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Bacterial profiles and drug resistance patterns of bacteria recovered from blood samples of Hospital admitted patients at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia.

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Abbreviations

AST	Antimicrobial Susceptibility Test
BC	Blood Culture
BSI	Blood Stream Infections
CLSI	Clinical and Laboratory Standard Institute
CONS	Coagulase Negative Staphylococcus
E.coli	Escherichia Coli
ESBL	Extended Spectrum Beta-Lactamase
MDR	Multidrug Resistance
AMR	Antimicrobial Resistance
MRSA	Methicillin resistance staphylococcus aureus
NICU	Neonate Intensive Care Unit
PCR	Polymerase Chain Reaction
PYR	Pyrrolidonyl Aryl Amidase
SPSS	Statistical Package for Social Science
TASH	Tikur Anbessa Specialized Hospital

// Abstract

Background: One of the leading causes of illness and mortality worldwide, bloodstream infections are frequently acquired in hospitals. As a result of the circulatory system's rapid spread of microorganisms and their toxins, it is a serious, perhaps fatal illness that gets worse with time.

Objective: To determine bacterial isolates and drug resistance patterns of bacteria recovered from blood samples of hospital-admitted patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Methods: A cross-sectional study was conducted between January 2021 and December 2022 among hospitalized patients at Tikur Anbessa Specialized Hospital in Addis Abeba, Ethiopia, who were suspected of having septicemia. To isolate the bacteria, blood samples were taken. Bacterial culture was conducted following standard microbiological procedures. Agar diffusion was used to isolate bacteria, and the Kirby-Bauer disc diffusion technique was used to determine drug susceptibility patterns on Muller Hinton agar. Data input and processing were conducted using version 26 of the Statistical Package for Social Science.

Results: The study included 218 paired samples in total, and 66 (30.3%) of the blood samples contained positive bacterial growth. Gram-positive and Gram-negative bacterial isolates were found in 62.2% and 37.8%, respectively, of positive blood culture findings. *Staphylococcus aureus* (34.8%) and *coagulase-negative Staphylococcus* (15.2%) were the two most commonly encountered gram-positive bacteria. Among gram-negative bacteria, the majority were *Escherichia coli* (15.2%) and *Klebsiella pneumoniae* (10.6%). *E. coli* and *Klebsiella pneumoniae* were resistant to most antibiotics, whereas *Staphylococcus aureus* was identified as being responsive to oxacillin (78.1%) and resistant to penicillin (73.9%).

Conclusion and Recommendation: This study identified a higher number of positive bacterial growths and resistance to penicillin and cephalosporin found for most bacteria. Therefore, there is a need to perform blood cultures and sensitivity tests before empirical treatment and also implementation of infection prevention should be strengthened.

Keywords: bacteremia, septicemia, drug resistance, blood culture, Tikur Anbessa.

1. Introduction

1.1 Background

Infections called bloodstream infections (BSIs) are those brought on by the presence of live microorganisms in the blood [1]. A contributing factor to the high death rate is bacterial infection [2, 3,]. Concerns about public health are raised by the emergence of resistance among the bacterial pathogens that cause diseases [4]. Bacterial bloodstream infections are characterized by the presence of live, immune-stimulating bacteria in the bloodstream. Different pathways exist for bacteria to invade the circulation and other generally sterile bodily areas. Due to the rapid spread of bacteria and their poisons in the blood, it is a dangerous, perhaps fatal illness [5].

Bloodstream infection (BSI) continues to be one of the primary causes of illness and death worldwide [3-7]. Three million babies and 1.2 million children worldwide have sepsis each year due to a bloodstream infection, which kills six million people worldwide [7]. In both the United States and Europe, BSI constitutes one of the top ten causes of death [3, 8]. Septicemia, which has a death rate of 53% in sub-Saharan nations like Ethiopia, is a serious medical problem [3]. 40% of instances of community- and hospital-acquired (CA/HA) sepsis and septic shock, as well as about 20% of ICU-acquired cases, have been triggered by bloodstream infections. If source management and suitable antibiotic treatment are put off, BSI tends to be related to poor clinical results [9].

Medical emergencies like septicemia pose a hazard to all of the body's organs since microorganisms in the blood might circulate continually or occur occasionally. Having bacterial colonization in the blood circulation is often a serious ailment that poses a threat to life [10]. Many kinds of bacteria have been identified from bloodstream infections, and illnesses attributed to these bacteria require immediate antimicrobial treatment with medication [11, 8].

Escherichia coli, a gram-negative bacteria that was once reported to be frequently responsible for bacteremia, is currently being displaced by other multidrug-resistant (MDR) bacteria such as *Acinetobacter, Pseudomonas, Enterobacter, Salmonella, Citrobacter, and Klebsiella* [5,10]. Additionally, gram-positive bacteria linked to bloodstream infection include *Staphylococcus aureus, Enterococci, alpha-hemolytic Streptococci,* and coagulase-negative *Staphylococcus* [5, 12].

A stay in the hospital, health professionals' disregard for infection control procedures, the insertion of foreign objects like catheters into blood vessels, as well as other risk factors like critical care units, have all been implicated as key risk factors [12]. The recording of pathogens in blood-like bacteremia using blood cultures (BCs) is used to make the diagnosis of BSI. Blood cultures (BCs) are still essential for the diagnosis of BSIs and are now regarded as the gold standard for BSI diagnosis. [1, 13].

Drug Resistance bacteria are growing in admitted patients very rapidly because of nosocomial infection so there is a need for updated findings.

1.2 Statement of the Problem

One of the most widespread healthcare-associated conditions is bloodstream infection (BSI), which is also one of the top causes of morbidity and death worldwide [14]. Due to the rapid spread of pathogens and their poisons in the blood, it is a dangerous, perhaps fatal illness. It can result from infections in any part of the body, such as the organs, cavities, abdomen, and urinary system [15].

Bacteremia patients run the risk of acquiring septicemia, a condition that can be fatal. In septicemia, growing bacteria release toxins into the bloodstream and cause cytokines to be released, which results in fever, chills, malaise, and lethargy, as well as breathing difficulties, especially in young children. It elevates septicemia to the status of a significant clinical condition [10].

Annually, 31.5 million cases of bacteremia are reported worldwide, and between 20 and 50 percent of those instances result in death [12, 16]. In sub-Saharan countries, including Ethiopia BSIs occur commonly among admitted patients. In Eastern African countries, the proportion of patients with bloodstream infections is reported to range from 11% to 28% [7]. Elevated BSI mortality is frequently correlated with improper, inadequate, or late anti-infective therapy [1].

There's a need for more data regarding the identification of bacteria and drug resistance patterns of bacteria in Ethiopia specifically among admitted patients. Additionally, there is a relatively higher drug resistance pattern among admitted patients in Tikur Anbessa specialized hospital because of hospital-acquired infection, so there is a need to generate new evidence and update previous findings in the study setting.

1.3 Significance of the Study

The current research offered up-to-date proof of the bacteria retrieved from blood samples of patients hospitalized in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. Moreover, this study identified the antibiotic resistance patterns of bacteria in blood specimens from Tikur Anbessa specialized hospital. This evidence is important and will be of significant use for the treatment of patients. The information on drug resistance patterns will support clinicians or physicians to switch their antibiotic usage which will in turn improve treatment outcomes. Additionally, it will be useful for the patients and the community to get better treatment.

2. Literature Review

2.1 Epidemiology

Geographical differences affect the epidemiology of blood culture infections as well as their antimicrobial resistance. For example, *Salmonella paratyphi* is the predominant Salmonella group found in blood cultures isolated in Africa, whereas *Salmonella typhi* dominates in the Asian population [17]. The majority of BSIs in Malawi are caused by *Salmonella typhi, Streptococcus pneumonia,* and *non-typhoidal salmonella* [18].

2.2 Prevalence of Bacterial Sepsis and Antimicrobial Susceptibility Pattern

S.aureus, Salmonella spp., and *Acinetobacter spp.* were the most frequently identified bacteria in research done by Khanal LK from Nepal which revealed that 10.3% of samples had bacterial growth [11].

India’s Mohanty A, et al. conducted a study and found that 41.4% of the samples had positive blood cultures. *Coagulase-negative staphylococci* (35.2%), *Staphylococcus aureus* (22.8%), and *Escherichia coli* (19.3%) were often found in those isolates. *Staphylococci* had developed resistance to erythromycin and penicillin. Additionally, gentamicin, ciprofloxacin, and amoxyclav were ineffective against *Enterobacteriaceae* [19].

India's Gohel K, et al. found 9.2% bacterial growth. Gram-positive and gram-negative bacteria were present in 58.3% and 40.2%, respectively, and 1.5% of isolates were fungal. The most frequently detected species were Staphylococcus aureus and Enterobacteriaceae [8].

Gill MK, et al. performed a retrospective study and found 24.8% bacterial isolates. Gram-positive bacteria, Gram-negative bacteria, and non-albicans Candida were 53%, 39.3%, and 7.9%, respectively. 34.5% *Coagulase-negative staphylococcus*, 15.4% *Acinetobacter*, and 14% *Staphylococcus aureus* were most frequently identified [4].

According to a descriptive study from Pakistan by Sana F, et al., 172 of them had growth. The growth contained 98 gram-negative rods, 50 gram-positive cocci, and 24 fungi. *Klebsiella pneumonia* at 22.7%, *Candida species* came in second at 14.3%, followed by *methicillin-resistant coagulase-negative staphylococcus* at 11.6% [20].

Growth was detected in 11.15% of the samples, according to Mia AR, et al. from a hospital in Bangladesh. Gram-negative bacteria grew at a rate of 74% and gram-positive bacteria at a rate of 26%; the majority of bacterial isolates showed azithromycin resistance [17].

Ebrahim-Saraie HS, et al. from Iran isolated 15.1% growth. *Staphylococcus epidermidis* (48.6%) and *Acinetobacter spp*. (8.1%) were the two bacteria that were discovered most frequently. The most effective antibiotics against gram-positive cocci were vancomycin and chloramphenicol. Additionally, both ciprofloxacin and chloramphenicol were effective against gram-negative bacteria [21].

Leopold SJ, et al. conducted an extensive investigation of AMR in sub-Saharan Africa. A total of 190 studies on blood pathogens were examined. Chloramphenicol resistance prevalence in *Enterobacteriaceae* varied from 31.0% to 94.2%, while third-generation cephalosporin resistance prevalence ranged from 0.0% to 46.5%. *Salmonella enterica Typhi* had a prevalence of nalidixic acid resistance that varied from 15.4% to 43.2% [22].

According to surveillance research undertaken in Malawi by Musicha P, et al. 68.3% and 66%, respectively, of all bacteria found were gram-positive and gram-negative. 51.1% of bacterial growth was resistant to penicillin, chloramphenicol, and cotrimoxazole [23].

In Rwanda, 80 bacterial growths from children were detected by Ishimwe E, et al., 30% of the bacteria were *Klebsiella species*, 27.5% were *Staphylococcus aureus*, and 22.5% were *Escherichia coli*. For *S. aureus*, oxacillin resistance was 60%, while gentamycin resistance was 77.3% for *Klebsiella species* [24]. The Kenyan researchers Oduor OC, et al. identified 29.9% of the bacterial isolates. *Staphylococcus epidermidis* 43.1% and *Klebsiella pneumonia* 22.8% were the more prevalent bacteria detected [25].

In Ethiopia research done by Alemnew B, et al., 25.78% of bacterial isolates were identified. Gram-positive bacteria were seen in 15.5% of the growth and gram-negative bacteria in 10.48%. Among the bacteria that were often found were *S. aureus* (7.04%), *coagulase-negative Staphylococcus* (5.75%), *Klebsiella species* (7.04%), and *E. coli* (1.69%) [5].

According to Fentie A, et al. from Gondar, 19.4% of samples had bacterial growth. Most of the bacteria found were *Staphylococcus aureus* (28.6%), *coagulase-negative staphylococci* (26.2%), and *Escherichia coli* (21.4%) [26]. Another study performed in Gondar, Dagnew et al. identified 18.2% growth during a retrospective examination. Identification of 69% gram-positive and 31% gram-negative bacteria. 42.3% of the bacteria detected were *coagulase-*

negative staphylococci, 23.9% were *Staphylococcus aureus*, 12.9% were *Klebsiella spp*., and 7.0% were *E. coli* [3].

The bacterial isolates 15.8%, which were discovered by Kumalo A, et al. from Jimma Hospital. Gram-positive bacterial growth by 53.3% and gram-negative ones by 46.7%. *Staphylococcus aureus* and *Escherichia coli* were isolated more frequently.[27].

Mekelle Hospital's Wasihun AG, et al. detected 28% of bacterial pathogens. Of those isolates, gram-positive bacteria accounted for 72.2% and gram-negative bacteria for 27.8%. The most common bacteria were *Staphylococcus aureus* (37.5%), *coagulase-negative staphylococci* (30.6%), and *E. coli* (3.1%) [28].

From Addis Ababa, Kitila KT, et al. determined that 32.8% of them had positive blood cultures. Gram-positive and gram-negative bacterial growth in the positive samples at rates of 77.4% and 22.6%, respectively. *Staphylococcus aureus* was the most often found bacterium, followed by *coagulase-negative Staphylococci* (26.21%), *Klebsiella pneumonia* (14.02%), and *Escherichia coli* (3.6%) [6].

From a total of 201 samples, Negussie A, et al. from Addis Ababa found that 27.9% showed blood culture positives. 51.8% of them were gram-negative, whereas 46.4% were gram-positive bacteria. *Serratia marcescens* made up 21.4% of the identified bacteria, while *Staphylococcus aureus* made up 23.2% [15].

Bacterial isolates were discovered in 15.2% of blood culture samples, according to Bitew A, et al. from Tikur Anbessa Specialized Hospital. Gram-positive and Gram-negative bacteria made up 45.3% and 54.7%, respectively, of the isolates. *Staphylococcus aureus* and *Klebsiella pneumonia* were the two bacteria that were most commonly discovered. Penicillin was extremely resistant to gram-positive bacteria, whereas gram-negative bacteria were resistant to ampicillin and amoxicillin clavulanic acid [12].

2.3 Transmission and Risk Factor

Age, length of hospital stay, and patients with multidrug-resistant (MDR) infections all contribute to an increased risk of BSI [18]. Several predisposing factors of BSIs are also associated with chronic diseases such as diabetes mellitus, renal and liver disease, and intravascular catheters, patients on hemodialysis, intravenous drug abusers, and patients who have undergone invasive procedures in the ICU are some risk factors for BSI [29].

2.4 Pathogenesis

Different pathogenic bacteria and their toxins in blood flow are the cause of BSIs. Bacteremia is a condition where there are only a few temporary blood bacteria present without any evident toxemia [30]. The majority of occult bacteremia episodes resolve on their own, especially those brought on by Salmonella and Streptococcus pneumonia, and serious complications are becoming less common. But there are also significant bacterial infections that can be fatal, such as pneumonia, septic arthritis, brain abscesses, osteomyelitis, cellulitis, meningitis, and sepsis [31].

2.5 Detection and Diagnosis

Antibiotic susceptibility testing (AST) can help determine the proper antibiotic medication for individuals with BSI and is a highly specific indication of BSI when a pathogenic bacterium is cultured from blood [31, 32]. Targeted treatment of BSIs requires quick pathogen identification and AST. Standard microbiological techniques, however, take 48–72 hours to deliver a definitive species identification and AST report [33]. The degree of sepsis and continuous antibiotic therapy can have a major impact on BC positive rates, which fluctuate widely despite advancements in BC procedures (34). It has been claimed that, as compared to the conventional BC approach, nucleic-acid-based technology, such as PCR, is more sensitive and can produce results faster [35].

2.6 Treatment and Prevention

Early appropriate antibiotic therapy is a fundamentally important aspect of the therapy of patients with BSI. Adequate treatment requires that all organisms isolated from blood are susceptible in vitro to the antimicrobials chosen; a proper route and dose, and an early administration after blood culture collection are needed [32,36]. Antibiotic susceptibility tests and bacterial cultures take time; empirical antibiotic therapy should start right away. However, the development of antibiotic resistance is a significant issue brought on by empiric therapy [21]. Due to the urgency and widespread occurrence of bacteria resistant to the majority of the presently prescribed antibiotics, it has developed into a severe health issue with numerous economic and social ramifications throughout the whole world [5, 28].

MDR infections are more likely to necessitate the administration of more expensive antibiotics, lengthen hospital stays, and raise the risk of mortality [8, 28].

3. Objectives

3.1 General Objective

- To determine bacterial and drug resistance patterns of bacteria recovered from blood samples of hospital-admitted patients at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia.

3.2 Specific Objectives

- To isolate bacterial pathogens in blood samples from patients who had been admitted to Tikur Anbessa specialized hospital in Addis Ababa, Ethiopia.
- To identify drug resistance patterns of bacteria isolated from blood samples among Hospital admitted patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

4. Research Questions

- What is the magnitude of bacterial bloodstream infection from hospital-admitted patients in Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia?
- Which bacteria is more dominantly isolated from blood samples of hospital-admitted patients?
- What is the drug resistance pattern of bacterial isolates from blood samples of hospital-admitted patients in Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia?

5. Methods and materials

5.1 Study Area

Tikur Anbessa Specialized Hospital (TASH), in Addis Ababa, Ethiopia, served as the study's location. TASH has more than 700 beds, which makes it the biggest specialized hospital in Ethiopia. The hospital has several departments that treat patients, including orthopedics, gynecology, hematology, oncology, and others. It serves as the Health Science College's teaching hospital at Addis Ababa University. TASH serves as a training facility for undergraduate and graduate medical and health science students, including those pursuing degrees in dentistry, nursing, midwifery, pharmacy, medical laboratory technology, and other fields dealing with the nation's and the community's health issues [37]. The microbiology department is a well-developed section within the laboratory department, which is divided into many units.

5.2 Study Design and Period

A cross-sectional study was carried out between January 2021 to December 2022.

5.3 Population

5.3.1 Source Population

All patients, who were admitted to Tikur Anbessa specialized hospital during the study period

5.3.2 Study Population

All hospital-admitted patients who were requested for blood culture in the study site during the study period.

5.4 Inclusion and Exclusion Criteria

5.4.1 Inclusion Criteria

- For patients who chose to take part in the study and were thought to have septicemia.

5.4.2 Exclusion Criteria

- Severely sick adult patients who were unable to provide permission due to their condition.

- Patients who were taken antibiotics within the last 7 days.

5.5 Study Variables

5.5.1 Dependent Variables

The magnitude of bacterial isolates and Drug resistance pattern of bacterial isolates

5.5.2 Independent Variables

- Sex
- Age
- Duration of hospital stay
- History of hospital admission

5.6 Measurement and Data Collection

5.6.1 Sample Size Calculation

A single population proportion formula was used to establish the sample size for the investigation. The proportion of bacterial isolates (15.2%) was taken from a previous study done in 2018 by Bitew. A, et al. in Tikur Anbessa Specialized Hospital, on the Multi-Drug Resistance Profile of Bacteria Isolated from Bloodstream Infection [12]. A 95% degree of confidence and a 5% error of margin are also made as assumptions.

Where: n = sample size

$Z\alpha/2$ = level of confidence

P = proportion

d = margin of error

$$n = \frac{z^2 * p * q}{d^2}$$ p=0.152, q=0.848, d=0.05, $Z\alpha/2=1.96$

$$\frac{1.96^2 * 0.152 * 0.848}{0.05^2} = 198$$

The study's ultimate sample size was 218 when taking into account a 10% non-response rate.

5.6.2 Sampling Technique

From the admitted patients at Tikur Anbessa Specialized Hospital, the study subjects were chosen using a convenient sampling technique.

5.6.3 Data Collection Procedure

Data for this study were gathered through the use of a standardized questionnaire. Three components make up the questionnaire. Questions about socio-demographic traits (such as sex and age) and clinical presentation were included in Section One. Section two included questions about the history of hospital admission. The third section was a lab form which was completed at the laboratory. The unit doctors examined patients in the wards for septicemia or bloodstream infections. Those patients with symptoms were included in this study. Respondents were made aware of the study's purpose before giving their consent, and this information was shared with them before the study started.

5.6.4 Laboratory Analysis

5.6.4.1 Blood Sample Collection

The collection site for a venous blood sample was cleaned with 70% alcohol and then treated with a 10% povidone-iodine solution using an aseptic approach by qualified laboratory professionals and nurses. According to CLSI recommendations, blood samples of approximately 5 ml for children and 10 ml for adults were drawn, placed in an aerobic BacT/ALERT bottle, and then incubated in a BacT/ALERT machine. A patient with suspected bacteremia had at least two rounds of blood cultures taken before the antibiotic was administered.

5.6.4.2 Culture Isolation and Identification

For the initial isolation of the microorganism, venous blood was placed in a BacT/ALERT culture bottle and incubated in an automated BacT/ALERT® 3D for five days at a temperature of 37°C with 5% CO2. Each patient received two aerobic blood culture bottles, and growth in both bottles was regarded as favorable. The bacterial growth that was identified by the flag and the audible sound of the device was then subcultured on a 5% sheep blood agar, chocolate, and MacConkey agar plate (Oxoid Ltd., UK) and placed in an incubator at 37°C for 18–24 hours. While chocolate and blood agar plates were incubated in a candle jar with a microaerophilic environment (5–10% CO2), the MacConkey agar plate was incubated aerobically. Gram staining was also done to get early results. Biochemical tests, Gram stain results, and colony features were used to identify all positive blood sample cultures. Conventional biochemical testing and serological identification for Shigella spp. and Salmonella were carried out in the case of gram-

negative bacteria. At the end of the fifth day, the flag was examined to confirm a negative result before being discarded [38, 39].

5.6.4.3 Antimicrobial Susceptibility Test

For susceptibility testing, pure colonies of isolated bacterial organisms were combined with normal saline and tested at 0.5 McFarland standards. The widely used medications were evaluated on the bacterial isolates (Table 1). According to CLSI recommendations, the standard interpretive chart indicating the zone widths of each antibiotic was used to determine the susceptibility of the isolates on Muller Hinton agar and 5% sheep blood with Muller Hinton agar using Kirby-Bauer's disk diffusion technique [40].

Table 1:- Antibiotics that are used for AST for gram-positive and gram-negative bacteria at TASH, 2023.

	Antibiotics used for Gram-positive bacteria	Antibiotics used for Gram-negative bacteria
	Oxacillin (1µg), Clindamycin (2µg), Erythromycin (15µg), Trimethoprim-Sulfamethoxazole(1.25/23.75µg), Penicillin (10µg), Ampicillin (10µg), Vancomycin (30µg), Gentamycin (10µg), Ciprofloxacin (5µg), Tobramycin (10µg), and Cefotaxime (30µ g)	Ampicillin(10µg),Gentamycin(10µg),Tobramycin(10µg),Augmentin(20/10µg),Cefepime(30µg),Ceftriaxone(30µg),Meropenem(10µg),Amikacin(30µg),Ciprofloxacin(5µg),Piperacillin-Tazobactem(100/10µg),Trimethoprim-Sulfamethoxazole(1.25/23.75µg), Ceftazidime(30µg) and Cefotaxime(30µg).

5.7 Data Quality Assurance

The lead researcher regularly verified the correctness of the collected data. All collected samples were checked for adequacy, hemolysis, and proper labeling. All samples were properly registered and entered into the BACT/ALERT machine. The laboratory strictly adhered to its Standard Operating Procedures (SOP) for preparing the media, which were carried out by the manufacturer's instructions. The Clinical Laboratory Standard Institution's (CLSI) quality control standards and media expiration dates were evaluated. We completed labeling the media, containers, and forms. Visual checks for contamination, hemolysis, unbalanced fill, bubbles, signs of freezing, and cracks in media or plastic Petri plates were made. Use the ATCC control strain for every isolate of a

bacterium, such as *E. coli* 35218*, S. aureus* 25923, *Pseudomonas aeruginosa* 27853, and *Haemophilus influenzae* 10479. On a log sheet, record the findings and keep them for future reference. The sample was preserved in tryptone soya broth with glycerol at -70 °C.

5.8 Data Analysis

To evaluate the data, conclude the prevalence of bacterial infections, and demonstrate bacterial resistance to antibiotics, version 26 of the Statistical Package for Social Science (SPSS) was used.

5.9 Ethical Considerations

The research ethical committee of the Department of Medical Laboratory Sciences at Addis Ababa University granted its ethical clearance before the study could be carried out. Before the data and blood samples were collected, informed consent was obtained. Patients who had septicemia symptoms were admitted to the wards, or casualties were requested to participate in the study if they were willing to provide consent and could provide blood samples throughout the investigation. Any details on the patient and their clinical background were kept private. Patients got therapy depending on the test findings once the data were made accessible to them in a week.

5.10 Dissemination and Utilization of Results

The research was disseminated to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, and then submitted for scientific publication.

5.11 Operational definition

Drug resistance bacteria: The ability of bacteria and other microorganisms to resist the effects of an antibiotic to which they were once sensitive.

Sepsis: The body's extreme response to an infection which happens when an infection you already have triggers a chain reaction throughout your body.

Bacteremia: The presence of bacteria in the bloodstream.

6. Results

6.1 Clinical and Demographic Characteristics of Study Participants.

A total of 218 paired samples were gathered, of which 108 (49.5% of them) were female and 110 (50.5% of them) were male. In terms of age, 97 individuals (44.5% of the total) were between 1 and 14 years old, while 5 (2.3%) were above 65 years. most of the participants were children, 67(30.7%) from pediatric wards, and 60(27.5%) from casualty. The majority of participants, which were 64(29.4%) stayed in the hospital for 3-4 days. And from the total participants,174(79.8%) of them did not have a history of hospital admission (table 2).

Table 2:- Clinical and demographic characteristics of study participants from bloodstream infection in TASH, 2023.

		Blood culture result	
Variable	Categories	Positive n=66(%)	Negative n=152(%)
Sex	Male	32(48.5)	78(51.3)
	Female	34(51.5)	74(48.7)
Age group (year)	<1	11(16.7)	16(10.5)
	1-14	27(40.9)	70(46.1)
	15 -24	7(10.6)	31(20.4)
	25-44	11(16.7)	19(12.5)
	45-64	8(12.1)	13(8.6)
	≥65	2(3.0)	3(1.9)
Ward	Causality	17(25.7)	43(28.3)
	Pediatric ward	22(33.4)	45(29.6)
	Adult ward	27(40.9)	64(42.1)
Duration of hospital stay	1-2 days	7(10.6)	33(21.7)
	3-4 days	14(21.2)	50(32.9)
	5-6 days	15(22.7)	38(25)
	≥7 days	30(45.5)	31(20.4)
History of hospital admission	Yes	35(53.1)	9(5.9)
	No	31(46.9)	143(94.1)

6.2 Bacterial Pathogens Causing BSI

From a total of 218 paired samples, 66(30.3%) of them were identified as positive bacteria growth and the rest 152(69.7%) of them were negative. From those positive samples, 41/66 (62.2%) had gram-positive bacteria and 25/66 (37.8%) had gram-negative bacteria. From Gram-positive bacteria, the predominate was *Staphylococcus aureus* 23/66(34.8%) and followed by *Coagulase-negative Staphylococcus* 10/66(15.2%), *Enterococcus species* 4/66(6.1%), *Staphylococcus lugdunensis* 3/66(4.5%) and the least only had 1/66(1.5%) which was *Streptococcus viridians* (figure 1). And also from Gram-negative bacteria, *E.coli* comes in first place with 10/66(15.2%), and next *Klebsiella pneumonia* accounts for 7/66(10.6%) and *Acinetobacter species* 6/66(9.1%), and the least isolated gram-negative bacteria's were *Pseudomonas* and *Citrobacter diversus* species each accounts only 1/66(1.5%). (Figure 2).

Figure 1:- Isolation of gram-positive bacterial growth from bloodstream infection in TASH, 2023.

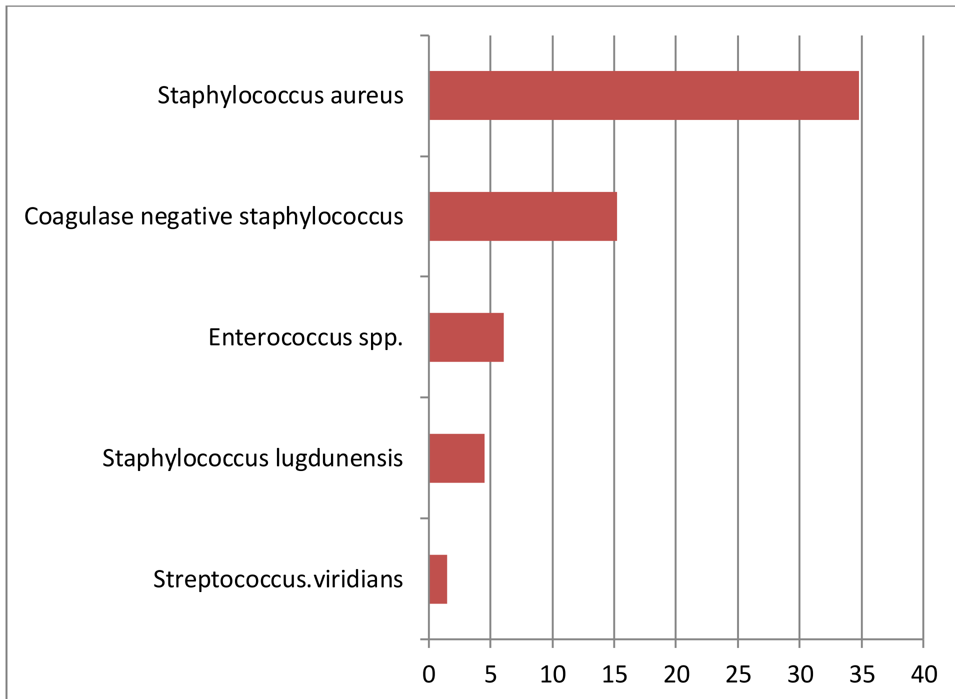
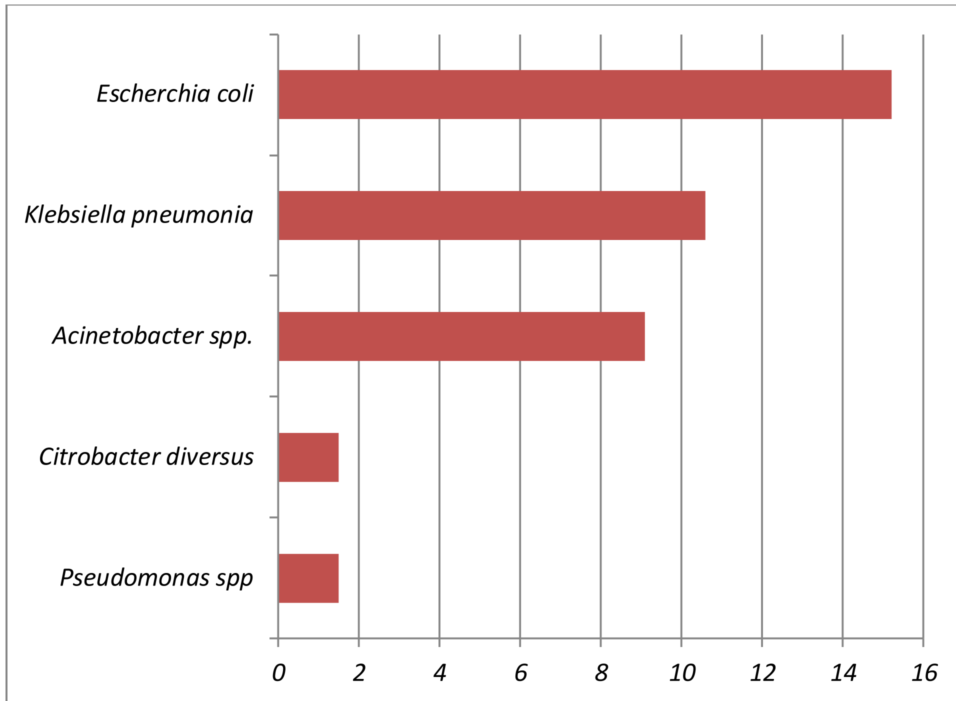


Figure 2:- Isolation of gram-negative bacterial growth from bloodstream infection in TASH, 2023.



6.3 Antimicrobial Susceptibility Testing

6.3.1 Antimicrobial susceptibility patterns of Gram-positive bacteria.

Staphylococcus aureus was the most commonly encountered gram-positive bacterium discovered to be responsive to oxacillin, ciprofloxacin, and trimethoprim-sulfamethoxazole (78.3%, 73.9%, and 65.2%, respectively), on the other hand, *Staphylococcus aureus* was high resistance to penicillin (73.9%).

The other gram-positive bacterial isolate was *coagulase-negative staphylococcus*, oxacillin was completely sensitive, clindamycin and erythromycin were (70%) sensitive, additionally, *coagulase-negative staphylococcus* was high resistance to penicillin (80%). Also, *Enterococcus species* were completely sensitive to vancomycin, 75% resistant to penicillin, and completely resistant to ampicillin (table 3).

Table 3:- Antimicrobial susceptibility patterns of Gram-positive bacteria among BSI in TASH, 2023.

Bacterial isolates		Antimicrobial susceptibility patterns (%)										
		OXA	CN	E	SXT	P	AMP	VAN	GN	CIP	TOB	CTX
S.aureus (n=23)	S	78.3	47.8	47.8	65.2	21.7	NA	NA	56.5	73.9	56.5	NA
	R	21.7	52.2	52.2	34.8	73.9	NA	NA	43.5	26.1	43.5	NA
CONS (n=10)	S	100	70	70	60	20	NA	NA	50	50	50	NA
	R	0	30	30	40	80	NA	NA	50	50	50	NA
Enterococcus spp (n=4)	S	NA	NA	NA	NA	25	0	100	NA	NA	NA	NA
	R	NA	NA	NA	NA	75	100	0	NA	NA	NA	NA
S. lugdunensis (n=3)	S	100	0	0	100	0	NA	NA	66.7	66.7	66.7	NA
	R	0	100	100	0	100	NA	NA	33.3	33.3	33.3	NA
Streptococci viridians(n=1)	S	NA	NA	NA	NA	NA	NA	100	NA	NA	NA	100
	R	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	0

OXA=Oxacillin, CN=clindamycin, E=erythromycin, SXT=trimethoprim+sulphamethoxazole, P=penicillin, AMP=ampicillin, VAN=vancomycin, GN=gentamycin, CIP=ciprofloxacin, TOB=tobramycin, CTX=cefotaxime, NA=not applicable.

6.3.2 Antimicrobial Susceptibility Patterns of Gram-negative Bacteria

From those gram-negative bacteria isolated, the major bacteria identified as *Escherichia coli* and highly sensitive to Amikacin 90%.on the contrary, highly resistant to ampicillin (90%), (80%) resistance to cefepime, ceftriaxone, ceftazidime, and cefotaxime. The other more frequent gram-negative bacteria isolated were *Klebsiella pneumonia*, and completely sensitive to Amikacin, However, they were resistant to ceftriaxone, ceftazidime, and cefotaxime by 85.7%, also ampicillin was completely ineffective. The subsequent isolate of bacteria, *Acinetobacter spp.,* was responsive to meropenem and ciprofloxacin, each contributing 83.4%. (Table 4)

Table 4:- Antimicrobial susceptibility patterns of Gram-negative bacteria among BSI in TASH, 2023.

Bacterial isolates		Antimicrobial susceptibility patterns (%)												
		AMP	GN	TOB	AGU	FEP	CRO	MEM	AMK	CIP	PZT	SXT	CAZ	CTX
E.coli (n=10)	S	10	60	60	40	20	20	50	90	60	40	70	20	20
	R	90	40	40	60	80	80	50	10	40	60	30	80	80
K.pneumoniae (n=7)	S	0	42.8	42.8	42.8	28.6	14.3	57.2	100	42.8	57.2	71.4	14.3	14.3
	R	100	57.2	57.2	57.2	71.4	85.7	42.8	0	57.2	42.8	28.6	85.7	85.7
Acinetobacter spp. (n=6)	S	NA	66.7	66.7	NA	66.7	66.7	83.4	66.7	83.4	66.7	NA	66.7	66.7
	R	NA	33.3	33.3	NA	33.3	33.3	16.6	33.3	16.6	33.3	NA	33.3	33.3
Pseudomonas spp. (n=1)	S	NA	0	0	NA	0	NA	0	100	0	0	NA	0	NA
	R	NA	100	100	NA	100	NA	100	0	100	100	NA	100	NA
Citrobacter diversus (n=1)	S	0	0	0	100	0	0	100	100	100	100	0	0	0
	R	100	100	100	0	100	100	0	0	0	0	100	100	100

AMP=ampicillin, GN=gentamycin, TOB=tobramycin, AGU=Augmentin, FEP=cefepime, CRO=ceftriaxone, MEM=meropenem, AMK=Amikacin, CIP=ciprofloxacin, PZT=piperacillin-tazobactam, SXT= trimethoprim+sulphamethoxazole, CAZ=ceftazidime, CTX=cefotaxime, NA=not applicable

7. Discussions

The number and diversity of the isolated bacteria and their patterns of antibiotic resistance were evaluated in this investigation. In this research, 30.3% of the 218 participants in total were found to have positive bacterial growth. In those positive samples, gram-positive bacteria comprised 62.2% of the isolates, whereas gram-negative bacteria accounted for 37.8%. *E. coli* and *coagulase-negative staphylococcus* each accounted for 15.2% of the bacteria isolated, with *Staphylococcus aureus* being the most common (34.8%).

In this investigation, the proportion of bacterial identification reached 30.3% and isolated bacterial growth is consistent with studies done in India which were 24.8% [4], Kenya 29.9% [25], Ethiopia 25.78% [5], Mekelle 28%[28], and two studies done in Addis Ababa reported magnitude of 32.8%[6] and 27.9%[15]. As opposed to research conducted in Asian nations, this study is higher, which ranged from 15.1% - 10.3% [21], [17], [11], and a study from Jimma reported 15.8% [27]. The increased magnitude of our study might have resulted from methodological differences, geographical differences, study population, study period, and study design differences. Additionally, the methods used in isolating the bacteria could have made a difference.

Among the positive samples, 41/66 (62.2%) had gram-positive bacteria which is in close accord with the Indian study 61.4% [19], and also higher than studies done in Jimma, Ethiopia 53.3%[27], in India 53% [4], two studies from Addis Ababa, Ethiopia 46.4% [15] and 45.3% [12] and finally in Bangladesh 26% [17]. Furthermore, our investigation is inferior to research conducted in Gondar, Ethiopia 69% [3], Mekelle, Ethiopia 72.2% [28], and 77.4% from Addis Ababa, Ethiopia [6].

On the other hand, In this study 25/66 (37.8%) had Gram-negative bacteria, thus comparable to both Indian studies 38.6% [19] and 39.3% [4], and higher than three studies performed in Ethiopia 27.8% [28], 22.6% [6], and 31%[3]. This study is inferior, Compared to research conducted in Jimma, Ethiopia 46.7% [27], and two research projects conducted in Addis Ababa, Ethiopia 51.8% [15], and 54.7% [12], and much lower than the study done in Bangladesh 74% [17]. In a few studies, the results of gram-positive bacteria and gram-negative bacteria isolations were parallel with our study. On the other hand, there were differences compared to our result,

therefore, there could be variations in the study population, sample size, research strategy, and geographic disparities.

Staphylococcus aureus, which comprised 34.8% of the Gram-positive bacteria, was the most common, and there is strong consistency with research conducted in Mekelle, Ethiopia (37.5%) [28], Also in Jimma, Ethiopia 40% [27]. Our study is higher than studies done in Rwanda 27.5% [24], Nepal 26.3% [11], India 22.8% [19], Gondar, Ethiopia 23.9% [3], and two studies conducted from Addis Ababa, Ethiopia 23.2% [15] and (23.4%) [12]. On the contrary, our study came behind a study conducted in Addis Ababa, Ethiopia, which was 50.0% [6]. The current study is higher than studies done in different places, it might be due to; Differences in study design, number of study populations, Seasonal variation, and Geographical variation.

Coagulation-negative Staphylococcus was the next-most common gram-positive bacterium, accounting for 15.2%, which is comparable to research conducted in Addis Ababa, Ethiopia, where 19.6% of the bacteria were present [15]. On the contrary, our study is inferior to one conducted in Addis Ababa, Ethiopia 33.8% [6], and substantially less than research conducted in India 35.2% and two studies done in Ethiopia 30.6 [28] and 42.3% [3]. and also, there were differences for the predominant isolate found in Iran which was *Staphylococcus epidermidis* 48.6% [21] and in Kenya *Staphylococcus epidermidis* 43.1% [25]. Predominant bacterial isolates varied throughout studies based on many variables, which could be caused by changes in research populations and study designs.

From gram-negative isolates, *E.coli* comes in first place with 15.2%, preceded by *Klebsiella pneumonia at* 10.6%, and *Acinetobacter spp.* 9.1%. In our study, *E.coli* is the predominant isolated gram-negative bacterium which was 15.2%, so there is a good agreement with studies done in India which was 19.3% [19], in Jimma, Ethiopia 13.3% [27]. Also, our study is higher than studies done in Mekelle, Ethiopia 3.1% [28]. Compared to our study, There were differences in predominating bacteria in different studies, in Addis Ababa, Ethiopia *Klebsiella pneumonia* was the predominant bacteria accounts for 17.27% [12], and in *Kenya Klebsiella pneumoniae* was 22.8% [25], furthermore, In Addis Ababa, Ethiopia *Klebsiella pneumonia* 14.02% and next *Escherichia coli* which was 3.6% [6]. And also Studies done in Asia the predominant isolates were *Acinetobacter spp.* 18.9% [11] and 8.1% [21]. In another study in Rwanda, The predominant isolated bacteria were *Klebsiella species* 30% and the second

common isolate was Escherichia *coli*, which was 22.5% [24], and also, in Addis Ababa Ethiopia the predominant isolated gram-negative bacteria found was *Klebsiella spp* which was 16% [15]. Our study is different from others in the predominating bacteria; it might come from variations in the research population, the sample size, or regional variations.

In the current investigation, *Staphylococcus aureus* was the most common gram-positive bacterium identified, which is susceptible to oxacillin (78.3%), trimethoprim-sulfamethoxazole (65.2%), clindamycin, and erythromycin each accounted for (47.8%) of sensitivity, furthermore (56.5%) for gentamycin sensitive. on the other hand, *Staphylococcus aureus* was highly resistance to penicillin (73.9%). In a study done in our continent, Rwanda oxacillin was 60% resistant which is higher than compared to this study [24]. Our research fits into an investigation conducted in Addis Ababa, Ethiopia that was resistant to Penicillin 82.9%, and also higher resistance to Erythromycin 75.6%, Trimethoprim-sulfamethoxazole 85.4% and Gentamycin 73.1% and least resistance to Clindamycin 4.8% [6]. Further research was conducted from Addis Ababa Ethiopia, *S.aureus* was resistant to gentamycin 40%, erythromycin 26.7%, clindamycin 20%, trimethoprim-sulfamethoxazole 33.34, penicillin 86.7%, compared to our study gentamycin, trimethoprim-sulfamethoxazole, and penicillin were in line with the above study in resistance pattern for *S.aureus*, on the other hand, erythromycin and clindamycin were lower resistance compared to our study [12].

The second predominant gram-positive bacterial isolate was *coagulase-negative Staphylococcus*, oxacillin was completely sensitive, clindamycin and erythromycin were (70%) sensitive, and *coagulase-negative Staphylococcus* was highly resistant to penicillin (80%) and (40%) nonresponsive for trimethoprim-sulfamethoxazole. This investigation runs concurrently with one that found substantial penicillin resistance (90.5%) in Addis Ababa, Ethiopia. On the contrary, compared to our study there was high resistance to Cotrimoxazole 85.7% and Erythromycin 83.3% [6]. In another study done in Addis Ababa, Ethiopia erythromycin, and clindamycin had 33.7% resistance so there is a similarity with our study. penicillin had 100% resistance, and trimethoprim-sulfamethoxazole had 62.5% resistance so there was a slightly higher resistance pattern compared to our study [12].

Another gram-positive bacterial isolate found in our study was *Enterococcus spp.* and there was 100% sensitivity for vancomycin, so the sensitivity pattern for vancomycin is greater than that of research conducted in Addis Ababa, Ethiopia, which was 40% [12].

From those gram-negative bacteria isolated, the most frequently found was *E.coli*, which was highly sensitive to Amikacin 90%, and also, extremely non-responsive to ampicillin (90%), 80% resistant to cefepime, ceftriaxone, ceftazidime, and cefotaxime. Additionally, this result is consistent with one conducted in Gondar, where ampicillin resistance was 100% [3]. In another study done in Addis Ababa, there is comparability in the resistance pattern of ampicillin and on the contrary, there is a lower resistance pattern for ceftriaxone and cefotaxime which was 50% resistance [12].

The second predominant gram-negative bacteria isolated was *Klebsiella pneumonia*, it was completely sensitive to Amikacin, and 71.4% sensitive to trimethoprim-sulfamethoxazole. On the other hand complete resistance to ampicillin, and 85.7% resistance to ceftriaxone, ceftazidime, and cefotaxime. Among two studies done in Ethiopia, there was a similarity with the resistance pattern in ampicillin which was 100% resistance [6, 12], and for ceftriaxone and cefotaxime which was 81.8% resistance [12]. On the contrary, there was a higher resistance pattern for Trimethoprim-sulfamethoxazole at 86.9% compared to our study [6].

Gram-positive and Gram-negative bacteria were similarly resistant to various medications in most studies, but there is also a difference compared to our study, this might be due to, the number of nosocomial infections among hospitalized patients being on the rise.

8. Strength and limitation

8.1 Strength

Our research has many strengths, which include;-

- we used a fully automated machine for the detection of bacterial photogenes.
- we included all age groups.
- Also, this study includes all isolated bacteria which were both gram-positive and gram-negative bacteria.

8.2 Limitation

- Our study identifies numerous bacterial isolates that cause bloodstream infection, but we were unable to identify anaerobic bacteria.

9. Conclusion and Recommendation

9.1 Conclusion

It had been found a high number of bacterial isolates, which was about 30%. More gram-positive bacteria than gram-negative bacteria were isolated, while the dominant bacterial isolates found in Bloodstream infection were *staphylococcus aureus*, *coagulase-negative staphylococcus, Escherichia coli,* and then preceding by *klebsiella pneumonia, acinetobacter spp,* and *Enterococcus spp*. In this study, Penicillin was identified to be the drug to which most gram-positive bacteria developed resistance. For the gram-negative, ampicillin was highly resistant and cephalosporins were resistant for most of the gram-negative bacteria.

9.2 Recommendation

Depending on this study, the physicians need to avoid the usage of last-line antibiotics, so we recommend that there should be a blood culture performed before antibiotics are used whenever possible. We also recommend the provision of health education mainly focusing on infection prevention for admitted patients and their attendants. Finally, there should be an implementation of infection prevention mechanisms within the hospital.

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11. ANNEXES

Annex I; Information sheet and Consent Form in English Version

Title of the Research Project: Bacterial profile and drug resistance pattern of bacteria recovered from blood samples of hospital-admitted patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Principal Investigator: Eleni Tamere (BSc, MSc candidate)
Name of the Organization: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntary. The research teams will include one principal investigator, and two advisors; please take as much time as you need to read or listen to the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to identify bacterial pathogens and AST patterns of bacteria in admitted patients.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimens collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staff as it is needed. The required clinical sample will be collected by laboratory professionals and nurses. Then, you are requested to give your consent to the sample collector. After consent, a sample will be taken from venous blood. Moreover, there will be a face-to-face interview for additional questions.

Potential risks and Discomforts

There might be some minimal risk and discomfort when we take venous blood. Nevertheless, we will try to minimize the discomfort as much as possible, as the blood samples will be taken by experienced laboratory professionals.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked about related to your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated. In addition, the result of the study will be beneficial for the detection and management of septicemia. Hence, you are indirectly benefiting other patients and society in this respect.

By participating in the study, you will directly benefit by being investigated for any pathogenic organisms and other clinical abnormalities. Establishing the reference will be used in the future to improve the general health status of Ethiopians

Participation and Withdrawal from the Study

Participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also refuse to give any sample. You can ask any questions regarding this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Name:- Eleni Tamere **Phone:+251912181453 E-mail:**elutam@gmail.com

Annex I; የተሳታፊዎችፈቃድናመተማመኛቅፅ

በአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስት/ክፍልበማስተርስድግሪተማሪየመመረ ቂያጥናትላይእዲሳተፉተጋብዘዋል።
እባክዎበዚህጥናትለመሳተፍከመስማማትዎበፊትከዚህቀጥሎየሚገኘዉንምንባብበጥሞናያንብቡናግልጽያ ልሆነልዎትንማንኛዉምሃሳብይጠይቁ።

መግቢያ

የጥናቱርዕስ Bacterial profile and drug resistance patterns of bacteria recovered from blood samples of hospital admitted patients at Tikur Anbesa specialized hospital, Addis Ababa, Ethiopia.

የእርስዎበዚህጥናትላይየሚኖርዎትተሳትፎሙሉበሙሉበበጎፈቃደኝነትላይየተመሰረተነዉ።
በዚህጥናትዉስጥላለመሳተፍወይምለመሳተፍከወሰኑበኋላለማቋረጥየሚወስኑቢሆንምእንኳዋበዚህሆስፒ ታልየሚሰጡዉማንኛዉምአገልግሎትአይቋረጥም።
በጥናቱለመሳተፍየሚስማሙከሆነየስምምነትቅጹላይበጹሁፍወይምበጣትፊርማማስቀመጥይጠበቅዎታ ል።

የጥናቱተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህጥናትለመሳተፍየሚስማሙከሆነናሙናዎለጥናቱእንዲሚዉልመስማማትይጠበቅብዎታል።
ከተወሰደዉናሙናላይየሚገኙመረጃዎችከዚህሆስፒታልዉጭለሚገኙናለስራዉአግባብነትላላቸዉሰዎችቢነ ገርየማይቃወሙመሆኑንመስማማትይጠበቅብዎታል።
ይሁንእንጅይህአይነቱመረጃየርስዎንማንነትየሚገልጡመረጃዎችንማለትምስም፤

አድራሻናየስልክቁጥርየመሳሰሉትንመረጃዎችንአይጨምርም፡፡

ይልቁንምለዚህአገልግሎትብቻየሚዉልእርስዎንለማወቅየሚያስችልመለያቁጥርጥቅምላይእንዲዉልይደረጋል፡

በተጨማሪምስለርስዎአጠቃላይየጤናሁኔታለሚቀርቡአንዳንድተጨማሪጥያቄዎችመልስመስጠትይኖርብዎትአል፡፡

በዚህጥናትመሳተፍየሚያስከትላቸዉችግሮችምንድንናቸዉ?

ናሙናበሚሰበሰብበትወቅትምንምአይነትየከፋችግርአያጋጥምዎትም፡፡

ሆኖምግንናሙናዉንለመሰብሰብልምድያለዉባለሙያስለሚመደብናአስፈላጊዉየጥንቃቄእርምጃስለሚወሰድየህመምስሜትአይኖርም፡፡

የህክምናመረጃበሚስጥርተጠብቆመቆየትየሚችለዉእንዴትነዉ?

ስለራስዎየሰጡትማንኛዉምመረጃናከተወሰደዉናሙናላይየተገኘዉየላበራቶሪዉጤትየሚዉለዉለጥናቱአላማብቻነዉ፡፡ይህንማህደርሊያገኙየሚችሉትየተወሰኑየጥናቱተባባሪሰዎችብቻናቸው፡፡

ከዚያምበላይስለእርስዎያለውንማንኛውንምመረጃየተለየየይለፍቃልባለውየኮምፒውተርየመረጃማህደርውስጥእንዲቀመጥይደረጋል፡፡

በዚህጥናትመሳተፍየሚያስገኛቸውጥቅሞችምንድንናቸው ?

ይህጥናትየማስተርስዲግሪመመረቂያእንደመሆኑመጠንበዚህጥናትበመካፈልዎበገንዘብየሚያገኙትጥቅምባይኖርምከጥናቱበሚገኘውውጤትግንተጠቃሚነዎት፡፡

በዚህጥናትተሳታፊየመሆንዎመብቶችምንድንናቸው ?

በዚህጥናትመሳተፍሙሉበሙሉበእርስዎፈቃደኝነትየተመሰረተበመሆኑበማንኛውምሰዓትናቦታየማቋረጥሙሉመብትየተጠበቀከመሆኑምበላይእራስዎንከጥናቱበማግለልዎምክንያትየሚቀርብዎትምንምአይነትየሆስፒታልአገልግሎትአይኖርም፡፡

ከዚህምበተጨማሪጥናቱንበተመለከተማንኛውንምአይነትጥያቄየመጠየቅናገለጻየማግኘትመብትአለብዎት

።የላብራቶሪምርመራውጤቱንምበነጻማግኘትይችላሉ።

ነገርግንእርስዎበሚሰጡንመረጃየችግሩንስፋትለመከላከልእናለመቆጣጠርጠቃሚስለሆነለሚቀርብልዎትጥ

ያቄቀጥተኛመልስይሰጡንዘንድበታላቅአክብሮትእንጠይቃለን።

ጥያቄካለኝወይምችግርቢያጋጥመኝምንማድረግይገባልይህንንጥናትበተመለከተወይምከዚህጥናትጋ

ርበተዛመደመልኩስለሚያጋጥሙድንገተኛአደጋዎችወይምጥያቄካለዎትበሚመለከተውአድራሻይጠቀሙ።

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Annex II; Informed consent form in English version

Card no…..…………………

I had been informed that the objective of this study is to identify bacterial pathogens and the AST patterns of bacteria. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing septicemia in Ethiopia. I was also informed about the confidentiality of this study. The principal investigator requested me to participate in the study which would require my willingness to provide the required data including blood samples, and filling questionnaire. Therefore, with a full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_______________________________________ hereby give my consent to provide the requested information and specimens as the doctors find best for me.

Signature: _________________________________ Date_______________________

Annex II; informed consent form in Amharic version

የተሳታፊዎችስምምነትማረጋገጫ

የሚስጥርቁጥር -----------------------------

የተሳታፊዉስም ------------------------------

እኔ ስሜ ከላይ የተጠቀሰውተሳታፊ “identify bacterial pathogens and AST patterns of bacteria.”ጥናትላይበቂገለጻተደርጎልኛል፡፡ለጥናቱምደምናሙናእንደሚያስፈልግተገልጾልኛል፡፡ የጥናቱንምአላማዎችምተረድቻለሁ፡፡

በቃለመጠይቁላይየገለጽኳቸውመረጃዎችበሙሉበሚስጥርየተጠበቁእንደሚሆኑተነግሮኛል፡፡ በጥናቱላይያለመሳተፍናማንኛውንምመረጃያለመስጠትእንዲሁምበማንኛውምጊዜከጥናቱራሴንየማግለል መብቴየተጠበቀእንደሆነተገልጾልኛል፡፡

ስለዚህለዚህጥናትመረጃናየስምምነትቃሌንየሰጠሁትበአጠቃላይሁኔታውንበመረዳትናበፍጹምፍቃደኝነትነ ው፡በተጨማሪምጥያቄለመጠየቅተፈቅዶልኝለማወቅየፈለኩትንያህልማብራሪያአግኝቻለሁ፡፡ የዚህጥናትተሳታፊበመሆኔየማገኘውጥቅምየሁሉንምምርመራውጤትበነጻማግኘትእንደሆነተረድቻለሁ፡፡

በአጠቃላይእኔከላይበመተማመኛቅፅየተጠቀሱትንሁሉበሚገባናበተረጋጋመንፈስአንብቤዋለሁኝ፡፡ ስለዚህበዚህጥናትለመሳተፍፈቃደኛመሆኔንበፊርማዬአረጋግጣለሁ፡፡

ፊርማ-------------------- ቀን ----/---/----------

(የስምምነትቅጹንማንበብለማይችሉተሳታፊዎች)

የአማካሪነርስስም --- ፊርማ -----------------

ቀን----------------------

Annex III; semi-structured questionnaire

1. Patients' age___________ years.
2. Sex: ☐ male ☐ female
3. patient's educational status: a) illustrate b) read and write c) elementary school d) high school e) college/university
4. The current unit of diagnosis; ☐ causality ☐ pediatric ward ☐ Adult wards
5. For how long did the patient admitted? ☐ 1- 2 days ☐ 3-4days ☐ 5-6 days ☐ ≥7 days
6. What was the cause of the high fever? ☐Suspected bacteremia ☐ malaria ☐ others specify……
7. Does the patient have a history of hospital admission? ☐ Yes ☐ no

Thank you!

8. Laboratory data

Fill in the following laboratory data according to the SOP and international guidelines for reporting results

Biochemical tests

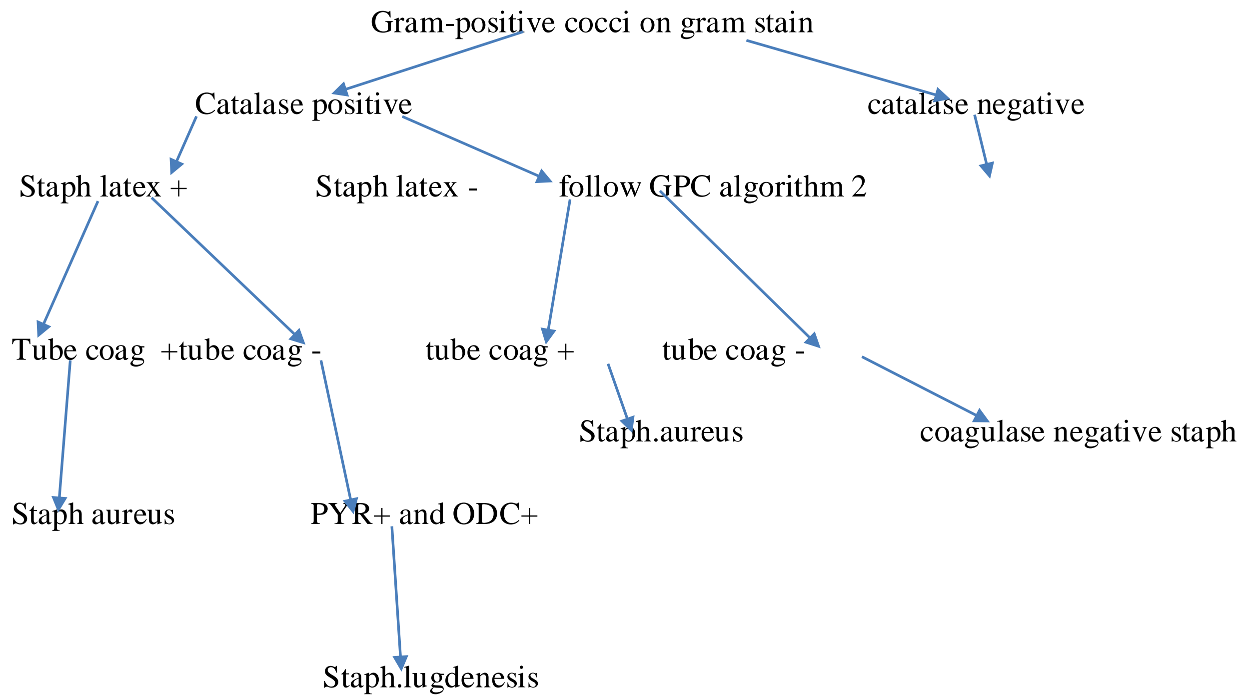
lactose	Indole	Urea	mannitol	H_2S	TSI	gas/ glu	citrate	motility	lysine	LDC	malonate

Antimicrobial susceptibility testing

isolated microorganism	Antimicrobial susceptibility result																
	Gentamicin	Ciprofloxacin	Ceftriaxone	Clindamycin	Amikacin	Cefoxitin	Ampicillin	Meropenem	Cefepime	Piperacillin	trimethoprim-sulphamethoxazole	Oxacillin	Amoxicillin+ clavulanic acid	Penicillin	Erythromycin	Vancomycin	Tobramycin

R-resistance, S-Sensitive, I-Intermediate

Annex IV; Gram-positive cocci algorithms



Annex V; Gram positive cocci algorithm2

Annex VI; Gram negative rods chart

I	Lactose	Ind Ol	UrEa	Mannitol	H_2S	Gas glu	Citrate	Motility	Lys In	Organism	Additional
LACTOSE			+	+	+	+	+	+	-	Citrobactor	Urea +
OR		-		+			+	+	-	Entrobactor cloacae	
ONPG (ortonitrophnile					-	+		-	+	Klebsiella pneumonia	Malonate +
galactosidase							-/+	-	-/+	Klebsiella ozenae	
)							+/-	+	-	Ent. agglomerans (Erwinia)	
POSITIVE NB:-	+					-	-	-	-	Klebs.rihinosclerom itus1 Shigella sonnei (2)	
					-		+	+	+	Serratia (3)	
1. Ornithine (-)			-	+		+	+	+	+	Ent.arogens (4) or Hafnia (5)	
2. Ornithine (+)									–	Ent.Cloacae (6) or Citrobactor (7)	
3.Gas variable					+	+	+	+	+	Citrobactor	
week. 4.Additional									+	Arizona	Malonate +
inositol (+)			+	+	-	+	+	-	+/-	Klebsella oxytoca	

5.Additional inositol (-)		+					+	+	+	Citrobactor diversus	
6.MR$^-$, VP$^+$				+	-	+	-	+	+	E. Coli	
7.MR$^+$, VP$^-$			-					-	+	E. Coli	
						-	-	-	-	E. Coli (A—D)	
				-	-	-	-	-	-	Sh. Dysente or E. Coli A-D	
II	Lactose	Indol	UrEa	Maitol	H_2S	Gas glu	Citrate	Motility	Lysin	Organism	Additional
LACTOSE AND ONPG NEGATIVE				+/-	-	+	+	+	-	Providencia rettgeri	PAD (+)
		+	+	–	-	+	-	+	-	Morganella morganii	PAD (+) LDC(-)
					+	+	-/+	+	-	Proteus vulgaris	PAD (+)
						+	+	+	-	Providenica alkalifaciens 4	PAD (+)
NB:-				-	-	-	+	+	-	Providinica Stuartii	PAD (+)
+ 90% or more positive	-						-	-	-	Shegella dysentriae	
			-		+	+	-	+	+	Edwardsiella	
-90% or more negative							-	-	+	E. coli (A—D)	
				+	-	-			-	Shigella spp.	

+/- majority negative							+	+	-	Providenicia stuartii (B)	PAD (+)
-/+majority negative				-	-	-	-	-	-	Shigella dysentriae	
		-	-		-	-	-	-	-	Shigella spp.	
				+		+	-	+	_	Salmonella Group A	
					+	+	+	+	+	Salmonella or Arizona	
						-	-	+	+	Salmonella thyphi	VC (+)
			+	-	+	+	+/-	+	-	Proteus mirabilis	PAD (+)
	d	-	D	+	-	+d	+	+	+	Seratia marcescers	Ox +/-
III	Lactose	Indol	UrEa	Maitol	H_2S	Gas glu	Citrate	Motility	Lysin	Organism	Additional
NON FERMENTATIVE	-	-	D	+/-	-	-	+	+/-	+/-	Pseudomonas Arogenesa	Cat + Oxi +
	+/-	-	-s	-	-	-	+/-	-	-	Acitinobactor	Cat + Oxi -
	-	-	-	-	-	-	d	+	-	Alcaligens spp.	Cat + Oxi -

ANNEX VII; AST

CLSI 2019 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 ^{o}c ,ambient air 16-18 hr

When test? #	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Ampicillin	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+Sulfame	1.25/23.75µg	≤ 10	11-15	≥ 16	

	thoxazole					
A	Ciprofloxacin (breakpoint for Salmonella only)	5 µg	≤ 20	21-30	≥ 31	

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 acid (PO only)	20/10 µg	≤ 13	14-17	≥ 18	
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

<u>Testing conditions</u>

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-	1.25/23.75 µg	≤ 10	11-15	≥ 16	

	Sulfamethoxazole					
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

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: 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 °c ,ambient air 16-18 hr. 24hr for vancomycin testing

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 ^{o}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</td></tr>
</table>

						clindamycin as R if "D-phenomenon" is seen (inducible clindamycin resistance) (EUCAST)
A	Trimethoprim-Sulfa	1.25/23.75 µg	≤ 15	16-18	≥ 19	
1	Tetracycline (PO only)	30 µg	≤ 24	25-27	≥ 28	If S for Tetracycline report S for Doxycyclin
3	Doxycycline (PO only)	30 µg	≤ 24	25-27	≥ 28	If S for Tetracycline report S for Doxycyclin
4	Vancomycin	30 µg	-	-	≥ 17	Resistant isolates are rare → retest isolate
2	Chloramphenicol	30 µg	≤ 20	-	≥ 21	ALWAYS TEST IN CSF ISOLATES
	Ceftazidime	DO NOT TEST	R			Report as R if requested (EUCAST)

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

<u>Testing conditions</u>

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

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university, and that all sources of materials used for the thesis have been fully acknowledged.

M.Sc. candidate: **Eleni Tamere (B.Sc.)**

Signature: ___________________

Date of submission: ___________________

This thesis has been submitted with our approval as advisors.

Advisor: **Adane Bitew (MSc, PhD)**

Signature: _____________________

Date: _____________________

Place: Addis Ababa, Ethiopia.

Advisor: **Mequanint Mitiku (B.Sc., MSc)**

Signature: ____________________

Date: ____________________

Place: Addis Ababa, Ethiopia.