1	Amoxicillin+clavulanic	20/10 µg	≤ 13	14-17	≥ 18	

	acid (PO only)					
7	Cefuroxime	30 µg	≤ 14	15-17	≥ 18	Do not report for Salmonella and Shigella spp.
4	Cefepime	30 µg	≤ 18	19-24	≥ 25	
5	Cefixime (PO only, only for uncomplicated UTI)	5 µg	≤ 15	16-18	≥ 19	Do not test or report Morganella spp. with cefixime
6	Imipenem or meropenem	10 µg	≤ 19	20-22	≥ 23	If R: Notify leader of Hospital Infection Control Team
2	Norfloxacin (PO only)	10 µg	≤ 12	13-16	≥ 17	Only test if ciprofloxacin is not available
9	Trimethoprim (PO only)	5 µg	≤ 10	11-15	≥ 16	Only test and report for urine isolate (drug N/A))
8	Aztreonam	30 µg	≤ 17	18-20	≥ 21	
3	Chloramphenicol	30 µg	≤ 12	13-17	≥ 18	DO NOT TEST IN URINE. ALWAYS TEST IN CSF.

Zone Diameter Interpretive Standards for *Pseudomonas aeruginosa*, in mm

Testing conditions
Media: Mueller-Hinton agar.
Use maximum 12 disks on a 150 mm plate;
Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center.
Number of disks to test: 6
Inoculum: direct colony suspension equivalent to 0.5 McFarland standards
Incubation: 35+/- 2 ^{o}c ,ambient air 16-18 hr

When test? #	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Ceftazidime	30 µg	≤ 14	15-17	≥ 18	
A	Gentamicin	10 µg	≤ 12	13-14	≥ 15	
A	Tobramycin	10 µg	≤ 12	13-14	≥ 15	
A	Amikacin	30 µg	≤ 14	15-16	≥ 17	
A	Imipenem or Meropenem	10 µg	≤ 15	16-18	≥ 19	If R: Notify leader of Hospital Infection Control Team
A	Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21	
1	Norfloxacin (PO only)	10 µg	≤ 12	13-16	≥ 17	Only test if ciprofloxacin is not available

2	Cefepime	30 µg	≤ 14	15-17	≥ 18	
	Ceftriaxone	DO NOT TEST	R			Report as R if requested (EUCAST)
	Cefotaxime	DO NOT TEST	R			Report as R if requested (EUCAST)
	Tetracycline	DO NOT TEST	R			Report as R if requested (EUCAST)
	Doxycycline	DO NOT TEST	R			Report as R if requested (EUCAST)

Zone Diameter Interpretive Standards for *Acinetobacter spp*, in mm

Testing conditions
Media: Mueller-Hinton agar.
Usemaximum 12 disks on a 150-mm plate;
Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center.
Number of disks to test: 6
Inoculum: direct colony suspension equivalent to 0.5 McFarland standards; Incubation: 35+/- 2 ^{o}c ,ambient air 20-24 hr

When test?	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21	
A	Gentamicin	10 µg	≤ 12	13-14	≥ 15	
A	Tobramycin	10 µg	≤ 12	13-14	≥ 15	
A	Amikacin	30 µg	≤ 14	15-16	≥ 17	
A	Trimethoprim-Sulfamethoxazole	1.25/23.75µg	≤ 10	11-15	≥ 16	
1	Imipenem	10 µg	≤ 18	19-21	≥ 22	See comment under #
A	Meropenem	10 µg	≤ 14	15-17	≥ 18	See comment under #
7	Ticarcillin	75 µg	≤ 14	15-19	≥ 20	
2	Ceftazidime	30 µg	≤ 14	15-17	≥ 18	
3	Cefepime	30 µg	≤ 14	15-17	≥ 18	
4	Tetracycline	30 µg	≤ 11	12-14	≥ 15	
5	Doxycycline	30 µg	≤ 9	10-12	≥ 13	
6	Gatifloxacin	5 µg	≤ 14	15-17	≥ 18	
8	Colistin	Gradient MIC	MIC ≥ 4	-	MIC ≤ 2	
9	Tigecycline	Gradient MIC	MIC ≥ 1	MIC = 0.5	MIC ≤ 0.25	Add comment: “AST based on non-clinical data, therefore interpret

						with caution"
	Cefotaxime	DO NOT TEST	R			Report as R if requested (EUCAST)
	Ceftriaxone	DO NOT TEST	R			Report as R if requested (EUCAST)
# IF MEROPENEM OR IMIPENEM RESISTANT DO THE FOLLOWING: 1: Perform AST for ALL remaining disks and gradient MIC strips in the table 2: Confirm ID of isolate (with API 20 NE, if available). 3. Perform Modified Hodge Test ("clover leaf test") for the presence of carbapenemase 4. Notify leader of Hospital Infection Control Team						

Zone Diameter Interpretive Standards for *Staphylococcus spp*, in mm

Testing conditions Media: Mueller-Hinton agar. Use maximum 12 disks on a 150 mm plate; Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center. Number of disks to test: 5 Inoculum: direct colony suspension equivalent to 0.5 McFarland standards Incubation: 33-35°c (testing at above 37°c may not detect MRSA), ambient air. Incubation time: 16-18 hr EXCEPT Coagulase-negative staphylococci and cefoxitin: 24 hours.						
When test? #	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A (NB! only S.aureus)	Penicillin G (only S. aureus)	10 unit	≤28	-	≥ 29 NB→	If ≥ 29 mm examine zone edge: report as R if sharp ("cliff"); report as S if fuzzy ("beach") Always R if cefoxitin R.
A	Cefoxitin (For S.aureus or S.lugdunensis)	30 µg	≤21		≥22	Do not report; report oxacillin and other betalactams based on cefoxitin result (see comments below)
A	Cefoxitin (For CoNS except S.lugdunensis)	30 µg	≤24		≥25	Do not report; report oxacillin and other betalactams based on cefoxitin result (see comments below)
A	Erythromycin (PO only)	15 µg	≤13	14-22	≥23	DO NOT TEST IN URINE OR CSF ISOLATES
A	Clindamycin (PO only)	2 µg	≤14	15-20	≥ 21 NB→	DO NOT TEST IN URINE OR CSF ISOLATES Place erythromycin disc and clindamycin disc 12-20mm apart (edge to edge). Report clindamycin as R if "D-

						phenomenon" is seen (inducible clindamycin resistance) (EUCAST)
A	Trimethoprim-Sulfamethoxazole	1.25/23.75 µg	≤ 10	11-15	≥ 16	
AU	Nitrofurantoin (PO only)	300 µg	≤ 14	15-16	≥ 17	Urine isolates only
	Ampicilin / Amoxicillin	DO NOT TEST				Report as S /R based on Penicillin G result. Always R if cefoxitin R.
	Oxacillin (Cloxacillin available PO and IV)	DO NOT TEST				Report as S /R based on Cefoxitin result
	Amoxicillin+clavulanicacid (PO only)	DO NOT TEST				Report as S /R based on Cefoxitin result
	Cephalotin	DO NOT TEST				Report as S /R based on Cefoxitin result
	Cefuroxime	DO NOT TEST				Report as S /R based on Cefoxitin result
	Cefotaxim	DO NOT TEST				Report as S /R based on Cefoxitin result
	Ceftriaxone	DO NOT TEST				Report as S /R based on Cefoxitin result
	Ceftazidim	DO NOT TEST	R			Report as R if requested
1	Gentamicin	10 µg	≤ 12	13-14	≥ 15	DO NOT TEST IN CSF UNLESS REQUESTED
3	Tobramycin	10 µg	≤ 12	13-14	≥ 15	DO NOT TEST IN CSF UNLESS REQUESTED
2	Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21	
5	Doxycycline (PO only)	30 µg	≤ 12	13-15	≥ 16	
6	Azithromycin	15 µg	≤13	14-17	≥18	DO NOT TEST IN URINE ISOLATES
7	Trimethoprim (PO only)	5 µg	≤ 10	11-15	≥ 16	Urine isolates only
4	Chloramphenicol	30 µg	≤ 12	13-17	≥ 18	DO NOT TEST IN URINE. ALWAYS TEST IN CSF.

Zone Diameter Interpretive Standards for *Enterococcus spp*, in mm

<u>Testing conditions</u>

Media: Mueller-Hinton agar.

Use maximum 12 disks on a 150 mm plate;

Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center.

Number of disks to test: Non-urine isolates : 2 disks + SULFA + TELLUR / Urine isolates: 3 disks + SULFA + TELLUR

Inoculum: direct colony suspension equivalent to 0.5 McFarland standards

Incubation: 35+/- 2 ^{o}c ,ambient air 16-18 hr. <u>24hr for vancomycin testing</u>

When test? #	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Ampicilin	10 µg	≤ 16	-	≥ 17	Report amoxicillin with the S/I/R result from ampicillin
A	Vancomycin	30 µg	≤ 14	15-16	≥ 17 NB→	If ≥ 17 mm examine zone edge: report as R if zone edge is fuzzy or colonies grow within the inhibition zone
A	SULFA DISK					Enterococcus spp are sulfa resistant
A	TELLUR DISK					Enterococcus faecalis: zone <12mm and black colonies close to the disk. Other enterococci/streptococci : zone>15mm
AU	Nitrofurantoin (PO only)	300 µg	≤ 14	15-16	≥ 17	Urine isolates only
1	Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21	Urine isolates only
2	Norfloxacin (PO only)	10 µg	≤ 12	13-16	≥ 17	Urine isolates only
	Penicillin G or ANY CEPHALOSPORINS (Ceftriaxone,cefotaxime, ceftazidime, ++)	DO NOT TEST	R			Report as R if requested (EUCAST)
	Erythromycin	DO NOT TEST	R			Report as R if requested (EUCAST)
	Tetracycline	DO NOT TEST	R			Report as R if requested (EUCAST)
	Doxycycline	DO NOT TEST	R			Report as R if requested (EUCAST)
	Chloramphenicol	DO NOT TEST	R			Report as R if requested (EUCAST)

Zone Diameter Interpretive Standards for *Haemophilus influenzae* and *H. parainfluenzae*, in mm

Testing conditions Media:Haemophilus test medium(HTM) or GC agar base and 1% growth supplement. (Chocolate agar: only if QC strain (Haemophilus influenzae ATCC 49247) is tested in parallel) Number of disks to test: 4 Use maximum 4 disks on a 100 mm plate. Use maximum 9 disks on a 150 mm plate. Inoculum: direct colony suspension equivalent to 0.5 McFarland standards. Incubation: 35+-2°c,5% Co_2,16-18 hr.						
When test? #	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Ampicilin	10 µg	≤ 18	19-21	≥ 22	Report amoxicillin as S/I/R from ampicillin
A	Amoxicillin+clavulanic acid (PO only)	20/10 µg	≤ 19	-	≥ 20	
A	Ceftriaxone	30 µg	-	-	≥ 26	
A	Trimethoprim-Sulfamethoxazole	1.25/23.75 µg	≤ 10	11-15	≥ 16	Do not test in CSF isolates unless requested.
2	Cefotaxime	30 µg	-	-	≥ 26	
6	Cefuroxime	30 µg	≤ 16	17-19	≥ 20	
3	Cefepime	30 µg	-	-	≥ 26	
4	Ciprofloxacin	5 µg	-	-	≥ 21	Do not test in CSF isolates unless requested.
1	Chloramphenicol	30 µg	≤ 25	26-28	≥ 29	Always test in CSF isolates
5	Tetracycline (PO only)	30 µg	≤ 25	26-28	≥ 29	Do not test or report if isolate is from CSF. If S for Tetracycline report S for Doxycycline
	Doxycycline (PO only)	DO NOT TEST				If S for Tetracycline report S for Doxycycline (Do not report if isolate is from CSF.)
	Ceftazidime	DONOT TEST	R			Report as R if requested (EUCAST)

Zone Diameter Interpretive Standards for *Streptococcus pneumoniae*, in mm

<table>
<tr><td colspan="7">Testing conditions
Media: Mueller-Hinton agar with 5%sheep blood. (Blood agar: only if QC strain (Str.pneum ATCC 49619) tested in parallel.)
Use maximum 9 disks on a 150 mm plate; use maximum 4 disks on a 100 mm plate.
Number of disks to test: 4
Inoculum: direct colony suspension equivalent to 0.5 McFarland standards. Incubation: 35+/- 2 °c,5% Co_2,20-24 hr.</td></tr>
<tr><td rowspan="2">When test? #</td><td rowspan="2">Antimicrobial Agent</td><td rowspan="2">Disk content</td><td colspan="3">Zone diameter nearest whole mm</td><td rowspan="2">Comments</td></tr>
<tr><td>R</td><td>I</td><td>S</td></tr>
<tr><td>A</td><td>Penicillin G NB! TEST WITH OXACILLIN DISK</td><td>1 µg Oxacillin
NB! DO NOT REPORT OXACILLIN RESULT !
SEE "COMMENTS" FOR INTERPRETATION!</td><td>-</td><td>-</td><td>≥ 20</td><td>Oxacillin zone ≥ 20mm means the following antibiotics can be reported as S:
Amoxicillin, ampicillin, augmentin, ceftriaxone, cefotaxime, cefuroxime. (EUCAST 2015)
If oxaxillin zone <20 mm and CSFspecimen :report Penicillin as R.
If oxacillin zone <20mm and not CSF specimen :
MIC (ETEST/MTS) must be determined for the relevant betalactam agents: No MIC, no report.</td></tr>
<tr><td>A</td><td>Erythromycin (PO only)</td><td>15 µg</td><td>≤ 15</td><td>16-20</td><td>≥ 21</td><td>DO NOT TEST IN CSF ISOLATES</td></tr>
<tr><td>A</td><td>Clindamycin (PO only)</td><td>2 µg</td><td>≤ 15</td><td>16-18</td><td>≥ 19 NB→</td><td>DO NOT TEST IN CSF ISOLATES. Place erythromycin disc and clindamycin disc 12-16mm apart (edge to edge). Report clindamycin as R if "D-phenomenon" is seen (inducible clindamycin resistance) (EUCAST)</td></tr>
<tr><td>A</td><td>Trimethoprim-Sulfa</td><td>1.25/23.75 µg</td><td>≤ 15</td><td>16-18</td><td>≥ 19</td><td></td></tr>
<tr><td>1</td><td>Tetracycline (PO only)</td><td>30 µg</td><td>≤ 24</td><td>25-27</td><td>≥ 28</td><td>If S for Tetracycline report S for Doxycyclin</td></tr>
<tr><td>3</td><td>Doxycycline (PO only)</td><td>30 µg</td><td>≤ 24</td><td>25-27</td><td>≥ 28</td><td>If S for Tetracycline report S for Doxycyclin</td></tr>
<tr><td>4</td><td>Vancomycin</td><td>30 µg</td><td>-</td><td>-</td><td>≥ 17</td><td>Resistant isolates are rare → retest isolate</td></tr>
<tr><td>2</td><td>Chloramphenicol</td><td>30 µg</td><td>≤ 20</td><td>-</td><td>≥ 21</td><td>ALWAYS TEST IN CSF ISOLATES</td></tr>
<tr><td></td><td>Ceftazidime</td><td>DO NOT TEST</td><td>R</td><td></td><td></td><td>Report as R if requested (EUCAST)</td></tr>
</table>

Zone Diameter Interpretive Standards for *Streptococcus spp, beta-hemolytic group**, in mm

*Includes large-colony group A, C and G strep., and group B strep. (Streptococcus milleri (="Strep anginosus group") should be tested with the "Strep, viridans group" table!)

Testing conditions

Media: Mueller-Hinton agar with 5% sheep blood. (Blood agar: only if QC strain (Streptococcus pneumoniae ATCC 49619) tested in parallel.)

Number of disks to test: 4

Use maximum 4 disks on a 100 mm plate;

Use maximum 9 disks on a 150 mm plate.

Inoculum: direct colony suspension equivalent to 0.5 McFarland standards

Incubation: 35+/- 2 ^{o}c ,5% Co_2,20-24 hr.

When test? #	Antimicrobial Agents	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Penicillin G	10 units	-	-	≥ 24	Resistant isolates are rare → retest isolate
A	Erythromycin (PO only)	15 µg	≤ 15	16-20	≥ 21	DO NOT TEST IN URINE OR CSF ISOLATES
A	Clindamycin (PO only)	2 µg	≤ 15	16-18	≥ 19 NB→	DO NOT TEST IN URINE OR CSF ISOLATES Place erythromycin disc and clindamycin disc 12-16mm apart (edge to edge). Report clindamycin as R if "D-phenomenon" is seen (inducible clindamycin resistance) (EUCAST)
A	Trimethoprim-Sulfa	1.25/23.75 µg	≤ 14	15-17	≥ 18	EUCAST 2015
3	Vancomycin	30 µg	-	-	≥ 17	If S for Tetracycline report S for Doxycycline
2	Chloramphenicol	30 µg	≤ 17	18-20	≥ 21	DO NOT TEST IN URINE. ALWAYS TEST IN CSF
1	Tetracycline (PO only)	30 µg	≤ 18	19-22	≥ 23	
	Doxycycline (PO only)	DO NOT TEST				If S for Tetracycline report S for Doxycycline
	Ceftriaxone/ Cefotaxime	DO NOT TEST				Report as S /R based on Penicillin G result
	Ampicilin / amoxicillin	DO NOT TEST				Report as S /R based on Penicillin G result

Zone Diameter Interpretive Standards for *Streptococcus, viridans group**, in mm

*this group includes (small-colony) betahemolytic Streptococcus milleri (="Strep anginosus group")
Testing conditions
Media: Mueller-Hinton agar with 5% sheep blood. (Blood agar: only if QC strain (Str.pneum ATCC 49619) tested in parallel.)
Number of disks to test: 4
Use maximum 4 disks on a 100 mm plate; use maximum 9 disks on a 150 mm plate.
Inoculum: direct colony suspension equivalent to 0.5 McFarland standards. Incubation: 35+/- 2 ^{o}c ,5% Co_2,20-24hr.

When test? #	Antimicrobial Agents	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Erythromycin	15 µg	≤ 15	16-20	≥ 21	DO NOT TEST IN URINE OR CSF ISOLATES DO NOT REPORT
A	Clindamycin (PO only)	2 µg	≤ 15	16-18	≥ 19 NB→	DO NOT TEST IN URINE OR CSF ISOLATES Place erythromycin disc and clindamycin disc 12-16mm apart (edge to edge). Report clindamycin as R if "D-phenomenon" is seen (inducible clindamycin resistance) (EUCAST)
A	Chloramphenicol	30 µg	≤ 17	18-20	≥ 21	DO NOT TEST IN URINE. ALWAYS TEST IN CSF.
A	Penicillin G	1 unit !	≤ 11	12-17	≥ 18	Perform gradient-MIC (ETEST/MTS) if Penicillin G 1 unit disk is not available.(EUCAST 2015)
	Ampicillin / amoxicillin	DO NOT TEST				Report ampicillin and amoxicillin as S/I/R from penicillin G
2	Cefotaxime DO NOT TEST ROUTINELY	30 µg	≤ 25	26-27	≥ 28	If S for Penicillin G report S for cefotaxime
1	Ceftriaxone DO NOT TEST ROUTINELY	30 µg	≤ 24	25-26	≥ 27	If S for Penicillin G report S for ceftriaxone
3	Cefepime DO NOT TEST ROUTINELY	30 µg	≤ 21	22-23	≥ 24	If S for Penicillin G report S for cefepime
4	Vancomycin	30 µg	-	-	≥ 17	Resistant isolates are rare → retest isolate
	Ceftazidime	DO NOT TEST	R			Report as R if requested (EUCAST)
	Tetracycline	DO NOT TEST	R			Report as R if requested (EUCAST)
	Doxycycline	DO NOT TEST	R			Report as R if requested (EUCAST)

Zone Diameter Interpretive Standards for *Neisseria meningitidis*, in mm

Testing conditions
Media: Mueller-Hinton agar with 5%sheep blood. (Blood agar or Chocolate agar: only if QC strain (Str.pneum ATCC 49619) tested in parallel)
Number of disks to test: 5
NB! Test maximum 2 disks on a 100mm plate and a maximum of 5 disks on a 150mm plate. That means that up to 3 small (100mm) plates may be necessary!
Inoculum: direct colony suspension equivalent to 0.5 McFarland standards
Incubation: 35+/- 2 ^{o}c ,5% Co_2,20-24 hr.

When test? #	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Cefotaxime	30 µg	-	-	≥ 34	
A	Ceftriaxone	30 µg	-	-	≥ 34	
A	Chloramphenicol	30 µg	≤ 19	20-25	≥ 26	
A	Rifampicin	5 µg	≤ 19	20-24	≥ 25	ONLY FOR PROPHYLAXIS; not for treatment
A	Ciprofloxacin	5 µg	≤ 32	33-34	≥ 35	ONLY FOR PROPHYLAXIS; not for treatment
	Penicillin G		-	-	-	Disk diffusion is unreliable for testing N.meningitidis vs penicillin and ampicillin, always perform gradient MIC (ETEST/MTS)
	Ampicilin		-	-	-	
	Ceftazidime	DO NOT TEST				Report as R if requested (EUCAST)
	Trimethoprim-Sulfa	DO NOT TEST				Report as R if requested (EUCAST)
	Azithromycin	DO NOT TEST				Report as R if requested (EUCAST)

Annex VIII: Declaration

The undersigned declares that this research paper complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. candidate: Mequanint Mitiku (B.Sc.)

Signature: __________________

Date of submission: __________________

This thesis research paper has been submitted with our approval as advisor.

Advisor: Kassu Desta (MSc, PhD candidate)

Signature: ___________________

Date: ___________________

Place: Addis Ababa, Ethiopia.