ADDIS ABABA UNIVERSTY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

BACTERIAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BLOOD CULTURE ISOLATES AT TIKUR ANBESSA SPECIALIZED HOSPITAL, ADDIS ABABA, ETHIOPIA

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Abbreviations

AST .....Antimicrobial Sensitivity Test

ATCC .....American Type Culture Collection

BSI .....Blood Stream Infection

CCU .....Coronary Care Unit

CLSI .....Clinical and Laboratory Standards Institute

CONS .....Coagulase Negative *Staphylococcus*

DIC .....Disseminated intravascular coagulation

DRERC .....Departmental Research and Ethics Review Committee

ESBL .....Extended Spectrum Beta-Lactamase

ICU .....Intensive Care Unit

MDR .....Multidrug-Resistant

MRCONS .....Methicillin Resistant Coagulase Negative *Staphylococcus*

MRSA .....Methicillin Resistant *Staphyloccus Aureus*

QC .....Quality Control

SD .....Standard Deviation

SOPs .....Operating Procedures

SPSS .....Statistical Package for Social Science

TASH .....Tikur Anbessa Specialized Hospital

TSI .....Triple Sugar Iron

WHO .....World Health organization

Abstract

Back ground: Bloodstream infections are major health problems that lead to morbidity and mortality of patients unless treated with appropriate antimicrobial treatment. Drug resistance of bacterial pathogens is challenging to alleviate sepsis. Blood culture is a gold standard technique which provides essential information for the diagnosis and proper medication.

Objective: The Aim of study was to assess bacterial profile and antimicrobial susceptibility pattern of blood culture isolates at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

Method: A cross-sectional study was conducted from April 2016 to September 2016. Using convenient sampling technique a total of 422 blood sample were collected from study participant who were suspected for blood stream infection. Bacterial isolates were identified by colony morphology, gram staining reaction, biochemical tests such as catalase, coagulase, optochin, PYR, triple sugar iron agar (TSI), citrate utilization, urease, motility, Indole were used for bacterial identification and susceptibility to antibiotics was done using Kirby Bauer disc diffusion technique method according to CLSI guidelines in Microbiology Laboratory of Tikur Anbessa Specialized Hospital. The data were analyzed by using SPSS version 20.

Result: About 66(15.6%) microbial were isolated. Out of this 64 (96.9%) were bacterial and 2(3.03%) were fungal (*Candida albicans*) isolates. The predominant bacterial isolates were *S.aureus* (22.72%) and *K.pneumoniae* (16.66%). Gram positive cocci were highly resistance to Penicillin (86.7%), followed by Ceftriaxone (46.7%), and 40% were MRSA.While clindamycin (80 %), erythromycin (73.3%), and Trimethoprim-Sulfamethoxazole (66.7%) were effective antibiotics. Most of gram negative rods were highly resistance to ampicillin (85.7%), amoxicillin clavulanic acid (77.14%), Ceftriaxone (75%), and Ceftazidimen (62.8%). Nevertheless amikacin (97.1%), meropenem (91.5%), and ciprofloxacin (77.1%) were sensitive antibiotic. Most of bacterial isolates (73.4%) were multidrug resistance to most frequently used antibiotics.

Conclusion: Overall, there was a high prevalence of blood stream infection with high resistance rate to the most commonly used antibiotics. Hence, timely investigation of blood stream infection and regular surveillance of antimicrobial resistance pattern is important to reduce morbidity and mortality due to blood stream infection.

Keywords: Blood stream infection, Bacterial profile, Antimicrobial sensitivity pattern

1. Introduction

1.1 Back ground

Blood stream infections cause significant mortality and morbidity worldwide [1,2]. BSIs are among the most common healthcare associated infections [3].That can lead to self-limiting infection to life threatening sepsis if not treated with appropriate antimicrobial treatment [4,5].The incidence of blood stream infections in patients has been associated with hospitalization, insertion of foreign bodies such as catheters into blood vessels, and other predisposing factors like ICU, lapses in hand washing, and non-adherence to infection control practices of medical staff. Genitourinary tract, intra-abdominal foci and respiratory tract are the common sources of blood stream infections [6,7].

In the United State, about 2 million patients per year acquire infections in the hospital; with mortality rates ranging from 20-50%. BSI accounts for 10-20% of all Nosocomial infections of these,4-5% BSIs were being fatal [8,7]. In sub-Saharan countries, including Ethiopia it is an important cause of illness and death. From which the mortality rate approaches 53% which makes it a significant health problem in developing countries [9].

A wide range of bacteria have been isolated in BSIs such as *K. pneumoniae, E.coli*, *Enterobacter* spp*, Salmonella* spp, *Citrobacter spp*, *Acinetobacter* spp, and *P. aeruginosa* were common gram negative bacteria isolates and gram positive bacteria includes; *S.aureus* ,Coagulase negative *staphylococcus* (CONS), *S.pneumoniae*, *S.pyogens*, *S.agalactiae,* and *E.faecium*. Among them, antibiotic resistant strains are emerging with great speed [10,11].Blood stream infection caused by gram negative bacteria are more severe than caused by gram positive bacteria [12]. Morbidity and mortality related to sepsis is high which need accurate detection of bacteraemia and fungemia . Blood culture remains the most practical and reliable method for diagnosis and management of blood stream infection though it is important to obtain blood cultures prior to starting empiric antibiotic therapy to optimize the chances of pathogen recovery [13, 14].

Organism isolated from blood culture varies according to geographical distribution. When blood stream infection is caused by multidrug resistant (MDR) bacteria ,it make therapeutic option

difficult and increase morbidity and mortality as well as leads to great economic loss since infection treated with more expensive antibiotics [15]. In almost all cases antimicrobial therapy initiated empirically before results of blood culture and antimicrobial susceptibility pattern of the isolates were available so making the right choice of empiric therapy is importance to reduce mortality and morbidity are associated with septicemia [16,17].

Periodic surveillance of antibiotic pattern is mandatory in each region; to provide effective empirical therapies, to be alert of the emerging pathogens, to develop rational prescribing practices, and to make policy decisions [6]. In Africa blood stream infection management is not as required due lack of bacteriological support needed for antimicrobial and background data needed for empirical treatment. As well as in Ethiopia there is elevated bacterial drug resistance due to the lack updated national guideline for antibiotic use, absence of good laboratory facilities to perform blood culture and antimicrobial drug susceptibility test [18,9].

1.2 Statement of the problem

Bloodstream infection (BSI) remains a global problem with high mortality and morbidity [1,12]. The problem is well pronounced in developing country. The disease may be short and self-limiting or may result in death, serious morbidity, and prolonged hospital stay in intensive care unit. The timely and appropriate use of antibiotics is currently the only way to treat blood stream infection. However, antimicrobial become resistance to many bacterial pathogens and become a serious public health concern with financial and social implications throughout the world [11, 16] .

Globally 31.5 million cases of bacteraemia and fungemia occur annually and 5.3 million people have died annually with mortality rates ranging from 20-50% [19,20,21]. Blood stream infection is the eighth leading cause of mortality, in the United States 17% of result in death [20,21]. It is much more in sub-Saharan countries including Ethiopia mostly in children, under 5 year old mortality rates of 100–250 per 1,000 compare with 10–30 per 1,000 in developed countries [12]. It is clear that persistent bacterial infections are a major contributor to this excess, with incidence rates confirmed to be much higher than those reported in developed countries [16,22].

Blood stream infection caused by multi drug resistance bacteria (MDR) become a serious public health problem due to economic and social implications all over the world. BSI result in prolonged hospital stay, higher cost and even death as compared to antibiotic susceptible bacteria [17,15]. In developing countries such as Ethiopia antibiotics resistance bacteria is a major problem [21]. Excess and arbitrary use of antibiotics as hit and trial method without proper sensitivity test ,unawareness of people about emergence of antibiotics resistance, unregulated sales of antimicrobials mostly for self-treatment without prescription lead to rapid spreading of antibiotic resistance bacteria in Ethiopia [20].

Currently, there are wide variations in bacterial drug resistance, while outcomes and reports of different studies in one region or in one period of time are not necessarily similar for other regions or periods of time though periodic observation of antimicrobial resistance pattern is vital in guiding physicians towards appropriate choice of antimicrobial agents to treat these blood stream infections, since it related with a series of social and environmental changes [6,23].

However, In Ethiopia few studies were conducted regarding bacterial profile and their antibiotic susceptibility patterns on the blood culture isolates. Most of the studies focused on blood stream infection caused by bacterial; therefore this study tries to determine BSI caused by both by bacteremia and fungemia/candidemia and antimicrobial susceptibility patterns of blood culture isolates at Tikur Anbessa Specialized Hospital, Addis Ababa.

1.3 Significance of the study

Knowledge of the distribution of blood stream infection and their drug susceptibility profiles:

- ✓ Increases the level of understanding on Blood stream infections and the common pathogens
- ✓ Provide updated information on susceptibility pattern of the isolates;
- ✓ Can be a source of information for policy makers or decision makers in this area;
- ✓ Health sector can design and implement preventive activities including expansion and strengthening of BSI prevention and monitoring.
- ✓ Can be used as a baseline for next studies in this area;

2. Literature Review

A study conducted in USA most frequently isolates were CONS (42.0%), *S.aureus* (16.5%), *Enterococcus faecalis* (8.3%), *E.coli* (7.2%), *K.pneumoniae* (3.6%), and *Enterococcus faecium* (3.5%) [24]. Also Studies in France, *E.coli, S.aureus* and CONS were the major isolate**.** Another Study in brazil shows, Most of the BSI episodes were caused by *Enterobacteriaceae* 46.5%, followed by non-fermentative gram-negative bacilli 34.6% [25,26].

A study done in Turkey, of these, 21.3% were positive, 22.8% were gram positive cocci 75.2% were gram negative bacilli and 2.0% were yeasts. From these 48.9% were CONS, 15.8% *S.aureus*, 3.1% *Brucella* spp [27]. Another Study done in Iraq by that Al-Charrakh et al., *Klebsiella* spp., *E.coli, S.aureus, Pseudomonas* spp., *Enterobacter* spp., *S. pyogenes, S.pnenmoniae, Enterococcus faecalis , Acinetobacter, Proteus* spp., *and Serratia marcescens* were isolated. *Klebsiella* spp. isolates were highly resistant to most of the antimicrobial agents studied. 59% to 83% of those isolates were resistant to pencillins (Amp, Amox), Cefotaxime, Gentamycin, Chloramphenicol and Trimethoprim-Sulfamethoxazole [28].

Based on study in Jordan, the most frequent pathogen found was *S.aureus* 86.2%, followed by *Klebsiella* spp. 9% [29]. Similar study done in Nepal by Shrestha et al. The overall incidence of bacteremia was 44.13%. The predominant gram posative isolate was *S.aureus* followed by CONS. In the gram negative *Klebsiella* was the most common followed by *Pseudomonas* and *Enterobacter*. *Klebsiella* had 87.5% and 78.3% resistance to ampicillin and gentamcin respectively. over all gram negative isolates were mostly sensitive to amikacin except *Pseudomonas* and *Acinetobacter* [30].

A retrospective study conducted at Qatar shows that gram positive organisms represented 66% while18% and 16% have developed fungal and gram negative sepsis respectively. All gram negative bacilli were sensitive to amikacin and meropenem. 21% of gram negative bacilli were multidrug-resistant [31]. Another Study conducted in Afghanistan by Tariq M, the frequency of positive blood culture was 12.2%.Out this 51.71% were gram-negative bacilli, 44.88% were gram-positive cocci and 14 (3.41%) *Candida species*. The predominant isolate was *Klebsiella* (16.1%), *Enterobacter* (10.2%) and *E.coli* (8.5%). Correspondingly, amongst gram-positive

cocci, the most frequently isolated species were *CONS* (26.34%) followed *S.aureus* (11.95%) and *Streptococcus* species (5.12%) [32].

According to study done in Pakistan, 16% shows growth, Out of these, 53% isolates were gram positive, 45% were gram negative while 2% isolates were yeast. *Staphylococcus* spp. was the leading pathogen followed by *Acinetobacter*, Pseudomonas spp., *E. coli* and *Salmonella* spp. *Enterobacteriaceae* revealed better susceptibility to amikacin (68.7%) and imipenem (64.3%) [33].

Based on study done by Sultana et al. in India, 61.75% and 35.90% were positive pediatric and adult respectively. *S.aureus* and *Klebsiella* species was the most common organism isolated in adult. Among the gram negative isolates the most common pathogen was *E.coli* 12 (14.29%), and the least common was *Candida albicans* (1.19%) isolated from adult blood cultures [34].

A Retrospective analysis by Latif et al. in Pakistan, total of 1824 blood cultures sample was collected from pediatrics/ neonatology wards and adult patients, 27.9% were culture positive . Of these, 97.6% bacterial isolates and 2.4% *Candida Spp* isolated. The most common organism was *Klebsiella* Spp. and *S.aureus*. 31.25% *S.aureus* isolates were resistant to Oxacillin, 93.7% of *Klebsiella Spp*. 6.49% of *Pseudomonas Spp*. and *Acinetobacter Spp*. Were resistant to Carbapenems [35].Similar study in India done by Manjunath et al. 216 microorganisms were isolated, out of which 19.94% were bacterial isolates and 0.955 were fungal isolates. Gram negative bacteria accounted for 74.09% cases with *Burkholderia cepacia* [37.5%] predominant, followed by *E.Coli*. Gram positive cocci accounted for 14.35% cases with *S.aureus* (80.64%) predominant, followed by *Enterococcus faecalis* (9.67%) [36].

Another study conducted by Usman et al. in Pakistan, 14.39% were culture positive, of these gram negative isolates were predominant with 68.42%. *S.aureus* followed by CONS *S.typhimurium*, *P.aeruginosa* , *E.coli* , and *K.pneumoniae* were common isolate [37].Another study done in Nepal by Karki et al., shows positive blood culture was 4.2%. Out of them, 269 65% were positive for *S.aures,* 29.3% E coli, 13.1% *K.pneumoniae*, 1.4% *S.pneumoniae* and 1.2% *S.viridence*. *S.aureus* was found most sensitive to chloramphenicol (88.8%) followed by amikacin (87.5%), ofloxacin (76.5%), ciprofloxacin (72%) and least sensitive to ampicillin, cloxacillin and penicillin. E.coli was found the most sensitive to amikacin (74.7%) followed by

ofloxacin (69.9%), ciprofloxacin (56.4%) and the least sensitive to cephalexin, gentamycin and ampicillin [38].

According to study done by Mustefa et.al in India, the most common isolated were *K. pneumonia 35%* followed by *S.aureus 24.1%, E.coli 22.5%,* CONS 11.2%, and *P.aeruginosa* 6.4%. Gram positive isolate were resistance to ampicillin 13.6% and gentamicin 45%, they showed moderate susceptibility to cefotaxime 73%, ceftriaxone 68%, cefoperazone 63%, amikacin 68%, and ciprofloxacin 63%. Among the gram negative bacteria, many of them were resistant to ampicillin (7.5%), gentamicin (45%) and third generation cephalosporins. They were moderately susceptible to amikacin (68%) and ciprofloxacin (70%), but highly susceptible to 100% meropenem [39].

A Cross sectional study carried out at India, Out of the 280 clinically diagnosed cases of bacteremia, 47(16.78%) was culture positive. Gram negative bacteria constituting 23/47 (51.06%) while gram positive isolates were 23/47 (48.93%). *Salmonella Typhi* (29.78%) was the most common organism, followed by CONS (27.65%), *S.aureus* (19.14%), *E.coli* (10.63%), *and Acinetobacter* spp (6.38%). This study showed that vancomycin, imipenem, gentamicin and ceftriaxone are the most effective antibiotics against most of the bacterial isolates [40].

According to study conducted in Zanzibar by Onken A et.al, reported around 14 % (66/469) pathogenic bacteria were isolated. The most frequently isolated microbes were *K.pneumoniae, E.coli, Acinetobacter spp. and S.aureus*. Infections were community-acquired in 56 patients (85%) and hospital-acquired in 8 (12%) [41]. Study done in Saudi Arabia Al-Towfiq et al., gram negative bacilli accounted for 46%, gram positive cocci constituted 48.7% and fungemia represented 5%. CoNS 23.7% followed by *S.aureus* 11.1% and *E.coli* 11.1%.Methicillin resistance was detected in 22% of *S.aureus* isolates and 71% of CONS isolate [42].

According to study done in Nigeria by Nwadioha et al. giving an incidence of 13.1% positive culture. There were 58.4% gram negative bacilli and 41.6% gram positive cocci. Bacteria isolated were *S.aureus* 34% and *Klebsiella* species 22% were common isolates. There was a (35%) higher occurrence of septicemia in neonates than in any other age groups in the hospital. Gram-positive organisms showed a higher antibiotic sensitivity ranging from 14% - 100% than the gram-negative bacteria (11% -80%) [43]. Similar study done in Tanzania, bloodstream

infection was 13.9%. The most frequent isolates were *klebsiella, salmonella, E.coli*, *Enterococci* and *S.aureus*. The mortality rate from gram-negative bloodstream infection (43.5%) was more than double that of malaria (20.2%) and gram-positive bloodstream infection (16.7%) [44].

A study conducted in south Africa, gram negative pathogens predominated (60% vs 33% Gram positives and 7% fungal); *K.pneumoniae(*154; 17%), *S.aureus* (131; 14%) and *E.coli* (97; 11%) were most prevalent [45]. Another study in south Africa shows, gram-positive 54.9% and gram negative 45.1%, most common isolate was CONS 19. 1%,followed by *K. pneumoniae* [46].

A cross sectional study was conducted in Jimma , Ethiopia with a culture positivity rate of 15.8 %. From which 53.3 % were for gram positive bacteria and 46.7 % for gram negative bacteria, the predominant isolates were *S. aureus* and *E. coli.* The ranges of resistance for gram positive isolates were from 0 % – 100 %. MDR was observed in 80 %. Of which 87.5 % were from gram positive and 71.4 % were gram negative bacteria [21].

A Cross sectional study conducted in Mekelle, Ethiopia 28% culture positive was isolated. *S.aureus* 37.5%, CONS 30.6%, *E.coli* 3.1%, *Citrobacter* spp. 1.7% and S.*typhi* 1.6% were the most dominant isolates. Antimicrobial resistance pattern for gram positive and gram negative bacteria was 0–83.3% and 0–100%, respectively. Multi drug resistant was 59%. Most bacterial isolates were sensitive to gentamicin, ciprofloxacin and amoxicillin clavulanic acid [9].

According to Study conducted in Gonder, Ethiopia the overall prevalence of bacteria isolate was 18.2%.The predominant bacteria isolate were CONS 42.3%, followed by *S.aureus* 23.9% and *Klebiesella* spp. 12.7%. The gram positive and gram negative bacteria constituted 69% and 31% of the culture isolates respectively. Antimicrobial resistance levels for the gram-negative were 20 to 100% and for gram positive bacteria 23.5% – 58.8% [20].

3. Objectives

3.1 General objective

- To assess bacterial profile and antimicrobial susceptibility patterns of blood culture isolates at Tikur Anbessa Specialized Hospital, Addis Ababa.

3.2 Specific objectives

- To assess bacterial profile of blood culture isolates.
- To determine drug susceptibility patterns of bacteria isolate

4. Materials and Methods

4.1 Study area

The study was conducted at Tikur Anbessa Specialized Hospital which is found in Addis Ababa, Ethiopia. Tikur Anbessa Specialized Hospital is now the main teaching hospital for both clinical and preclinical training of most disciplines. It is also an institution where specialized clinical services that are not available in other public or private institutions are rendered to the whole nation. It provides tertiary level referral treatment and is known to be open 24 hours for emergency services. The hospital is estimated to offer diagnosis and treatment for approximately 370,000-400,000 patients yearly with 800 beds, 200 doctors, 379 nurses and 115 other health professionals dedicated to providing health care services. The various departments, faculties and residents under specialty training in the School of Medicine provide patient care in the hospital. The hospital also has 950 permanent and contract administrative staff to support the hospital activities.

4.2 Study design and period

A cross-sectional study was conducted at Tikur Anbessa Specialized Hospital from April 2016 to September 2016.

4.3 Source Population

All admitted patients suspected of blood stream infection at Tikur Anbesa Specialized Hospital during the study Period was source population.

4.4 Study Subjects

All admitted patients suspected of blood stream infection at Tikur Anbessa Specialized Hospital during the study period.

4.5 Eligibility criteria

4.5.1 Inclusions criteria

- All patients suspected of blood stream infection

- All patients who gave blood sample and patients, parents or guardian volunteer to give consent and ascent to participate on the study.
- Patients not take antibiotic

4.5.2 Exclusions criteria

- Patients who had received antibiotics
- Out patients

4.6. Sample size determination and Sampling technique

4.6.1. Sample size determination

422 clinical samples were collected from April 2016 to September 2016. Therefore all willing patients who had blood stream infection were included in this study within the time allocated.

The sample size is calculated using the single population proportion formula

$$n = \frac{Z\alpha^2 P (1- P)}{d^2}$$

Where:

n is minimum required sample

Z is Z-score at 95% CI (1.96)

P is population proportion (50 %)

d is margins of error 5% (0.05)

$$n = \frac{(1.96)^2 (0.5 (1-0.5)}{(0.05)^2} = 384$$

Thus, the study was including 384 subjects, but Assuming 10 % non-response rate, the sample size was: n=384+10 %=384+38= 422

4.6.2. Sampling technique

Convenient sampling technique was used

4.7. Study variables

4.7.1 Dependent Variables

- Bacterial and *Candida* isolates
- Antimicrobial susceptibility pattern

4.7.2 Independent Variables

- Socio demographic factors; age and sex

4.8 Data collection and processing

4.8.1 Sample collection and transportation

About 10 ml of adults, 5 ml for children and 1m1-3ml of neonate blood were collected from different wards and critical care unit from suspected patients by nurses and clinician under strict aseptic conditions using 70% alcohol and povodine iodine, bottles were labeled with patent's identification number and date of collection. blood was transferred to blood culture bottle containing 90ml of brain heart infusion broth for adult and 45 ml for children and neonate (1:10 dilution) [43]. Transported to Tikur Anbessa Specialized Hospital Microbiology Laboratory with minimal delay (15 minutes).

4.8.2 Culture and identification

All blood cultures were processed in Laboratory using standard procedure by conventional method. Blood culture bottle was incubated aerobically at 37˚C for 24hr then sub-cultured. Blood culture bottle which shows no sign of growth was further incubated at 37˚C then checked for sign of growth (turbidity, haemolysis, clot formation) daily up to 7 days. Bottles which showed signs of growth was sub-cultured on Blood agar, Chocholate agar, MacConkey agar, and Sabouraud dextrose agar (all Oxoid Ltd, UK). The Blood agar , MacConkey agar and Sabouraud dextrose ager plates was incubated in aerobic atmosphere. whereas chocolate agar was incubated

in a carbon dioxide atmosphere using a candle jar at 37°C for 24 to 48 hrs. Blood culture broths with no microbial growth after 7 days were sub-cultured for 48hrs before being reported as a negative result. Bacterial isolates were identified by colony morphology, gram staining reaction, biochemical tests using catalase test, coagulase test, optochin, PYR, triple sugar iron agar (TSI), citrate utilization test, urease test, motility, indole test using the standard procedure for bacterial identification. Yeasts (*candida*) were identified by colony morphology on Sabouraud dextrose agar and germ tube test [47].

4.8.3 Drug Susceptibility Patterns

The *in vitro* susceptibility patterns of bacterial isolates were performed based on CLSI guideline. Suspension was prepared using a sterile wire loop; 3-5 pure colonies were picked and emulsified in nutrient broth standard inoculums were adjusted to 0.5 McFarland and swabbed onto Muller-Hinton agar. While for *S.pneumoniae* Muller-Hinton agar with 5% sheep blood is used. Drug susceptibility testing of all bacterial isolates was performed using Kirby Bauer disk diffusion method and incubating at 37^0C for 18-24 hours. The zone of inhibition was measured to the nearest millimeter and isolates were classified as sensitive, intermediate and resistant according to the standardized table supplied by CLSI. The following antibiotics which are currently recommended by CLSI version 2015 were tested such as Ampicillin(10µg), Amoxicillin-clavulanic Acid (10µg), Penicillin (10µg), Gentamicin (10µg) ,Tobramycin (10 µg), Erythromycin (15µg), Clindamycin (2µg) ,Trimethoprim-sulphamethoxazole (1.25/23.75µg), Vancomycin(30µg) Ceftriaxone(30µg), Cefoxitin(30µg), cefotaxime(30µg), Ciprofloxacin(5µg), Ceftazidime (30 µg), oxacillin (1µg) Amikacin (30µg), and Meropenem (10 µg) [48].

4.8.4 Quality Control

To maintain the quality from sample collection up to final laboratory identification and data management the standard operating procedure of sample collection and laboratory analysis were followed strictly. All the equipment was checked for their functionality. The prepared culture media were checked for sterility by incubating the five percent of prepared media for overnight and observe for the presence of any growth. Abilities of the prepared media supporting the growth of organisms were checked by inoculating control strains. We also used these control strains before we performed culture and sensitivity tests in the whole process of this study. The

known control organisms used were *S.aureus* ATCC 25923, *E.coli* ATCC 25922 and *Pseudomonas* ATCC 27853

4.8.5 Operational definition

Multidrug resistance – bacterial isolate resistant for two or more classes of antibiotics tested.

Resistant – bacterial isolates that are not inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used.

Susceptible – bacterial isolates that are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection.

4.8.6 Data quality Assurance

Socio-demographic characteristics of the patient were collected appropriately after getting consent and ascent. Samples were collected in accordance with SOPs. Culture results were recorded before entry to statistical tool. Before analysis the data was checked.

4.8.7 Statistical analysis and interpretation

Collected data was processed, edited, and analyzed using *SPSS version 20* (Statistical Package for social sciences, SPSS) statistical. During analysis frequencies of the different variables was determined, cross- tabulations; chi-square test, and binary logistic regression were used for statistical analysis of data. A 'P' value less than 0.05 was considered as statistically significant.

4.9 Ethical consideration

Before starting the research work, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences. Permission letter was also obtained from the study site. All eligible subjects were informed as their participation was voluntary and as the aim of this study is only to collect necessary information which is helpful to assess prevalence blood stream infection and participant results were

provided. All the information obtained from the study subjects were coded to maintain confidential.

4.10 Dissemination of results

After conducting the research, the results of the study was submitted to Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences. Therefore, it can serve as a reference in the library. In addition, it will be presented for annual conferences of professional societies and other concerned bodies. The finding of the study was also being presented to the medical scientific community and manuscript will be submitted to peer reviewed journals for publication.

5. Result

Socio-demographic characteristics

A total of 422 blood samples were collected from patients suspected for blood stream infection from different wards of TASH. The socio-demographic characteristic of study participants is summarized in as shown in **(Table 1)**. Out of 422 patients, 236(55.9%) were male and 186(44.1%) were female. The mean age of the study participants was 16.63 years SD (+17.69%). Majority of participants 191 (45.3%) were aged between 1-14 years. In our study, most of the patients who developed sepsis were females 36(54.5 %) even though there were no statically significant difference (OR= 1.68, 95% CI =0.972-2.79, P = 0.064). The range of bacteria varies with the age of patients. High proportion of bacteria was isolated from children (47.0%). However, it was not statistically significant (COR=2.034, 95%CI=0.454-9.12, P = 0.354)

Table1 :- Association of socio-demographic variables with blood culture results at Tikur Anbessa Specialized Hospital from April 2016 – September 2016

Culture result (%)					
Variable		Negative	Positive	COR(CI;95%)	P-value
Age	<1 years	49(13.8%)	12(18.2%)	2.571 [.529-12.50]	0.242
	1-14 years	160(44.9%)	31(47.0%)	2.034 [0.454-9.122]	0.354
	15-24 years	57(16.0%)	7(10.6%)	1.289 [0.248-6.709]	0.763
	25-44 years	57(16.0%)	14(21.2%)	2.57 [0.540-12.31]	0.235
	45-64years	21(5.9%)	2(3.0%)	1	
	>65 years	12(3.4%)	0(0.0%)		
Gender	Female	150(42.1%)	36(54.5%)	1.68[0.972-2.79]	0.064
	Male	206(57.9%)	30(45.5%)	1	

*WHO age classification standard [49].

1-Reference category, COR-Crude Odds Ratio, CI- Confidence Interval

Microbial isolates

Among 422 blood sample collected from patients suspected of blood stream infection 66 (15.6%) were found to be culture positive. All infections were mono microbial. Out of this 64 (96.9%) were bacterial isolates and 2(3.0%) were yeast *(Candida albicans)* isolates. Gram positive and gram negative bacterial isolates constitutes 29/66 (44 %) and 35 /66 (53.0%) respectively. As it is shown on (**Figure 1).**

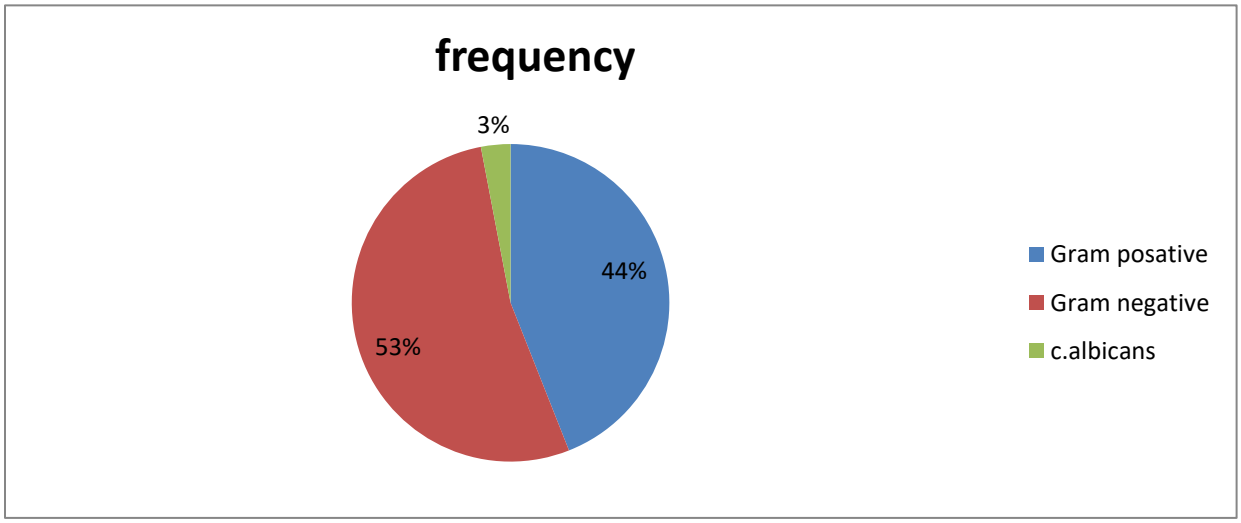

Figure 1:- Frequencies of Microbial isolates from blood cultures of patients attending TASH from April to September 2016**.**

Among gram positive *S.aureus* were predominant bacterial isolate followed by *CONS* , *Enterococcus* spp , *S.pneumoniae* Whereas from gram neg*ative* bacteria the predominant isolate were *K.pneumoniae,* followed by *Acinetobacter* spp, *Enterobacter cloacae,E.coli* , *P.aeruginosa* , *K.oxyotica, citrobacter* spp , and *Serratia marsenses.* Frequency of microbial isolates shown on (**figure 2**).

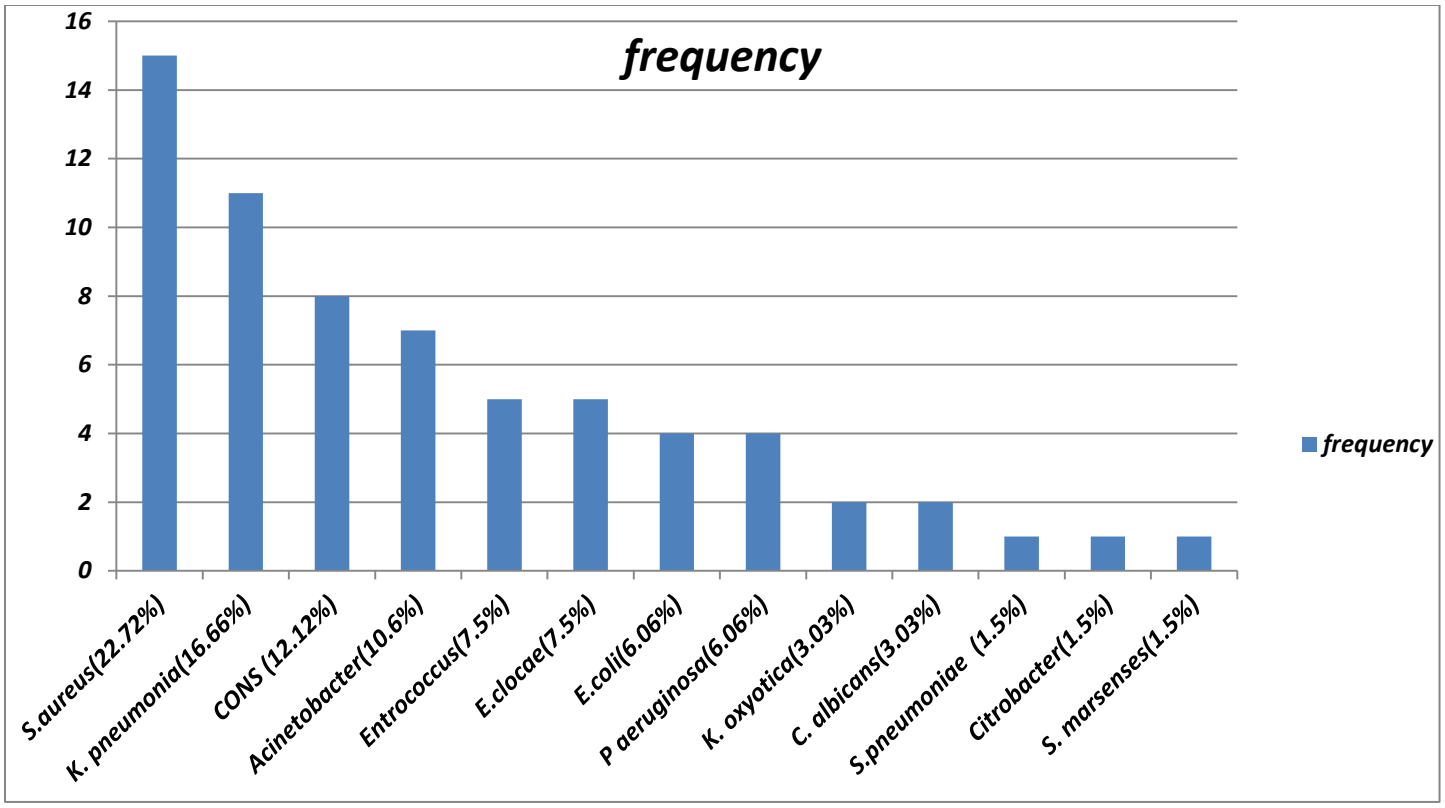

Figure 2:- A bar graph shows frequency and types of microbial isolates from blood cultures of patients attending TASH from April–September 2016.

In age group of <1years *S. aureus* 4(26.7%) and *K. pneumoniae* 4(36.4%) were most isolated bacteria. *S.aureus* was the commonest bacteria in the age group of 1-14 years 7(46.7%) followed by CONS 4(50%) and *K. pneumoniae* 4(36.45%). In age groups of 14-24years and 25-44 years *Acenetobacter* spp 2(28.6 %) and 3(42.9%) respectively was the dominant pathogen isolated. On the other hand the two *Candida albicans* were isolated form age group of <1 years and 14-24 years one each. Age group >65 years was not affected. Overall highest number of bacteria were isolated from age group 1-14 years 31(47%) followed by the age group 25-44 years 14(21.2%). Age wise distributions of microbial isolate are shown on **(Table 2**).

Table 2:-Age wise distribution of microbial isolates at TASH, from April 2016-September 2016

Isolated Microbes	**Age category**				
	<1years	1-14years	14-24years	25-44years	45-64years
S.aureus	4(26.7%)	7(46.7%)	1(6.7%)	3(20.0%)	0
CONS	1(12.5%)	4(50%)	0	3(37.5%)	0
Enterococcus spp	0	3(60%)	1(20)	1(20%)	0
S.pneumoniae	0	1(100%)	0	0	0
K. pneumonia	4(36.4%)	4(36.45)	1(9.1%)	2(18.2%)	0
Enterobactercloacae	0	2(40%)	1(20%)	1(20%)	1(20%)
E.coli	0	3(75%)	0	1(25%)	0
K.oxyotica	0	2(100%)	0	0	0
Citrobacter spp	0	1(100%)	0	0	0
Serratia marsenses	1(100%)	0	0	0	0
P.aeruginosa	1(25.0%)	2(50.0%)	0	0	1(25%)
Acinetobacter spp	0	2(28.6%)	2(28.6%)	3(42.9%)	0
C.albicans	1(50%)	0	1(50%)	0	0
Total =66	12(18.2%)	31(47.0%)	7 (10.6%)	14(21.2%)	2(3.0%)

Antimicrobial susceptibility pattern

The susceptibility pattern of bacteria isolates (n=64) was done using CLSI 2015 guideline. The range of resistance for gram positive isolates was from 26%-87.5%, presented in **(Table 3**). The highest degree of resistance was seen in *S.aureus* for Penicillin (86.7%) followed by ceftriaxone (46.7%). From a total of 15 *S. aureus* 6 (40%) were MRSA (Methicillin Resistant *S. aureus*) cefoxitin disc used. On other hand CONS were 100% resistance to penicillin, ceftriaxone (75.0%) and 50% was MRCONS (Methicillin Resistant Coagulase Negative *Staphylococcus*). *Enterococcus* spp isolates were resistance to ampicillin (80%) and vancomycin (60%). *S.pneumoniae* was 100% resistance to Trimethoprim-Sulphamethoxazole. While Clindamycin (80%), Erythromycin(73.33%) and Trimethoprim-Sulphamethoxazole (66.6%) was susceptibility to *S.aureus*.

Antimicrobial resistance levels for the gram negative organisms, ranged from 2.85% to 85.7% Over all most of gram negative isolate was highly resistance to ampicillin (85.7%), and amoxiciln-clavulanic acid (77.14%), ceftriaxone (75%), and Ceftazidimen (62.8%). While amikacin (97.1%), meropenem (91.4%), ciprofloxacin (77.1%), and gentamicin (62.86%) were effective antibiotic for gram negative bacterial isolates. However, *P.aeruginosa* and *Acinetobacter* spp were resistance to meropenem (25.0% and 28.65%) respectively. *Entrobactercloacae* were resistance to Amikacin (20%), as shown on, **(Table 4)**.

Table 3:- Antibiotic susceptibility pattern of gram positive bacterial isolates from blood culture of patients attending TASH, from April to September2016

Bacterial Isolates	Antibiotics									
	P	CRO	CXT	VA	CN	E	DA	SXT	P	AMP
S.aureus (n=15)	S	53.33	60.0	NT	60.0	73.33	80.0	66.66	13.33	13.33
	R	46.7	40.0	NT	40.0	26.7	20.0	33.34	86.7	86.7
CONS (n=8)	S	25	50.0	NT	100	62.5	62.5	37.5	0	0
	R	75	50.0	NT	0	37.5	37.5	62.5	100	100
Enterococcus(n=5) *Spp*	S	NT	NT	40.0	NT	NT	NT	NT	NT	20.0
	R	NT	NT	60.0	NT	NT	NT	NT	NT	80.0
S.pneumoniae (n=1)	S	100	NT	NT	NT	100	100	0	100	100
	R	0	NT	NT	NT	0	0	100	0	0
Total (29)	S	45.9	56.52	40.0	75.0	70.9	75.0	54.2	12.5	13.8
	R	54.1	43.47	60.0	25.0	29.1	25.0	45.80	87.5	86.2

Where: CRO:Ceftriaxon; CXT: Cefotixin; VA:Vancomycin; CN:Gentamicin; E:Erythromycin, DA: Clindamycin; SXT:Trimethoprim-Sulphamethoxazole; P:Penicillin;AMP:Ampicillin CONS,Coagulase negative *Staphylococcus*, NT; Not tested

Table 4:- Antibiotic susceptibility pattern of gram negative bacterial isolates from blood culture of patients attending TASH, from April-September 2016

Bacterial Isolated	Antibiotics										
	P	CTX	AMP	AMC	CRO	AK	MEP	CAZ	CIP	CN	TOB
K.pneumoniae (n=11)	S	18.2	0.0	9.1	18.2	100	100	27.3	90.9	54.5	45.5
	R	81.8	100	90.9	81.8	0	0	72.7	9.1	45.5	54.5
Enterobacter cloacae(n=5)	S	20.0	20.0	40.0	20.0	80.0	100	60.0	80.0	40.0	80.0
	R	80.0	80.0	60.0	80.0	20.0	0	40.0	20.0	60.0	20.0
E.coli(n=4)	S	50.0	25.0	25.0	50.0	100	100	75.5	75.5	100	75.0
	R	50.0	75.0	75.0	50.0	0	0	25.0	25.0	0	25.0
K.oxyotica (n=2)	S	50.0	0	0	50.0	100	100	0	0	100	50.0
	R	50.0	100	100	50.0	0	0	100	100	0	50.0
Citrobacter (n=1)	S	0	0	0	0	100	100	0	100	100	100
	R	100	100	100	100	0	0	100	0	0	0
Serratia marsenses (n=1)	S	0	0	100	0	100	100	0	100	0	100
	R	100	100	0	100	0	0	100	0	100	0
P.aeruginosa (n=4)	S	NT	25.0	50.0	NT	100	75.0	50.0	100	75.0	50.0
	R	NT	75.0	50.0	NT	0	25.0	50	0	25.0	50.0
Acinetobacter (n=7)	S	NT	28.6	14.3	NT	100	71.4	28.6	57.1	57.1	57.1
	R	NT	71.4	85.7	NT	0	28.6	71.4	42.9	42.9	42.9
Total (35)	*S*	25.0	14.3	22.85	25.0	97.15	91.5	37.5	77.1	62.86	60.0
	R	75.0.	85.7	77.14	75.0	2.85	8.5	62.8	22.9	37.14	40.0

Where: CTX:Cefotaxime; AMC:Amoxicillin-Clavulanic acid; AK:Amikacin; MEP: Meropenem; CIP: Ciprofloxacin; TOB:Tobramycin; CAZ: Ceftazidimen.

Multidrug resistance level of bacterial isolates

In this study, the overall prevalence of multidrug resistance (bacteria resistant for two or more antibiotics tested) was 73.4%. However, 7.8% bacterial isolates were sensitive for all antibiotics tested. Among gram negative bacteria 31/35 (88.5%) and gram positive bacteria 16/29 (55.1 %) was MDR. Among gram positive bacteria CONS (100%*)* and *S.aureus* (46.6%) were multidrug resistance. Among gram negative bacteria *Enterobactercloacae, K.pneumoniae* (100 % each), *E.coli, P.aeruginosa* (75% each), and *Acinetobacter* spp (71.42%) were MDR. High level of resistance to different antibiotic particularly to ampicillin in almost all gram negative bacterial isolate were observed **(Table 5)**. *K.pneumoniae* 3(27.2%), *Acinetobacter* spp 2(28.57%), *S.aureus* 3 (20 %), and *K.oxyotica* 2(50%) were resistance for seven antibiotics tested

Table 5:- Multiple antibiotics resistance pattern of bacterial isolates from blood culture of patient at TASH, 2016

Bacterial Isolate	**Resistance antibiotics (%)**						
	R1	R2	R3	R4	R5	R6	R7
S.aureus (n=15)	7(46.)	0(0)	2(13.3)	1(6.6)	1(6.6)	0(0)	3(20)
CONS (n=8)	0	3(37.5)	1(12.5)	2(25	2(25)	0	0
Enterococcus (n=5)	3(60)	1(20)	0	0	0	0	0
S.pneumoniae (n=1)	1(100)	0	0	0	0	0	0
K.pneumoniae (n=11)	0	1(9.1)	0	3(27.2)	2(18.2)	2(18.2)	3(27.2)
*Enterobactercloacae (*n=5)	0	0	1(20)	3(60)	0	1(20)	0
E.coli (n=4*)*	0	0	0	2(50)	1(25)	0	0
K.oxyotica (n=2)	0	0	0	1(50)	0	0	1(50)
Citrobacter (n=1)	0	0	0	1(100)	0	0	0
Serratia marsenses (n=1*)*	0	0	0	1(100)	0	0	0
P.aeruginosa (n=4)	0	1(25)	0	2(50)	0	0	0
Acinetobacter (n=7)	1(14.28)	0	2(28.57)	0	0	1(14.28)	2(28.57)
Total (59)	12(18.75	6(9.37)	6(9.37)	16(25)	6(9.375)	4(6.25)	9(14.06)

R1-resistant for 1 antibiotic, **R2**- resistant for 2 antibiotics, **R3**- resistant for 3 antibiotics, **R4**- resistant for 4 antibiotics, **R5**- resistant for 5 antibiotics **R6** resistant for 6 antibiotics **R7** resistant for 7 antibiotics.

6. Discussion

Blood stream infection is the main cause of morbidity and mortality. Infection with antibiotic-resistant bacteria has made the therapeutic options difficult. Antibiotic use and misuse in humans, animals and agriculture. In addition, clustering, and overcrowding, and poor infection control were reason for emerging antibiotic- resistance bacterial strain [12,15].

In present study the isolate rate was 66/422 (15.6%) which is similar with reports in Jimma 15.8% [21], Zanzibar 14% [41], Pakistan 16% [33], Nigeria13.1% [43] ,and Dhaka 14.38% [50]. However, higher than another study conducted Jimma 8.8% [11] ,Nepal 7.28% [15], India (8.3%,9.25%) [6,17],and Iran 5.6% [23]. It was, lower than from study in Mekelle 28% [9], Gonder 18.2% [20], India 22.3% [7], Nigeria 19.3% [8],Turkey 21.3% [27] ,and Lebanon 18.6% [51]. Possible explanation for the difference in prevalence rate of BSI between regions and countries could be due to variation number of study population , variation in study design and method [17,9].

In our study, 53.0% of BSI was caused by gram negative bacteria and 44% were by gram positive bacteria pathogen. This finding is agreement with previous studies done in Addis Ababa 46.4% and 51.8%, and Afghanistan 44.88% & 51.71%, accounted for gram positive and gram negative bacteria respectively [14,32] Similarly, other studies reports gram negative bacteria as commonly isolate but higher prevalence than our finding; Iran (13.5% ,86.5%), Nigeria (22.9% , 77.1%), Nepal (7.28%,78.69%) , and Turkey (22.8% , 75.2%) were gram positive and gram negatives respectively, [3,8,15,27]. This study finding dissimilar with other studies were gram positive bacteria were the predominant isolate such as Mekelle, Gonder ,and Jimma [9,20, 21]. This difference may be due Summer season (seasonal variation) and temperature are associated with substantially increased frequency of BSIs, particularly among clinically important gram negative bacteria [52].

In other studies the incidence of Candidemia was much higher 11.1% [7]. While in present study 3.0% of positive blood culture gave growth of *C.albicans* which, in accordance with other studies in India 4.15%, Turkey 2.0%, Afghanistan 3.41%, and Lahore 2.4% [6,27,32,35]. Difference in prevalence could be due to the underlying condition of patients, variation of

etiologic agents, geographical locations, epidemiological difference of the etiological agents, and variation in study design.

The commonly isolated bacteria from our study includes: *S.aureus* 22.72% ,CONS 12.12%, *S.pneumoniae* 1.5%, *Enterococcus* spp 7.5%, *K.pneumoniae* 16.66%, *Acinetobacter* spp10.6%, *Enterobactercloacae 7.5%,E.coli,*and *P.aeruginosa* (6.06%each), *K.oxyotica* 3.03%, *Citrobacter* spp, and *Serratia marsenses* (1.5% each). More or less similar observations have been made in study done in different part of Ethiopia and among other countries however, the proportion and occurrence of the organisms were varied. Jimma; S.aureus 40%, CONS 13.3% , *E. coli 13.3*, *K. pneumoniae*, *P.aeruginosa ,Salmonella species, Enterobacter spp* and *Citrobacter spp* (6.7% each) were isolated [21], Addis Ababa*; S.aureus* 23.2% ,CONS 19.6%, *Enterococcus spp* and ,*Streptococcus spp* (accounts only one isolate each), *Serratia marcesence* 21.4% *Klebsiella spp*16%,*Salmonella* 5.4% and *Enterobactercloacae* 3.6%, E.coli, Acinetobacter, *Pseudomonas spp* (one isolate each) [14], India; *S. aureus* 72%, *Enterococcus faecalis* 22.1%, *Streptococcus* 5.5%, *E.coli* 35.6% *Salmonella typhi* 25.7%, *K.pneumoniae* 14.0%, *P.aeruginosa* 8.7%, *Acinetobacter* 12.2%, *Enterobacter* spp 33% were common isolates [6].

In our current study we found all cases of blood stream infections were due to mono microbial which in lines with earlier reports by Dagnew et al. and Nwadioha et al.[20,43] Unlike to our study; septicemia of poly-microbial etiology were reported by Dramowski et al., and Ballot et al. [45,46]

Among gram positive isolates *S. aureus* was the predominant isolate accounts 22.72%. This result is in accordance with the findings obtained in other study even if there is prevalence difference. USA 22.8%, Mekelle 37.5%, Cameron 20.9%, Addis Ababa 21.1%, India 47.7%, Jimma 40%, French 9.5 %, and Nepal 65% [2,9,12,14,16,21,20, 25,38], and dissimilar to the other studies that report CONS as major isolate [5,11,20,24]. In most studies CONS were considered contaminant, but now they are potentially important pathogens and their increasing incidence has been recognized. In recent years, CONS have become the major cause of nosocomial bloodstream infections to some extent as results of the increasing use of intravascular devices and increased number of hospitalized immune compromised patients [14,21].

Among gram negative isolate the predominant isolates were *K. pneumoniae* 16.66%,this finding is in agreement with other studies in Negeria 22% [43], Afghanistan 16.1% [32], South Africa 12.1% [46] and Iran 33.5% [3].However different from previous finding in Addis Ababa which report second isolate were *Serratia marcesence* [14]. The difference is due to variation in age group of study participant. Since, their participant were children (≤12 years) so these bacteria emerging as nosocomial pathogen in the area of NICU. Furthermore, other studies report predominant isolate were *E.coli* [6,9,21,19] *P.aeruginosa* [4], *S.paratyphi A* [15], *S. Typhi* [23,40,51], *Acinetobacter* spp [33], and *Providencia* spp [38]. In our findings there were isolates of *Enterococcus* spp, and *S. pneumoniae*. Unlikely, other study done in other parts of Ethiopia report *S.pyogenes*, and *Salmonella species* were isolated [20,11]. The probable justification for these differences could be, geographical location, and difference of the etiological agents, and seasonal variation

Overall the resistance range of gram positive bacteria was from 26 to 87.5%, whereas from 2.85 to 87% to gram negative which unlike from other study done in different part of Ethiopia and other country ; Jimma 0–85.7% and 0–100% , Gonder 23.5–58.8% and 20–100%, another study in Jimma 0-100 and 14.3 - 85.7 % and Nigeria 14% to 100% and 11% to 80% for gram positive and negative respectively [11,20, 21, 43]. The increased resistant blood isolates may due to continuous use of commonly available antimicrobials, this could challenge the management of patients very complicated [11].

We observed pencillin (87.6%) resistance to S.*aureus*. which was similar to other studies, in Iran, Jimma , French, Afghanistan, and Nigeria [3,11,25,32,43]. High pencillin resistance was seen in many part of the word, probably due to indiscriminate use of antibiotics [10], and production of B-lactamase enzyme by bacteria. *S.aureus* were resistance to ceftriaxone (46.7%), which comparable with other studies conducted in Addis 46.2% [14] and Mekelle 57.4% [9]. However, in contrast with other findings [21,1129]. Besides, 40% of S.*aureus* were MRSA (Cefoxitin disc used). More or less similar observation was seen in other studies done, India (33.3 %), Addis Ababa 38.5%, India70.6% Jimma (33.3%), Turkey 60%,and Afghanistan 51% [6,14, 17,21, ,27,32] were MRSA. This could be due to the indiscriminate use of these drugs especially in hospitals as an emergency empirical therapy [7]. *S.aureus* was sensitive for clindamycin (80%), erythromycin (73.3%), and Trimethoprim-Sulfamethoxazole (66.7%).

More or less Comparable with pervious study done by Negussie et.al., [14] Rajeevan et.al. [19], and Usman et.al. [37]. However, dissimilar with other finding done by Wasihun et al. [9] and Dagnew et al. [20].

In current study, most gram negative isolates were resistant for ampicillin, amoxicillin-Clavulanic acid, ceftriaxone, and Ceftazidimen 85%,77.1%,75%,62.8% respectively, which is in accordance with the study findings in Jimma and Nigeria ampicillin (82.6% and 88.9%) respectively, India ampicillin (75%) and cephalosporins (ceftazidime-55%, cefotaxime-50%, ceftriaxone-45%) were resistance antibiotic [11,8,39]. In contrast to other study in Mekelle and Jimma Amoxicillin clavulanic acid was reported effective against gram negative isolate [9,21]. It's may be due to antimicrobial resistance pattern varies by geographical location between country [15]. Whereas Meropenem (91.4%), Amikacin (97.1%), ciprofloxacin (77.1%), and gentamicin (62.8%) were effective antibiotic against gram negative isolates. Which in lines with earlier study done in Turkey meropenem (100%), amikacin, (91%) and ciprofloxacin (81%) [27], also study in India report meropenem and amikacin as effective antibiotics [33]. Other studies in Ethiopia and other country reported ciprofloxacin effective antibiotic [9,11,20].The high cost of this group of drugs precludes their use as first choice in the treatment.

Most bacterial isolates showed resistance to the most commonly used drugs. Infection with multidrug-resistant (MDR) organisms is becoming more common, making the choice of empirical antimicrobial therapy challenging. In our study 73.4% multiple drug resistance (bacteria resistant for two or more antibiotics tested) observed. These finding is comparable with study conducted Jimma 80% were MDR [21]. However, reports from Mekelle shows low MDR isolates 59% [9], and Addis Ababa shows high MDR which is 92.7% when compared to our finding [14]. The high frequency of MDR might be a reflection of inappropriate use of antimicrobials, shortage of laboratory diagnostic tests, and unavailability of updated guideline for proper selection of antibiotics [21].

Generally, the present results indicated that meropenem ,amikacin, and ciprofloxacin are highly active against gram negative bacteria and clindamycin, erythromycin, and Trimethoprim-Sulfamethoxazole are active against gram positive organisms causing blood stream infections ,though these antibiotics should not be used indiscriminately and kept as reserve drug because if

resistance is developed then treatment will be complicated. Therefore, it is advisable to continuously evaluate the sensitivity-resistance pattern of isolates in each region so as to make a rational use of antibiotic.

7. Limitation of the study

- ✓ Unable to isolate anaerobic bacteria pathogens because of lack of facilities needed.
- ✓ Lack of MIC for Vancomycin
- ✓ Using one blood culture bottle

8. Conclusion

In our study, the prevalence of bacterial pathogens in blood stream infections was found to be high. Which is caused by both gram positive and negative bacteria and also demonstrates the presence of fungaemia due to *Candida albicans* . The emergence of drug resistance and multiple antimicrobial resistances bacteria to most commonly used antibiotic was high. Therefore, timely investigation of the bloodstream infections and monitoring of antibiotic susceptibility pattern and proper use of antibiotic is important to reduce mortality and morbidity due to bloodstream infections.

9. Recommendations

- The antimicrobial resistance pattern should be assessed by large scale surveillance to identify and control the spread of resistant bacteria strains.

- The study on Candidemia in Ethiopia is lack, further study need to be carried out

- Antibiotic restriction, combination therapy, and infection control programs can help to prevent the spread of multidrug resistant strains.

- Unregulated sales of antimicrobials mostly for self-treatment without prescription should be controlled.

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Annex I:

1. **Participant Information Sheet (English version)**

Department of Medical Laboratory Science, school of allied health sciences and dental health service, and training center, School of Medicine , College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Title: Bacterial profile and antimicrobial susceptibility patterns of blood culture isolates at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

First of all we would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please feel free to ask.

Background information

Blood stream infections are a major cause of mortality and morbidity both in developed and developing countries. A proper antimicrobial treatment can help to reduce mortality due to BSI, Knowledge of common organism and their antimicrobial susceptibility pattern in vital. However, in appropriate use of antibiotic causes increase drug resistance to commonly used drugs that challenge controlling of blood stream infection. Since blood stream infection is one of the major problem in our country the result of the study can be helpful in planning and intervention to solve the problem.

Aim of the study: The purpose of this study is to *assess Bacterial profile and antimicrobial susceptibility patterns of blood culture isolates at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.*

Expected from participants

As a participant of this study, you are expected to give blood. Being asked to give sample does not neccessarly mean that you have the disease.When you are found to be positive for the micro-organism, you will be informed by the health worker and receive proper treatment. You need to know that your results might be discussed with other appropriate individual out of this hospital.

But your name, address will not be disclosed rather an identification code will be used in such conditions.

Time required participating

You will spend 10-15 minutes until the specimen is collected and permission form is signed.

Risks of participant

Specimen collection will have minimal effect and you will not get any risk as the sample will be collected by well trained professionals. But you may fill minor temporary pain during sample collection.

Confidentiality

The information in your records is strictly confidential. All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional will have access the information. The information will be encoded in a computer and saved with password protection.

Benefits of participation

By participating, you will get no financial benefits. Even though there is no direct benefit due to participation in this study, the findings of the study is useful for better understanding of the problems of ***blood stream infection***. You will also obtain all the results of the analysis for free and communicated to your physician for the appropriate management.

Rights of participants

Your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

Communication

In case if you have any questions, unclear ideas and doubt about the project, contact addresses are:

Investigator: Seneshat Eshetu (BSc), DMLS; AAU, +251912009798/0979720102

Email- senamar.b@gmail.com

Advisor: Adane Bitew (PhD), DMLT, AAU +251911039162

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251112755170

2. **Participant Information Sheet (Amharic version)**

ለጥናቱ ተሳታፊዎች

1. **የጥናቱ አላማ**

“ብለድ ስትሪም ኢንፌክሽን የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ ነው።

2. **የተሳትፎ ሁኔታ**

- **ለሚወሰዱ ናሙናዎች ፍቃደኛ መሆንና በሚወሰድበት ጊዜ ትብብር ማድረግ**

3. **ሊከሰቱ የሚችሉ ስጋቶች**

- **ናሙና በሚወሰድበት ወቅት እጅግ በጣም ዝቅተኛ የሆነ የህመም ስሜት ሊሰማ ይችላል። ነገር ግን ይሄ ነው የሚባል ችግር የሚያስከትል አይደለም።**

4. **ጥቅሞች**

- **በጥናቱ ተሳታፊዎች “ብለድ ስትሪም ኢንፌክሽን የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና እና ህክምና ማግኘት።**
- **በጥናቱ ተሳታፊዎች ላይ በሽታ ከተገኘባቸው ተገቢዉን ህክምና እንዲያገኙ ማድረግ።**
- **የጥናቱን ውጤት ለሆስፒታሉ በመስጠት ተገቢዉን መረጃ አግኝቶ የአገልግሎት ማሻሻያ ለማድረግ ያስችለዋል።**

5.**ሚስጥር ስለመጠበቅ**

- **የተሳታፊ ስም አይገለጽም**
- **የሰጡት ናሙና ለተናቱ አላማ ብቻ የሚያገለግል ይሆናል**

6. **በጥናቱ ያለመሳተፍ ወይም ራስን የማግለል መብት**

- **የርስዎ ተሳትፎ በፈቃደኝነት ላይ የተመሰረ ነዉ።**

- **እርስዎ በጥናቱ ላይ መሳተፍዎን ለመሰረዝ ከፈለጉ በማንኛውም ሰአት መሰረዝ ይችላሉ፡፡ ይህንንም ከወሰኑ ማንም ምክንያቱን እንዲገልጹ ሊያስገድድዎት አይችልም፡፡**
- **በጥናቱ ዉስጥ አለመሳተፍ በጤና እንክብካቤዎ ላይ ምንም አይነት ተጽእኖ ይኖረዉም፡፡**

1. ***መረጃ ስለማግኘት***

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

የተመራማሪዉ አድራሻ : ስልክ፡ 0912009798/0979720102

ኢ-ሜይል፡ senamar.b@gmail.com

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

3. Informed consent (English version)

I undersigned the purpose of this study. I have been informed there is no harm except little discomfort during sample collections. I have been informed that other people will not know my results. I understand that there may be no benefit to me personally apart from clinical service I get from these results .I have been told that participation in this study is voluntary and I may refuse to be in the study. The study has been explained to me in the language I understand. I give consent to participate after a clear understanding of the objectives and conditions of the study.

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

Participant code: _____

Signature: _____

Date: _____

For those who can't read the information

Advisor nurse name……..

Signature ……

Date ……..

4. **Informed consent form (Amharic version)**

የተሳታፊ ስምምነት ቅጽ

ይህ ገጽ. ማለትም “ብለድ ስትሪም ኢንፌክሽን የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ” በሚል ርዕስ የተሳታፊ ስምምነት ቅጽ ነዉ፡፡ በመሆኑም እባክዎን በዚህ በታች የተዘረዘሩትን ነጥቦች ይረዱና፤ ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈርሙ እጠይቃለሁ፡፡ከእኔ የሚወሰደዉ ናሙና ለጥናቱ አላማ ብቻ እንደሚዉል ተረድቻለሁ፡፡ሁሉም መረጃዎች እና የናሙና ዉጤቱ ምስጢራዊ መሆኑን ተገንዝቤአለሁ፡፡በጥናቱ ላይ በመሳተፌ ምንም የገንዘብ ክፍያ እንደማላገኝ ተረድቻለሁ፡፡በጥናቱ ያለመሳተፍ እንዲሁም በማንኛዉም ጊዜ የማቃረጥ መብት እንዳለኝ አዉቄአለሁ፡፡ሁሉም መረጃዎች በአስተባባሪዉ/ዎች ተገልጸልኝ በደንብ ተረድቻለሁ፡፡

የተሳታፊ ፊርማ፡ ----------

የተሳታፊ አድራሻ፡----------

ቀን፡----------

የስምምነትቅጹንማንበብለማይችሉተሳታፊዎች

የአማካሪነርስስም ----------

ፊርማ ----------

ቀን----------

5. Informed assent Form (English version)

I undersigned the purpose of this study. My child has to say to choose if I want to be in the study. I have been informed there is no harm except little discomfort during sample collections. I have been informed that other people will not know my child results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered .I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child. By signing below I agree to let my child to participate in this research study.

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

Participant code: _____

Signature: _____

Date: _____

For those who can't read the information

Advisor nurse name……..

Signature ……

Date ……..

6. **Informed assent Form (Amharic Version)**

የስስምምነት መጠየቂያ ቅጽ

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

የተሳታፊ ፊርማ፡ ----------

የተሳታፊ አድራሻ፡----------

ቀን፡----------

የስምምነትቅጹን ማንበብ ለማይችሉ ተሳታፊዎች

የአማካሪነርስስም ----------

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Annex II: Standard Laboratory procedures

1. Blood sample collection

Aseptic blood collection procedure is important in order to avoid contamination of the blood by normal skin flora during the sample collection which can make difficult to differentiate between false and true infection.

Procedure

1. Cleanse the vein puncture site with 70% isopropyl alcohol, starting at the middle of the site, swab with 10% povidine-iodine solution and allow the site to air dry wipe the top of the bottle using an ethanol-ether swab.
2. Using a sterile syringe and needle, withdraw about 1 ml of blood from neonate , 5 ml from a children and 10 ml from adult.
3. Dispense the blood into the Brian Heart infusion culture medium bottle containing 90ml of broth or in proportion of 1ml of blood to 9ml of BHI broth containing bottle.
4. Label each bottle with number of the patient, and the date of collection.
5. Incubate at 35–37 °C for up to 7 days, examining and sub-culturing Blood agar, Chocholate agar, MacConkey agar, and Sabouraud dextrose agar .

2. Gram stain

Principle

Following staining with a crystal violet and treatment with iodine, the dye–iodine complex is easily removed from the more permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria. Retention of crystal violet by Gram positive organisms may also be due in part to the more acidic protoplasm of these organisms binding to the basic dye

Procedure

1. Prepare smear on clean slide then air-dry

2. Flood slide with crystal violet; leave for 1 minute

3. Rinse slide in clean running water

4. Flood slide with Gram's iodine; leave for 1 minute

5. Rinse slide in clean running water

6. Apply acetone and rinse immediately under running water (exposure to acetone 5 seconds)

7. Counter-stain with carbol fuschin/safranine for 1minute

8. Rinse in clean running water then dry with blotting paper

1o. Place a drop of immersion oil on the slide and view with 100x oil-immersion objective.

3. Culture media preparation and inoculation

3.1. General protocol of Culture media preparation

1. Weighing and dissolving of culture media

2. Sterilization

3. Addition of heat sensitive ingredients

4. PH testing of culture media

5. Dispensing of the culture media

6. Sterility testing

7. Quality assurance of culture media

8. Storage of culture media

Prepare media made from dehydrated products in as damp-free an environment as possible. To prevent the risk of inhaling fine particles of dehydrated media, wear a dust mask while handling dehydrated media, powder or use granulated media

- Wash the hands immediately after preparing media.
- Once the ingredients are weighed, follow exactly the manufacturer's instructions.
- Use completely clean glassware, plastic or stainless steel equipment that has been rinsed in pure water. The container in which the medium is prepared should have a capacity of at least twice the volume of the medium being prepared.
- Use distilled water from a glass still. Deionized water can also be used providing the exchange resins do not contain substances inhibitory to bacteria. Water containing chlorine, lead, copper, or detergents must not be used. Besides containing substances harmful to bacteria, impure water can alter the pH of a medium or cause a precipitate to form.
- Add the powdered or granular ingredients to the water and stir to dissolve. Do not shake a medium but mix by stirring or by rotating the container.
- When heating is required to dissolve the medium, stir while heating and control the heat to prevent boiling and foaming which can be dangerous and damage the medium,over heating a medium can alter its nutritional and gelling properties, and also its pH.
- Autoclave a medium only when the ingredients are completely dissolved. Always autoclave at the correct temperature and for the time specified.
- Dispense medium in bottles or tubes in amounts convenient for use.

3.2 Dispensing media into Petri dishes

1. Mix the medium gently by rotating the flask or bottle. Avoid forming air bubbles. Flame sterilizes the neck of the flask or bottle and pours 15–20 ml of medium into each dish (90–100 mm diameter). Air bubbles enter while pouring, rapidly flame the surface of the medium before gelling occurs. Rotate the dish on the surface of the bench to ensure an even layer of agar.
2. When the medium has gelled and cooled, stack the plates and seal them in plastic bags to prevent loss of moisture and reduce the risk of contamination. Do not leave the plates exposed to bright light especially sunlight.
3. Store at 2–8 ^{o}c.

3.3 Inoculate culture media

Immediately before inoculating a culture medium check the medium for visual contamination or any change in its appearance which may indicate deterioration of the medium, e.g. darkening in color. When inoculating, or seeding, culture media an aseptic (sterile) technique must be used. This will:

Prevent contamination of cultures and specimens,

prevent infection of the laboratory worker and the environment.

Aseptic techniques

3. Flame sterilizes wire loops, straight wires, and metal forceps before and after use. Whenever possible, use a Bunsen burner with a protective tube.
4. Flame the necks of specimen bottles, culture bottles, and tubes after removing and before replacing caps, bungs, or plugs.
5. When inoculating, do not let the tops or caps of bottles and tubes touch an unsterile surface. This can be avoided by holding the top or cap in the hand. Always use racks to hold tubes and bottles containing specimens or culture media.
6. Make slide preparations from specimens after inoculating the culture media.
7. Decontaminate the work bench before starting the day's work and after finishing.
8. Use a safety cabinet when working with hazardous pathogens.
   Wear protective clothing; wash the hands after handling infected material

4. Biochemical tests

- **Catalase test**

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as *streptococc*i. Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. Add 2–3 ml of hydrogen peroxide solution into a slide and using a sterile wooden stick pick colonies of the test organism and mix with hydrogen peroxide solution then look for bubbling.

- **Coagulase tests**

The coagulase test differentiates strains of *Staphylococcus aureus* from other coagulase-negative species. *S.aureus* strains are capable of coagulating plasma. The coagulase test can be performed using two different procedures Slide test and tube test. For both tests, clumping or clots of any size indicate a positive response.

Procedure

Slide test method

1 Place a drop of distilled water on slide

2 Emulsify a colony of the test organism to make suspension.

3 Add a drop of plasma to the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds.

Test tube method

1. Take small test tubes and add plasma into tube.

3. Add the test broth culture to tube.

4. After mixing gently, incubate the tubes at 35–37^{o}C. Examine for clotting after 24 hour.

Note: When looking for clotting, tilt each tube gently.

Results

Clotting of tube contents . . . . . *S. aureus*

No clotting or fibrin clot . . . . . Negative

- **PYR Test (Pyrrolidonyl Aminopeptidase)**

It is used for the presumptive identification of group A streptococci (*Streptococcus pyogenes*) and *Enterococcus spp*

Principle: The enzyme L-pyrrolidonyl arylamidase hydrolyzes the L-pyrrolidonyl- β-naphthylamide substrate to produce a β-naphthylamine. The β-naphthylamine can be detected in the presence of N,N-methylaminocinnamaldehyde reagent by the production of a bright red precipitate.

Result: Positive: Bright pink or cherry-red color within 1-2 minutes.

- **Optochin Test**

For identification of alpha-hemolytic streptococci as *S. pneumoniae*

Procedure: Using an inoculating loop, streak two or three suspect colonies on 5% sheep blood agar plate then Place a P disk(5 μg) and Incubate at 35-37°C with ~5% CO2 for 18 to 24 hours.

Result: zone of inhibition of 14 mm or greater indicates sensitivity (*S. pneumoniae)*

- **Indole test**:

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. It is important in the identification of *Enterobacteriaceae* . Few colonies of the culture will be inoculated into peptone water and incubated at 37oC for 24 hours. Few drops of indicator (Kovac's reagent) will be added and the Colour change will be then observed. If the layer of indicator reagent turns to red within 1 minute, it is Indole positive and if it remains yellow it is Indole negative

- **Urease test**

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive bacteria from other *Enterobacteriaceae*. Urea agars will be inoculated heavily over the entire surfaces of the slant and then incubated at 37^0c for 3-12 hours. A urease-positive culture produces an alkaline

the medium color become red and Urease-negative organisms do not change the color of the medium, which is pale yellow-pink.

- **Triple Sugar Iron (TSI) Agar Slant**

Triple sugar iron agar is used for the differentiation of enteric pathogens by ability to determine carbohydrate fermentation and hydrogen sulphide production. using a sterile inoculating needle, stab the butt of the LIA slant twice then streak back and forth along the surface of the agar with the organism. Incubate at 37^0c for 18 to 24 h. If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented. If alkaline slant–acid butt (red–yellow): glucose fermented only. If alkaline slant–alkaline butt (red–red): glucose not fermented. The presence of black precipitate (butt) indicates hydrogen sulfide production, and presence of splits or cracks with air bubbles indicates gas production.

- **Citrate utilization test citrate using Simmon's agar**

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator. Pick a single isolated colony and lightly streak the surface of the slant, then incubated at 37^oc aerobically for 18 to 48 hours. Blue color indicates a positive reaction and green color indicate negative reaction.

- **Motility Test (using motility agars)**

This medium is used for checking the motility of organisms. Low agar concentration allows free movement of bacteria. Motility agar will be prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility will be examined after 35-37^0c for 24 hour. Motility will be indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation.

- **Lysine decarboxylase(LDC)**

The acids produced by the bacteria from the fermentation of glucose will initially lower the pH of the medium and cause the pH indicator to change from purple to yellow. The acid pH

activates the enzyme that causes decarboxylation of lysine to amines and the subsequent neutralization of the medium. This results in another color change from yellow back to purple. Bacteria that decarboxylate lysine turn the medium purple. In addition bacteria that produce H2S appear as black colonies.

- **Oxidase test**

The oxidase test is used to assist in the identification of bacteria which produce the enzyme cytochrome oxidase. Piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple color.

- **Germ Tube Test**

Principle

Germ tubes are short outgrowth, non-septate germinating hyphae. They are ½ the width and 3 – 4 times the length of the cell from which they arise. When cells of Candida are incubated in serum at 370C for 2-4 hours *Candida albicans* produce short, slender, tube like structures called germ tubes. Formation of germ tubes is associated with increased synthesis of protein and ribonucleic acid. Various media like fetal bovine serum may be used as a substitute to human pooled serum.

Requirements:

- Culture: Suspected Candida colonies from culture plate
- Reagent: Serum (human, sheep, fetal bovine)or other commercially produced media for germ tube testing
- Others: Test tubes, loop or wooden applicator, Pasteur pipettes, slides, cover slips

Procedure:

1. Aliquot 0.5 ml (12 drops) of serum or media in a test tube.

2. Make a light suspension of the suspect yeast colonies (by touching 1-2 large colonies or 3-4 smaller colonies with a sterile wooden applicator stick or loop) on serum.

3. Incubate the tube for 2-3 hours in a 35 – 37°C incubator.

4. Place a drop of the suspension on a slide using a Pasteur pipette and cover with a cover slip.

5. Examine the wet mount microscopically (at 40X) for production of germ tubes (long tube-like projections extending out from the yeast cells).

Result and interpretation:

9. Positive Result: A short hyphal (filamentous) extension arising laterally from a yeast cell with no constriction at the point of origin. May be confirmed as *C.albicans*

10. Negative Result: No hyphal extension arising from a yeast cell or a short hyphal extension with constriction at the point of origin.

5. Antimicrobial susceptibly tests

- **Disc diffusion susceptibility tests**

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc.

Test Inoculums Preparation

- 3 to 5 pure colonies of the same morphological type will be selected from blood agar plate. The colonies are transferred into a tube containing 4 to 5 ml of tryptone soy broth.

- The turbidity of the broth culture will be adjusted with 0.5 McFarland standards.
- The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface.
- Left for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Application of Disks to Inoculated Agar Plates

- The predetermined series of antimicrobial disks is dispensed onto the surface of the inoculated agar plate.
- The plates are inverted and placed in an incubator at 37 ^{0}c

Interpreting Results

- After 16 to 18 hours of incubation, each plate is examined. The diameters of the zones of complete inhibition are measured, including the diameter of the disk. Zones are measured to the nearest whole millimeter, using sliding calipers which is held on the back of the inverted plate.

Annex III: Blood sample collection format

Lab.code	Age	Sex	Time of collection	Date of collection	Name& Signature

Annex IV: Annex Assurance of Principal Investigator

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

Name of the Investigator: _____

Signature: _____

Date: _____

Approval of the primary Advisor

This thesis has been submitted for examination with my approval as University Advisor.

Name: - Dr. Adane Bitew, Associate Professor

Signature: _____

Date: _____

This thesis has been submitted for examination with my approval as External Advisor.

Name: - Miss Tigist Getachew

Signature: _____

Date: _____

This thesis has been submitted for examination with my approval as External Advisor.

Name: - Mr.Solomon Gizaw

Signature: _____

Date: _____