Addis Ababa University

College of Health Science

Department of Medical Laboratory Sciences

Bacterial profile, antimicrobial Sensitivity pattern and outcome of blood stream infection among febrile patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Wubet Tesfaye, entitled:

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Abbreviations

AAHB	Addis Ababa Health Bureau
AAU	Addis Ababa University
AST	Antimicrobial Sensitivity Test
ATCC	American Type Culture Collection
BSI	Blood stream infection
CDC	Centers for disease control and prevention
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
CoNS	*Coagulase negative Staphylococcus*
DRERC	Departmental Research and Ethics Review Committee
GNB	Gram negative bacteria
MDR	Multidrug-Resistant
QC	Quality Control
SD	Standard Deviation
SOP	Standard Operating Procedures
SPSS	Statistical Package for Social Science
TSB	tryptic soy broth
TSI	Triple Sugar Iron
VRSA	*Vancomycin Resistance staphylococcus*
WHO	World Health organization
ZMH	Zewditu Memorial Hospital

Abstract:

Background: Throughout the world infectious disease like bloodstream infection become a major cause of morbidity and mortality. The excessive and irrational use of antibiotics has led to an increase in the multidrug-resistant and thus worsened the condition.

Objective: To determine Bacterial profile, antimicrobial susceptibility pattern of blood stream isolates, and mortality of febrile patients admitted at Zewditu Memorial Hospital, Addis Ababa, Ethiopia

Methods: A cross-sectional and prospective observational study was performed in 198 febrile patients at Zewditu Memorial hospital from May to Dec, 2019. Demographic and clinical data were collected by interviewing patients, reviewing medical records and outcome was followed until their discharge or death. Venous blood was collected aseptically into TSB, incubated at 35 +2°C and checked for sign of bacterial growth. Bottles which showed signs of growth were further sub-culturedon chocolate agar, blood agar,macconkey agar. Bacterial isolates were identified by colony morphology, gram staining reaction, and biochemical. Kirby Bauer disc diffusion technique was used to test Antibiotic susceptibility. Multiple Logistic regression analysis was used to see the association between variablesusing SPSS software version 20.

Results: A total of 198 blood cultureswere performed fromfebrile inpatients of ZMH of those 93(47%) were females and 105(53%) were males with the age range of 1 day to 8 years. From the total 43(21.7%) showed bacterial growth. The gram positive and negative bacteria accounted for 69.7% (30/43) and 30.3 %(13/43) respectively. The commonest gram positive organisms were *S.aureus* 20 (66.66%).Among gram negative isolates the most predominate organisms were *K.pneumonia* 6(46.1%). For gram positive bacteria Penicillin and Trimethoprim-sulphamethoxazole showed highest resistance andComplete Sensitivity were seen for Vancomycin in all the isolate.Relatively 80(%) of gram negative isolates expressed susceptibility towards Amikacin andhighest resistancewere seen in Trimethoprim- sulphamethoxazole. Mortality among patients with positive culture was 51.1% (22/43), which was higher than mortality recorded for patients with negative blood culture 14.8% (23/155).

Conclusion: In this study high prevalence of septicemia and greater resistances of antibiotic which increase the mortality risk three fold was found in the hospital settings. Therefore special attention has to be given to infection prevention control and antibiotic prescription policy

Key words: blood stream infection, bacterial isolate, drug resistance, Mortality

1. Introduction

1.1 Background

Blood infection defined as the presence of viable bacteria or fungal and other microorganisms confirmed by laboratory as positive by performing blood culture in the blood (i.e. bacteremia) [1].Blood stream infection(BSI) is one of the mostserious situations in infectious disease constituted by invasion of the blood stream by microorganisms. Since blood is a normally sterile site, continuous, intermittent, or transient presence of microorganism in circulating blood is a threat to every organ in the body [2, 3].

Bloodstream infection is characterized by a high morbidity and mortality, which is directly related with the delay in administration of the first adequate anti-infectious agent [4, 5]. It has serious consequences like shock, disseminated intravascular coagulation, multiple organ failure, and even death[2]. Major clinical presentations of septicemia patients are fever, difficulty in breathing, tachycardia, malaise, refusal of foods or lethargy which required medical emergency of urgent rational antibiotics therapy [6].

Primary BSI (i.e., blood stream infection that is not documented with primary source of infection) can be distinguished from secondary BSI (i.e., blood stream infection often as a result oflocalized focus of infection, such aswound infection, biliary tract infection pneumonia, skin and soft-tissue infection) and may cause high fever. So, the detection of BSI is greatly assisted by the management of febrile inpatients [1, 3].

Knowing frequentlyisolated microorganisms and their antimicrobial resistance patterns is necessary for treatment of bloodstream infection. Organismisolation and studying their antimicrobial sensitivity patterns is needed to start specific therapy. The procedure is lengthy and needs growing of the microorganisms in culture media [2].However, until microbiological documentation, the adequacy of anti-infectious agent cannot be guaranteed especially in the context of an increasing rate of multidrug-resistant organisms [7, 8, 9].

The empirical use of antibiotics results in a 15±30% rate of inappropriate treatment, Which is associated with a two to five fold increase in the mortality risk of septicemic patients and a contributing factor in the recent increases in antibiotic-resistant organisms [10,11].

Blood cultures are one of the most frequently performed microbiological tests in hospitals worldwide and still remain gold standard for detecting bacteremia [12].

Therefore, up to date etiological data for major pathogens causing blood stream infections and determining pathogen antibiotic susceptibility patterns to evaluate the changing trend ofantimicrobial susceptibility is very crucial. Hence, this study was carried out to provide the update information in the study area which can improve clinical practice by guiding empirical antibioticChoice and play a positive role in better health care management.

1.2 Statement of the problem

Throughout the world infectious disease like bloodstream infections become a challenge for public health which cause high morbidity and mortality [4].In Europe an estimated burden of BSI is 1,200,000 episodes each year and 157,000attributable deaths and it became a growing public health concern [13].In the United States generally there are around two million infectious disease are acquired per year while in hospitals, from this infectious disease approximately (10–20%) involve the blood infection, and (4.5%) are fatal [14, 15, 16].In Germany Blood Stream Infection have been declared the third most common cause of death[17].

Increasing prevalence of blood stream infection in developing countries such as in Nigeria 41% [33] Uganda 37% [34]in Zambia 33%[35] is a major health problem which cause the biggest challenge for the health care providers in the selection of appropriate antimicrobial agents. In addition to that it become complicated since most of the organism develop resistance to most of the antimicrobial agents, which is the main stay of treatment.

In Ethiopia there are many studies conducted in different cities which shows high prevalence of the infectionin Gonder 18.2%[26], in Jimma 8.8% [37], in Mekele 37.5%[38] and in Addis Ababa different hospitals 42% [39] 15.2 % [40].

Blood stream infection may be causedby infections which areself-limiting infections to the worst life threatening sepsis that needed to be treated rapidly and aggressively. The excessive and irrational use of antibiotics has led to an increase in the multidrug-resistant and thus worsened the condition[20].The infection caused by MDR organism is more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics[21].

Resistance pattern of antibiotics increase from time to time so timely surveillance of antimicrobial resistance must be done, and updated knowledge of local pathogens is essential to start prompt and appropriate empirical therapy. So that the results of this study may show the current burden of the disease, microbiological features, drug resistance data and the mortality rate of the infection ,at Zewditu Memorial Hospital. Such data can provide updated information for clinician to choose the right antibiotic therapy for proper timely treatment of patients , important to guide development of algorithms for empiric treatment of BSI in these regions. It also provides more data on the burden and mortality rate of the infection in the hospital.

1.3 Significance of the Study

Since the prevalence of bacterial profile and antimicrobial resistance varies in accordance with geographical and regional location and also there is variability between hospitals in different countries it requires continuous analysis of local trends. Therefore, updating data on etiological agent for major pathogens responsible bloodstream infections and patterns of antibacterial suitability in this Hospital help clinician choose the right antibiotic therapy.

Microbiologic data obtained from such studies are very essential to provide information in development of algorithms to treat blood stream infection empirically in thishospitalgiven the wide variation in results of other studies.

This study designed to determine the epidemiology and etiology of blood stream infection in patients who are with fever; this study also provides more data on the morbidity, mortality, microbiological features, and drug resistance data at Zewditu memorial hospital. In addition, the results of this study can be used as reference for further similar studies that will be conducted in Ethiopia in the future

2. Literature review

Since blood stream infection being one of the challenging problem, many research have been done in the world .These research showed the prevalence of etiologic and their antimicrobial pattern has been changed from place to place and from time to time. so, it needs to updated epidemiological data for agiven place and time .

As observational retrospective study done in Indonesia the general study participant were 95, fromwhich 15(15.8 %) showed bacterial growth, 53.3 % of them were gram positive. Higher number was recorded from *Staphylococcus aureus* .The remaining 46.7 % wasgram negative recorded with highest number of *Escherichia coli*. Resistance was observed to most antibiotics tested in-vitro with a range for gram positive bacteria 0 % to vancomycinand 100 % to penicillin G, and for gram negative 14.3 %to Amoxacillin clavulanic acidand 85.7 %to Ampicillin . Eighty percent of pathogens were multi drug resistant. Out of which 87.5%, for gram positive and 71.4 % forGram negative [23].

In atertiary care hospital of North India 2231 blood culture samples were studied retrospectively. From the total, 447 (79.1%) were culture positive; Gram-positive account for 306 (54.2%) of the result, of them *Coagulase-negative staphylococci*were 208 (67.9%) and Gram negative were 141 (24.9%) . The most sensitive drugs for gram-positive isolates were vancomycin, and linezolid while gram-negative isolates showed 100% sensitivity to colistin and tigecycline [24].

On another similar study at Dhaka, Bangladesh, 2017 samples were cultured, 13.6% of the cultured blood samples showed growth. Gram-negative bacterias were identified in higher number (72.1%) and from which*Salmonella Typhi* accounts the highest number (36.9%) and most of the strains were multidrug-resistant (MDR). Overall, Gram positive bacteria were more resistant to most of the commonly used antibioticsAmpicillin, erythromycinthan Gram-negative bacteria likeAmpicillin cotrimoxazol, but the MDR level was high in both groups[25].

A Study conducted at teaching hospital in Telangana, India in 2015 revealed that from 629 blood samples, 117 (18.6%) showed positive result. Out ofthese Gram-Negative were 58.1% and Gram-Positive were 41.9%. From the total*Klebsiella* and *Staphylococcusaureus*account the highest number respectively. Carbapenems were effective drug for Gram Negative Bacilli and Vancomycin and Linezolid were effective for Gram Positive. Other drugs showed variable resistant pattern with common drugs like Ampicillin, Penicillin, Amoxycillin, Cephalosporins being highly resistant [26].

Retrospective cross sectional study in North India includes 565 blood samples of these140 showed growth. From the total,Gram positive and negative bacteria accounts for 74(53%) and 55(39.3%) respectively. *CoNS* were 49 (34.5%)which showed highest frequency followed by*Staphylococcus aureus*22 (15.4%) .From both gram positive and negative bacteriaresistance was higher [27].

In Kanpur, North India prospective study include 121 patients. Twenty seven (22.3%) showed growth from the 27 isolate, 24(88.9%) showed bacterial growth, ande the remaining showed fungal growth. From the total growth 16(59.3%) were gram negative bacilli, 8(29.6%) were gram positive cocci and 3(11.1%) were gram positive budding yeast cells. *E.coli* (22%), *Klebsiella pneumoniae* (22%), *Coagulase negative Staphylococcus*(15%) were the most frequently isolated followed by *Pseudomonas aeruginosa* (11%) *S.aureus* (11%), *Klebsiella oxytoca* (4%), *Enterococcus* (4%) and Candida *spp* (7%) [28].

In New Delhi India a research conducted prospectively the most frequently isolated gram positive were*CoNS* 63.5%, *S. aureus* 23.1%, alpha-hemolytic*streptococci* and e*nterococci* 5.8%.Gram-negative bacteria identified were*Salmonella Typhi* 24.1%, *Escherichia coli* 23.3% and *Pseudomonas aeruginosa* 13.8% identified. Maximum resistance to beta-lactam antibiotics were observed in *Coagulase-negative staphylococci*.*Staphylococcus aureus*showed higher rate of resistance to most of the tested antibiotics 100% resistance to penicillin. From gram negative maximum resistance was showed for Ampicillin and minimum to Amikacin[29].

A study conducted at Belgium explained that Methicillin resistance observed in around 55–75% of nosocomial organism. The first organisms which showed glycopeptide resistance was *CoNS*. Now a day *CoNS* become the major cause of nosocomial and health-care related infection due to mainly the use of indwelling and invasive medical devise [30].

On single-center retrospective study in Japan a total of 2,105 patients with BSIs were included; 1,786 survived and 319 died, and the 30-day mortality rate was 15.2% over the 5-year study period [31].

A retrospective nested case control study in Brazil categorize participants in two: the one who stayed to hospital or death due to BSI as Group one and those with BSI, but that did not show same outcome as Group two .The rate of death or hospitalization raised to 1.05 times for all age group with blood stream infection, 2.75 fold increment of mortality and morbidity were observed for patient with hemodialysis from which multi drug resistance are isolated[32].

A prospective study conducted at Lagos,Nigeria recruited 100 children in its study the overall positive culture rate was35% .Neonate showed the highest rate (41%) .In the older infants 7% prevalence reduction was observed, and also 5% reduction were noted in less than 5years but in older children1.4 increment observed [33].

A prospective research conducted in Uganda indicates that neonatal septicemia was confirmed in about 110(37%) of the 293 neonates. From the 110 neonates with confirmed sepsis, 20 (18.1%) died and 17 (85%) died in the first 2 days of admission [34].

In a cross sectional observational study inZambia 313 neonates were included; (103/313)33% showed bacterialgrowth, from which 85% (88/103) were early-onset sepsis. *Klebsiella*species 57% (77/103) showed the highest rate, coagulase-negative staphylococci6% (7/103)] showed the second rate,*Escherichia coli* and Candida species 5% (5/103)] showed the rest. For Antibiotic resistance ranged from 96%-99% for*Klebsiellaspecies*, Cultureresults were available for only 25% (26/103) of participant before discharge or death [35].

In Gonder ,Ethiopia a total of 390 blood culture performed in 2013 from which 71 (18.2%) were culture positive. *CoNS* 30 (42.3%), *S.aureus* 17 (23.9%) from the gram positive and *Klebiesella* (12.9%), *E.coli*(7.0%), were from gram negative. Resistance were seen to most antibiotics. The range ofsusceptibilitywere 23.5% – 58.8% for commonly used antibiotics like Penicillin,Ampicillin,Tetracycine and Gentamycin for gram positive, and 20%– 100% forgram - negative and highest resistance were observed in Ampicillin and Chloramphenicol f[36].

On study conducted at Jimma university specialized hospital in 2011 260 blood specimens were tested only 23(8.8%) showed growth to seven different types of bacteria. .High rates of resistance were observed to most antibiotics tested. Resistance for gram positive bacteria range from 0% to 85.7% high resistance was observed to penicillin and Ampicillin , and for gram negative from 0% to 100% 100%resistance was seen against Ampicillin 88.9% to Tetracyclin . None of the isolates were resistance to ciprofloxacin and ceftriaxonin [37].

In 2015 A Study conducted atMekelle Hospital, Northern Ethiopia 514 febrile patients were included, 144(28%) were culture positive. *Staphylococcus aureus*54 (37.5%), *Coagulase-negative staphylococci* 44 (30.6%),*Escherichia coli* 16 (3.1%), *Citrobacterspp*. 9 (1.7%) and *Salmonella typhi*8 (1.6%) were the frequent isolates, Antimicrobial resistance pattern for gram positive and gram negative bacteria was 0–83.3% high resistance were seen to Trimethoprim-sulphamethoxazole and Doxycylin and Vancomycin were effective antibiotic and 0–100%

overall high resistance was seen in ceftriaxone . Amoxicillin clavulanic acid and ciprofloxacin were effective , respectively[38].

On other study conducted at yekatit 12 hospital burn center, Addis Ababa, Ethiopia in 2013 a total of 50 patients were included and 21(42%) of them were found bacteremic .*CoNS* 9(42.8%), *S.aureus*, 8(38.2%), Bacillus spps, 2(9.52%), *K. pneumoniae*, 1(4.8%), and *P.aeruginosa*, 1(4.8%), wereisolates mostly. Antimicrobial resistance was observed for most of common antibiotics like Ampicillin, Doxycycilline Nalidixic acid and Penicillin G[39].

At TikurAnbessa Specialized Hospital, Addis Ababa, Ethiopia in2018, a total 422 sample diagnosed, 64 (15.2%) samples showed growth. *Staphylococcus aureus*and *Klebsiella pneumoniae* were the frequent ones. Penicillin (86.7%) showed high resistance against Gram-positive bacteria while ampicillin (85.7%) and amoxicillin clavulanicacid (77.14%) were showed high resistance against Gram-negative bacteria. Clindamycin and amikacin (80%), (97.1%) showed greater sensitivity antibiotic against Gram positive and Gram-negative bacteria, respectively [40].

On study conducted at black lion hospital in 2015 292 adult patients have been studied .thirty eight blood cultures were positive patients with positive blood culture showed higher death rate than those without bacteriemia ie, [50.0% vs. 9.8%].Eleven patients who showed growth in *Enterobacteriaceae* were resistant to third generation cephalosporins all died. Most of gram-negative enteric bacteria,Showed resistance to this antibiotics third generation cephalosporin, gentamycin chloramphenicol and cotimoxaxol[41].

3. Objective

3.1 General Objective

To Determine Morbidity, Mortality, Bacterial profile and antimicrobial Susceptibility pattern of blood stream isolates among febrile patients admitted in Zewditu Memorial Hospital, from May to Dec, 2019 G.C. Addis Ababa, Ethiopia

3.2 Specific Objectives

- To describe the bacterial isolate of blood stream infections in febrile patients
- To determine the antibiotic Susceptibility pattern of bacteria isolate causing blood stream infection
- To describe the Mortality of blood infection in the study site

4 Hypotheses

The Morbidity, Mortality, prevalence of bacterial profile and antimicrobial susceptibility pattern of isolates on blood stream are similar with the finding of other study conducted atTikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia by Eshetu et al

5 Material and method

5.1 study area

The study was carried out at Zewditu Memorial Hospital. Zewditu Memorial Hospital is found in Addis Ababa, Ethiopia. It is established in 1925E.C. first the hospital was constructed by Seventh-day Adventist Church and ruled by them, but during the Derg regime it was nationalized to the country in about 1976. Currently the Hospital is operated by Addis Ababa Health Bureau. The Hospital is found under the Kirkos Sub-City of City Government of Addis Ababa. According tothedata obtained from the hospital it give servicefor around 102,749 patients annually and from this 14,531 are inpatients and average 111 patients are examined as febrile monthly

5.2 Study design and period

A cross sectional and prospective observational study was conducted at Zewditu Memorial hospital Addis Ababa, Ethiopia from Mayto Dec, 2019 G.C.

5.3 populations

5.3.1. Source population

All febrile patients visiting inpatients of Zewditu memorial hospital during the study period

5.3.2 Study population

All febrile patients admitted to wards during the study period and full filling the inclusion criteria were the study population

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

All febrile patients suspected of blood stream infection and volunteer to participate.

5.4.2 Exclusion criteria

Patients who receive any antimicrobial treatment before two week

5.5 Study variable

5.5.1 Dependent variable

- Bacterial isolate of blood stream infections in febrile patient
- Antibiotic resistance of bacteria isolate from blood stream Infection
- Mortality of blood stream infection

5.5.2 Independent variable

Sex

Age

Source infection

Indwelling medical devise

5.6Measurement and data collection

5.6.1 Sample size determination

The sample size (n) is calculated using formula the single proportion

$$n = \frac{Z\alpha^2 P (1- P)}{d2}$$

By using 95% confidence level, Z value will be 1.96

5% margin of error (d)

Proportion from other = 15.2% from prevalence of study conducted at Tikur Anbesa [28]

$$n = \frac{(1.96)^2 1.52 (1- 1.52)}{(0.05)2} = 198$$

Thus, the study includes 198 subjects,

5.6.2 Sampling Method

Convenient sampling technique was used

5.6.3 Data and Sample collection procedure

Data collectors (experienced nurse and laboratory technologist) were identified, and informed to collect the data as per the pre-structured questionnaire. The purpose of the study as well as any related harm and benefit was explained to the study participants . Demographic data and potential risk factor including presence of,site of infection, indwelling medical device, , length of hospital staywere collected by, interviewing the patients, patients guardian, reviewing different

medical records, nursing chart and consulting the physicians in charge of patients .And the outcome of the patient was followed until their discharge or death. Data collected for each patient were recorded on the pre structured questioner

On the basis of patient age10 ml of venous blood for adultsand 2–3 ml for children was collected aseptically using 70% alcohol and2% tincture of iodine and transferred into a bottle containing tryptic soy broth (TSB) sterile culture medium in a 1: 5 proportion and EDTA tube for CBC, every sample container had a label which include information about: subject code, ID number, Date and time and then transported to the microbiology laboratory of ZMH within 5-10minutes

5.6.4 Principle of each laboratory analysis

Tryptic soy broth (TSB) containing the sample was incubated at 35 ±2°C.Blood culture broths were checked for sign of bacterial growth (turbidity, daily up to7 days. Bottles which showed signs of growth were further sub-cultured and incubated at 35 ±2°C for 24 h. After 7 days the broth was checked for growth and if it doesn't show growth it was further subculture and again if no growth it is reported as negative. Bacterial isolates were identified by colony morphology, gram staining reaction, biochemical tests, using Catalase test, Coagulase test, Triple Sugar Iron agar (TSI), citrate utilization test, Urease test and Motility Indole test using the standard procedure for bacterial identification [22]

Antibiotic SusceptibilityTesting

Kirby Bauer disc diffusion technique was used to test Antibiotic susceptibility the media used were Muller Hinton agar (MHA) plate. From a fresh non-selective agar plate pure colonies were selected and transferred to 5 mL sterile normal saline and thoroughly mixed to make the suspension homogenous and turbidity was adjusted using McFarland densitometer to match with a 0.5 McFarland standard, then inoculated following the standard over the entire surface of a MHA plate using a sterile swab. Then Using sterile forceps, the antibiotic discs were placed on MHA by considering 24 mm distance between each disk and 15 mm from the border.), zone of inhibition was measured and reported as susceptible (S), intermediate (I), or resistance (R) using the 2018 CLSIguide line .The tested antibiotic discs included are shown in the tables below

Table 1.Antibiotics used for Gram negative bacterial pathogens

Antibiotic	Concentration(µg)	Antibiotic	Concentration (µg)
Ampicillin	10	Meropenem	10
Gentamycin	10	Ciprofloxacin	5
Amoxicillin-clavulunate	10/20	Piperacillin tazobactam	100/10
cefazolin	30	Trimethoprim-sulfamethoxazole	1.25/23.75
Ceftriaxone	30	Ceftazidime	30
Cefotetan	30	Chloramphenicol	30
cefuroxime	30	tetracycline	30

Table 2.Antibiotics used for Gram positive bacterial pathogens

Antibiotic	Concentration(µg)	Antibiotic	Concentration(µg)
Penicillin	10	Tetracyclin	30
Cefoxitine	30	Ciprofloxacin	5
Clindamycine	2	Chloramphenicol	30
Erythromycin	15	Gentamycin	10
Trimethoprim-sulfamethoxazole	1.25/23.75	Vancomycin	30
Doxycyclin	30		

5.7 Data Quality Assurance:

Pre-analytical

Socio-demographic characteristics of the patient were collected appropriately after getting consent and asent. Samples were collected in accordance with SOPs. Aseptic technique was implemented in all the steps of specimen collection and inoculation on the broth media to minimize contamination.

Analytical

All materials equipment and procedures were adequately controlled. All culture media was prepared according to the direction of the manufacturer. Sterility of media was checked by incubating overnight at 35 ± 2^{o}c and performance of media was tested using the known strain growth, biochemical test and antimicrobial susceptibility test results were confirmed by experienced microbiologists working in the microbiology unit of the study site.

Post-analytical

Results were checked for legibility, completeness and recorded before entry to statistical tool. All laboratory isolates were stored as per the SOP of the study site.

5.8 Data Analysis and Interpretation

Collected data were processed, edited, and analyzed using *SPSS version 20* statistical. During analysis frequencies of the different variables were determined; chi-square test, and logistic regression were used for statistical analysis of data. when 'P' value were less than 0.05considered as statistically significant.

5.9 Ethical Consideration

The project proposal were defended to College of Health sciences Department of Medical Laboratory Sciences of Addis Ababa University. And ethical clearance were obtained from Departmental Research and Ethics Review Committee (DRERC) of Medical Laboratory Sciences, from Addis Ababa Health Bureau Ethical Review Committee and permission letter were obtained from hospitals administration. The purpose and procedure of the study were explained for hospitals administration and study participant. Those who agreed to participate were asked to sign consent form and documented. For the participants found to have bacterial isolates, results were sent to the responsible physician as soon as possible. Codenumbers are used to assure the confidentiality of participant's information.

5.10 Dissemination of results

The findings of this study will be presented to the department of Medical Laboratory Sciences for public defense. The result will also be communicated to Zewditu hospitals. Effort will be made to publish the findings in journals

5.11Operational definition

Multiple drug resistant (MDR), resistant to one or more antibiotics in three or more classes of antimicrobials agents by bacterial isolate defined as MDR.

BSI: Blood stream infection defined as the presence of viable bacteria confirmed by laboratory as positive by performing blood culture in the blood (i.e., bacteremia)

A febrile patient: when a single auxiliary temperature of >37.5°C and sustained over a 1-hour period.

Mortality during hospital stay: mortality which is followed until their discharge .

Community-onset infection:refers to an infection acquired in the community which is Occurring 48 hours or more before admission**.**

Nosocomial infection:refers to hospital acquired infection which is occurring within 48 hours or more after admission.

6 .Results

6.1 Socio-demographic characteristics

A total of 198 study participants clinically suspected cases of bacteremia were included in the study. Of these participant , 93(47%) were females and 105 (53%) were maleswith the age range of one day to 80 years. The mean age was 11.2 $\pm$ 17.8 years, (See table 3). In the age groups of <1 year-old recorded the highest participation 106(53.5%) and the >50 years had the least of them 12 (6%) . The majority of the patients 99 (50%) were admitted to the pediatric ward while 45 (22.7%) wereNICU and the remaining patients, 27 (13.6%) were admitted to the medical ward and 13 (6.6%) were in emergency branch of the medical admission unit. Eight (4%) and 5(2.5%) were included from the departments of surgical operation and gynecology, respectively (see table 3)

Table 3.Sociodemographic characteristic of febrile patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia (May–Dec 2019)

variables	Categories	Frequency	Percent
Sex	Female	105	53
	Male	93	47
Age	<1 year	106	53.5
	1-14	38	19.2
	15-30	23	11.6
	31-50	19	9.6
	>50	12	6.1
Ward	Pediatric ward	99	50
	Neonatal ICU	45	22.7
	Medical ward	27	13.6
	Emergency	13	6.6
	Surgical ward	8	4
	Gynecology	5	2.5
	Medical ICU	1	0.6

6.2 prevalence of blood stream infection and bacterial profile

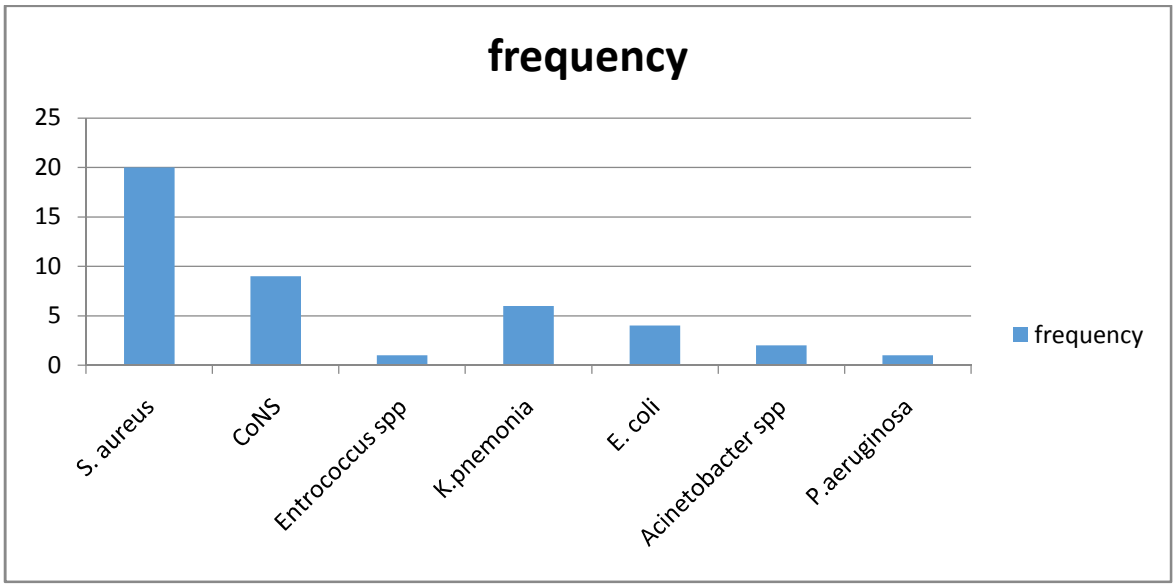
During the study period, a total of 198 blood samples were analyzed and bacteremia was confirmed in 43 (21.7%) cases from these culture positive patients 27/105 (25.7) were males and 16/93 (17.2%) were females. Culture positivity rate was highest in the age group of >50 5/12 (41.6%) followed by those in the age group of <1 years 28/106(26.4) (Table 4)

Table 4.Prevalence of bacteremia among sex and age groups of febrile patients at zewditu memorial hospital, Addis Ababa, Ethiopia (May–Dec 2019)

Age group		Number of tested n	Positive result n (%)
<1	Male	55	18 (32.7)
	Female	51	10 (19.6)
	Total	106	28 (26.4)
1-14	Male	21	2 (9)
	Female	17	2 (11)
	Total	38	4 (10.5)
15-30	Male	12	3 (25)
	Female	11	1(9)
	Total	23	4 (17)
31-50	Male	10	1 (10)
	Female	9	1 (11.1)
	Total	19	2 (10.5)
>50	Male	7	3 (42.8)
	Female	5	2 (40)
	Total	12	5 (41.6)
Total		198	43(21.7)

Out of 198 blood cultures 43(21.7 %) were positive for different bacteria species, The gram positive and gram negative bacteria accounted for 69.7 % (30/43) and 30.3 %(13/43), respectively .From the total of 43 bacterial isolates the commonest gram positive organisms were *Staphylococcus aureus* 20 (66.6%) and *Coagulase negative Staphylococcus* (*CoNS*) 9 (30%), while *Enterococcus* accounts only one isolate 1(3.3%).Among gram negative bacterial isolates the most predominate organisms were*Klebsiella pneumonia* 6 (46.1%) followed by*Escherichia coli 4(30.7%)* and *Acinobacter spp*2 (15.3%) (Fig 1)

Figure 1. The frequency of bacterial isolates from patients with bacteremia at Zewditu Memorial Hospital, Addis Ababa, Ethiopia from(May –Dec 2019)



6.3 Antimicrobial resistance of blood culture isolates

In antibiotic susceptibility of the bacterial isolates showed resistance for gram positive bacteria ranges from zero to 100%. *S.aureus*isolates had shown highest resistance toTrimethoprim-sulphamethoxazole16 (80%), and lowest to Vancomycin .The other isolates *CoNS*showedhighest resistance to five antibioticspenicillin7 (78%)Trimethoprim-sulphamethoxazole7 (78%) Doxycycline 7(78%)Tetracyclin7 (78%)and Gentamycin7(78%)and the lowest was seen in vancomycin .Complete Sensitivity were seen for to Vancomycin in all the gram positives. Clindamycin became highly sensitive antibiotic for gram positive bacteria next to Vancomycin. (Table 5)

Table 5. Antibiotics resistance patterns of Gram positive bacteria isolated from febrile patients, at zewditu memorial hospital, Addis Ababa, Ethiopiafrom May to Dec2019

Organisms No	**Resistance of Antibiotics tested No (%)**										
	P	OX	CLD	ERY	SXT	DOX	T	CIP	C	GEN	VA
S. aureus (n=20)	15 (75)	6 (30)	1 (5)	14 (70)	16 (80)	13 (65)	14 (70)	8 (40)	8 (40)	14 (70)	0
CONS (n=9)	7 (78)	3 (33)	1 (11)	6 (66)	7 (78)	7 (78)	7 (78)	4 (44)	4 (45	7 (78	0
Enterococcus(n-1	1 (100)	NA	NA	NA	NA	1 (100)	1 (100)	0 (0.0)	0 (0.0)	NA	0 (0.0)
Total (n=30)	23 (76.7)	9 (30)	2 (6.6)	20 (66.6	23 (76.7)	21 (70)	22 (73.3	12 (40)	12 (40)	21 (70)	0 (0.0)

Key: *CONS* : Coagulase negative Staphylococcus;; P: Penicillin; Ox: Oxacillin:CLN: Clindamycin; ERY: Erythromycin; SXT: Trimethoprim-sulphamethoxazole;T: Tetracycline; DOX: Doxycycline; CIP: Ciprofloxacin; CHL: Chloramphenicol GEN: Gentamycin;; CTX: Cefoxitin;VA: Vancomycin

The range of resistance for gram negative bacteria in this study was from zero to 100%. *Klebsiella pneumonia* was highly resistant to Amoxicillin clavunate, cefazolin ,Ceftriaxoneand Trimethoprim-sulphamethoxazole (83% each),. *Escherichia coli* showed 25% resistance

toTrimethoprim-sulphamethoxazole and 100% sensitivity to Amikacin.Relatively80(%) of isolates expressed susceptibility towards Amikacin**.** Piperacillin/Tazobactam was effective for gram negative bacteria next to Amikacin. (Table 6)All infections isolated during study periodwere monomicrobial.

Table6.Antibiotics resistance patterns of Gram Negative bacterial isolated from febrile patients From May to Dec 2019, at Zewuditu Memorial Hospital, Addis Ababa, Ethiopia

Organisms No.	**Resistance of Antibiotics tested No (%)**													
	AMP	GEN	AUG	KZ	CRO	CXM	MRP	CIP	TZP	SXT	CAZ	C	TE	AK
K. pneumonia (n=6)	6 (100)	3 (55)	5 (83)	5 (83)	5 (83)	4 (66)	3 (50)	2 (33	2 (33)	5 (83)	4 (66)	3 (50	4 (66)	1 (17)
E.coli (n=4)	2 (50)	2 (50)	2 (50)	3 (75)	3 (75)	3 (75)	2 (50)	2 (50	1 (25	3 (75)	2 (50)	2 (50	3 (75)	0 (0.0)
Acinetobacter spp (n =2)	NA	2 (100)	NA	NA	1 (50)	NA	2 (100)	0 0.0	O (0, 0	2 (100)	2 (100	NA	1=I (50)	1 (50)
P. aeruginosa (n=1)	NA	1 (100)	NA	NA	NA	NA	0 (0.0)	1 100	0 0.0	NA	1 (100	NA	NA	0 (0.0)
Total (n=13)	8 61.5	6 45.2	7 53.8	8 61.5	9 69.2	7 53.9	7 53.9	5 38.5	3 23.1	10 76.9	9 69.2	5 38.5	7 53.1	2 15

Key: AMP: Ampicillin; GEN: Gentamycin: AUG: amoxillin-Clavunate; KZ; Cefazolin; CRO: Ceftriaxone CTT: Cefotetan. CXM: Cefuroxime; MRP: Meropenem; CIP: Ciprofloxacin, TZP: Piperacillin Tazobactam; SXT: Trimethoprim-sulphamethoxazole; CAZ: Ceftazidime; C: Chloramphenicol; TE: Tetracycline .AK; Amikacin

6.4 Mortality rate of blood stream infection

Overall mortality observed during the study period was 23% (45/198) and the rest of 77 % (153/198) survived cases and were discharged. Mortality among patients with positive blood culture was 51.1% (22/43), which was significantly higher than mortality recorded for patients with negative blood culture results14.8% (23/155)P=0.002. Patients with positive blood culture had a threefold higher mortality than those without bacteremia (50.1% vs. 14.8%). (Table 7)

Table 7. Summary of outcomes by sex and age from febrile patients at zewditu memorial hospital, Addis Ababa, Ethiopia (May-Dec 2019)

Ages categories	Number (%)	Sex(No)	Death		Discharged (n)
			Culture Positive (n)	Cultures Negative (n)	
< 1 yrs.	106 (53.5)	Female(51)	5	6	40
		Male (55)	11	6	38
1-14 yrs.	38 (19.2)	Female(17)	1	1	15
		Male(21)	2	3	16
15-30 yrs.	23 (11.6)	Female(11)	0	1	10
		Male(12)	1	2	9
31-50 yrs.	19 (9.6)	Female(9)	1	1	7
		Male(10)	0	1	9
>50 Yrs.	12 (6)	Female(5)	0	1	4
		Male(7)	1	1	5
Total	198	Female(93)	22	23	153
		Male(105)			

The case fatality rate associated with an episode of *S. aureus* bacteremia was 36.4% (8/22). It followed by *CoNS , K. pneumonia* and *E.coli* which was observed in 22.7% (5/22). 13.6% (3/22) and 13.6% (3/22), respectively .Over half 23/43(53.5%) of the patients in our study acquired their bloodstream infection in the hospital settings and 20/43(46.5%) were community acquired.

The mortality of bloodstream infection was 51.1% (22/43) ,27.3 %(6/22) for community-onset versus 72.7 %(16/22) for nosocomial bloodstream infections.(Table 8)

Table 8 Summary of mortality rate by organism for both community-onset and nosocomial bloodstream infection at zewuditu memorial hospital, Addis Ababa, Ethiopia (May-Dec 2019)

Sr. no	Organism	Number of deaths due to:			No deaths	
	Gram positive	Community-onset	Nosocomial infection	Total no. of deaths	Community-onset	Nosocomial
1	*Staphylococcus aureus*	2	6	8	9	3
2	*CoNS*	1	4	5	1	3
3	*Enterococcus*		1	1	-	-
	Gram negative					
4	*Klebsiella pneumonia*	2	1	3	3	
5	*Escherichia coli*	1	2	3	1	
6	*Acinobacter spp*	0	2	2	-	-
7	*Pseudomonas aeruginosa*	0	0	0	-	1
	Total	6	16	22	14	7

6.5 Risk factors and Clinical variables

An abnormal temperature was found in 95.5 %(189) of patients. The most prevalent clinical features were tachypnea manifested by a raised respiratory rate in 194 out of 198 cases (98 %) and tachycardia in 192 out of 198 (97 %) patients.

The main infection sites were: respiratory tract, (69/198; 32.6 %), followed by gastrointestinal tract, (29/198; 15 %) and urinary tract, (23/198; 12 %). More than sixteen percent (31/198) of cases had more than one site infection involved.

Indwelling medical device such as intravenous (IV) usage were observed in 96 (65%) 54(36%) other different device of the study participant(table 9)

Among admitted cases the length of hospital stay ranges from 2 to 45 days. The median length of stay among culture positive patient is 16 days while the culture negative patient was 7 days which shows excess (9 days) length of stay this may added proportion of hospitalization due to the infection complication The mean of length of time From the date of admission to blood sample collection was 2.44 days

Table 9. Risk factor and clinical variablesof febrile at zewditu memorial hospital, Addis Ababa, Ethiopia (May-Dec 2019)

variables	Category	Total (n)	Culture positive	Culture Negative	COR	AOR	P value
Sex	Male	105	27	78			
	Female	93	16	77	0.71	-	0.31
Age	<1	106	28	78	5.08	0.216 (0.001-4.06)	0.03
	1-14	38	4	34	3.7	0.645 (0.040-10.42)	0.758
	15-30	23	4	19	2.3	0.290 (0.025-3.43)	0.92
	31-50	19	2	17	2.6	0.851 (0.051-14.25)	0.91
	>50	12	5	7			
Infection sites	Respiratory tract	69	19	50	.000	-	0.999
	Intestinal tract	29	5	24	.000	-	0.999
	Urinary tract	23	4	19	.000	-	0.999
	Nervous	32	4	28	.000	-	0.999
	Soft tissue	7	1	6	.000	-	0.999
	Cardio vascular	3	0	3	1.000	-	1.000
	> one site infection	31	10	21	.000	-	0.999
	unknown	4	0	4			
Indwelling device	Yes	150	42	108	4.2	4.094(1.639-10.229)	0.003
	No	48	1	47			
Length of hospital stay	>10day	65	23	42	52.1	0.016(0.001-0.207)	0.001
	5-10 day	63	19	44	22.2	0.049(0.006-0.393)	0.005
	<5 day	70	1	69			
Source of infecton	Community	20	20	-	0.021	15.7 (3.5-2-70.5)	0.000
	Nosocomial	23	23	-	0.010	144.4(12.4 -1676.6)	0.000
	Negative	155		155			
Hospital out come	death	45	22	23	2.28	1.772(1.642-10.268)	0.002
	survival	153	21	132			

7. Discussion

In this study there were 198 study participant who meet the inclusion criteria, from 198 study participant the number of male were 105 (53%) and female 93(47%) the number of males were more than female which was consistent with previous studies in Ethiopia [52, 53] , Nigeria[54,55] and Kenya[56] Although, this study showed that males were more infected than females (25.7% vs. 17.2%) respectively, there was no statistically significant difference in gender variation in this study. This slight variation has been previously reported by various studies[57, 51]. The mean age of this study was 11.2 $\pm$ 17.8 years, ranges from a one day to eighty years. In the age groups of <1 year-old recorded the highest participation 106(53.5%) and the >50 years had the least of them 12 (6%) The results of this study showed that BSI was more prevalent in under one year old ages and > 50 year adults. This finding is supported by different other studies [37, 54]. There was a statistically significant association between age of patients and BSI (P= 0.03) indicating that high BSI was seen in less than one year age group. This is comparable with study reported from different area [57, 51] and also >50 years old [49]. This might be due to fact that extreme ages are susceptible to infection because of lower immunity. And also the age group of <1yearare exposed to infection because they are poor hygiene practices, may be bottle feeding which may contaminate the infants easily and get infections [25, 4849].

In this study the overall frequency of bacteria isolated from blood culture was 43 (21.7%). This was comparable with study conducted into different area of Ethiopia, 21.4% [48] in Addis Ababa, 18.2% in Gonder, [58]. However, it was lower compared to the study done other developing countries where routine blood culture was performed such as in Nigeria (35%) [31] and Uganda (37%) [34],it may be due to the use difference in blood culture system, duration of study period as well as patients probably received clinical care, including antimicrobial agents, in other health care setting before coming to the study referral hospital infection prevention practice or the increment of multidrug resistant organisms for different reasons and difference intime of the studies.

Sixty nine percent of our findings showed that BSI was caused by gram positive bacteria and 30.3% was caused by gram negative bacteria. Similar Study findings also reported that higher BSI caused by gram positive bacteria than gram negative bacteria reported from Ethiopia (72.2% vs. 27.8%) and (69% vs. 31%) [25, 57]. Another study from India reviled that (53% vs. 39%)

and (59.3% vs. 29.6%) also showed higher BSI caused by gram positive bacteria than gram negative bacteria respectively [26,40].

This study revealed that *S.aureus* and *CoNS were* the first and second most prevalent gram positive bacteria agents isolated in this study area. Different studies also showed similar result [25, 58, 49, 26].This can be explained by the fact that this pathogens are commonly found in the hospital environment which might be contaminate among admitted patients and increase the infection rate [41]. In most studies *CoNS* were considered as contaminant [42,44], but now they are potentially important pathogens and their increasing incidence has been recognized due to the routine use of indwelling medical devices such as vascular grafts, prosthetic heart valves and joints now a days *CoNS* consideredas nosocomial pathogens and health- care related infection [46, 44]. *KlebsiellaPneumonia* and *Eschersia coli* were the predominant isolated gram negative bacteria with prevalence rates of (13.95%) and (9.3%) respectively. This results showed similarity with previous study in Addis Ababa, Ethiopia where the gram negatives *Klebsiella spp*.(9.7%) and *E. coli* (8.1%) were the predominant[48].

Increase in resistance of antimicrobial agents was the second result revealed by this study and this is become the major problem for the treatment of common infections. It has also indicated that most resistance range for both gram positive and gram negative organisms was ranges from0-100% which was comparatively similar with the study findings in Ethiopia [52] The commonly used drugs showed high resistance by the bacteria identified, it makes empirical treatment difficult and challenging. In our study multiple drug resistance (resistance to three or more drugs) was 90.7% which is comparable with the study done in Ethiopia [52, 53] with 89.1% and 92.7% respectively but the study done in South Africa, Johannesburg [56] was relatively low (32.5%). This difference might be due to difference in antibiotic prescription policy, inappropriate use of antimicrobials, lack of laboratory diagnostic tests, and unavailability of updated guideline for the selection of antibiotic.

In this study 93.4% of gram-positive bacteria were sensitive to Clindamycin and gram negative bacteria was sensitive to Ciprofloxacin (61.5%) and to Cefuroxime (46.1%) which was comparable with other studies in Ethiopia [58], in Zambia [35], in India [26], and in Nigeria [50] In the present finding, Vancomycin was a highly active drug against Gram positive organisms with 100 % sensitivity. Similarly in other studies Vancomycin was highly effective drug against Gram positive bacteria [46,47]. However, this should not be expected that Vancomycin activity

continues for a long time as, there have been reports of Vancomycin resistant*S. aureus* (*VRSA*) from studies [23, 49].

In general, ciprofloxacin was shown to be the effective drug against the tested Gram positive and Gram negative bacteria isolates. Similar findings have been reported in different studies done in Ethiopia and in Iran [58, 58] Most of the GNB were multi drug resistance 83% to Amoxillin-Clavunate, 69.2.% resistant to Ceftazidime and 57.1% resistant to Ceftriaxone. Meropenem; resistance was detected in 100%isolates of *Acinetobacter50*% of *K. pneumonia* and 50% of *E.coli*. This high resistance of pathogens could be explained by the frequent use of these drugs, since this drugs being the first line drugs in infections cases, inappropriate use of antibiotics and few people self-prescribing antibiotics and treatment by the patients due to availability of antibiotics on the market in the study area [51,59].

Mortality observed during the study period was 23% (45/198) and the rest of 77 %(153/198) survived cases was discharged. Mortality among patients with positive blood culture was 51.1% (22/43), which was higher than mortality recorded for patients with negative blood culture results14.8% (23/155) and has significant association P=0.002with blood stream infection and this finding was comparative with other study conducted inAddis Ababa, Ethiopia which was 50.0% with positive culture result vs 9.8% with negative blood culture result [27]. Medical device that are inserted in different body parts such as intravenous (IV) usage were observed in 96 (65%) of the study participant and it has association with bacteremia p = (0.003) this could be due to the fact that the indwelling device can get accesses to the blood stream and it has a risk of contamination, this result was supported by other study conducted in Ethiopia [53]Site of infection identified in this study include respiratory tract, (69/198; 32.6 %), followed by gastrointestinal tract, (29/198; 15 %) and urinary tract, (23/198; 12 %) More than sixteen percent (31/198) of cases had more than one site infection involved another study conducted at UK has also comparable results pulmonary 76/146(52%) abdominal 45/146(30%) urinary 16/146 (11%) others 10/146(7%)[61] but association was not seen in this study for this specific site of infection Since this study has been done on one health care location and the shorter study period employed, we performed relatively lower number of cultures. As a result, the finding may not be truly representative. Nevertheless, the data are of value as a basis for future study with respect to bacterial profile and antimicrobial susceptibility of sepsis in Ethiopia.

8 Limitation of the study

Mortality of the patient was followed until their discharge which can be considered as mortality during hospital stay

Isolation of fungi and anaerobe was not done due to hospital set up

9 Conclusion

In this study, the overall prevalence of blood stream infection was 21.7%.High incidence of septicemia has found in the hospital settings. The most obvious finding of this study is that most of the isolates were high rate of resistance to most commonly used antibiotics to treat bacterial infections and mortality was strongly associated with bloodstream infection which increase mortality risk was increase three fold .Therefore up to date etiological data for major pathogens causing blood stream infections and determining pathogen antibiotic susceptibility patterns to evaluate the changing trend of antimicrobial is very important to reduce the incidence of blood stream infections and special attention has to be given regarding to infection prevention control and antibiotic prescription policy

10. Recommendations

On the bases of these prospective crossectional study findings, the following are recommended

- ✓ Periodic and continuous surveillance of etiologic agent and their antibiotic susceptibility pattern must be studied to prevent further emergence and spread of resistant bacterial pathogens.
- ✓ Treatment of bacteremia should be based on culture and sensitivity rather than on empirical antibiotic treatment
- ✓ Because of high incidence of septicemia found in the hospital settings special attention has to be given regarding to infection prevention control and antibiotic prescription policy.
- ✓ Efforts should be made to improve the quality of the bacteriological laboratory diagnosis services.
- ✓ Further research to determine the most feasible combination of antibiotics for the management of blood stream infection is urgently needed

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Annex

Annex I. General information for the study participants (English version)

Introduction

My name is WubetTesfayeand I am MSc student of Addis Ababa University, School of Medical Laboratory Sciences. I am doing research entitled morbidity, mortality,Bacterial profile and antimicrobial Sensitivity pattern of blood stream isolates among febrile patients at zewditu hospital, Addis Ababa, Ethiopia With advances in health care system, threat to Blood stream infection (BSI) still remains high. Currently Blood stream infection (BSI) continues to affect hospitalized patients and results in morbidity, mortality and additional costs as different study indicates. So this study will indicate bacterial profile from Blood stream infection suspected patients in Zewditu memorial hospitals in Addis Ababa, Ethiopia, commonly isolated bacteria and antimicrobial drug resistance patterns

What is the reason of this study?The objective of this research To Determine Bacterial profile and antimicrobial Suseptability pattern of blood stream isolates among febrile patients admitted in zewditu Memorial Hospital, Addis Ababa, Ethiopia. If you agree to participate in the study, about blood collection blood will be collected for culture, as per the standard. Additionally some clinical information will be interviewed from patients

Will the information be confidential All the data obtained will be kept confidential by placing in looked rooms/ cabinets, only the study personnel will have accesses to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names

What are the costs?All the investigations performed for the participants of this study will be free of charge. **.**

What about compensation?You will not be compensated for your participation in this study but the patient may benefit from the study .

What about my rights to decline participation or withdraw from the study Yourparticipation in this study is purely voluntary, and you may stop the participation or you may refuse to answer

some of the questions if you feel uncomfortable. You are free to participate in this study or you can withdraw your consent anytime, which will not incur/involve any penalty or loss of benefits to which you are entitled.

What about the harm which may happen in the study? This research involvesbloodcollection from patients in this hospital. There will be a little bit of pain while collecting the sample otherwise are no major risks and will not cause the patientsno harm.

Assurance of the principal investigation put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of reports for all stakeholders of the research project.

(PI): signature_____ Date_____

Note: if you have any questions about this study, feel free to ask now or anytime throughout the study by contacting:

PI Address: WubetTesfaye: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

E-mail: wubet2004@gmail.com: Tele: +251929012503

Annex II. Information sheet (Amharic version)

መግቢያ

በአዲስ አበባ ዩኒቨርስቲ በጤና ሳይንስ ኮሌጅ በሜዲካል ላቦራቶሪ ትምህርት ክፍል ማስትሬት ዲግሪ ተማሪ ስሆን በዘውዲቱ ሆስፒታል ውስጥ የተኙ ከፍተኛ ሙቀት የላቸው ታካሚዎች የሚጋለጡዋስያን መኖራቸውና ለበሽታው የሚጋለጡዋስያን ለመድሀኒት ያላቸው ምላሽ ላይ ምርምር እሰራለሁ፡፡ ስለዚህ ይህ ጥናት በዘውዲቱ ሆስፒታል አዲስ አበባ ከተማ ያለውን ዋና ዋና የተዋህሲያን አይነት ለይቶ ለማወቅና ተዋስያን ለመድሀኒት ያላቸው ምላሽ አፋጣኝ ምላሽ ለመስጠት ይረዳናል ::

የጥናቱ ምክንያት

በዘውዲቱ ሆስፒታል ውስጥ የተኙ ከፍተኛ ሙቀት የላቸው ታካሚዎች በዚህ ጥናት ላይ ለመሳተፍ ፈቃደኛ ከሆኑ ከእያንዳንዱ ዋርድ ከፍል ካለ የተኙ ከፍተኛ ሙቀት የላቸው ታካሚዎች ላይ የደም ናሙና የሚወሰድ ሲሆን ለበሽታ የሚጋለጡዋስያን መኖራቸውን ምርመራ ማወቂያ ይሰጣል በተጨማሪ አንዳንድ መረጃዎች በጤና ባለሞያዎች ላይ ሊወሰዱ ይችላሉ፡፡

የጥናቱ ሚስጥራዊነት

የተገኘው መረጃ ሁሉ ሚስጥራዊነት በተቆለፈ ክፍል ወይም ሚስጥር ውስጥ በማስቀመጥ የጥናቱ ባለቤት ብቻ እንዲገኘው ይደረጋል፡፡ በምርመራ ወቅት የተለየ ኮድ በመጠቀም የተመረመሪው የታካሚው ስም እንዳይታወቅ እንከላከላለን፡፡

የክፍያ ጉዳይ

በጥናቱ ላይ በሚሳተፉ ላይ ለሚደረጉ ሁሉም አይነት የላቦራቶሪ ምርመራዎች ውጤት ክፍያ ነፃ ሆኖ በተመራማሪው ይሸፈናል፡፡

የማካካሻ ጉዳይ

በጥናቱ ላይ ስለተሳተፉ ምንም አይነት ማካካሻ አያገኙም ነገር ግን ህመሙ ከሚደረገው ውጤል ቅምርመራ በሽታው በጊዜ ተገኝቶ ለተሻለ ህክምና ትይታከሚል፡፡

ከጥናቱ ላይ ያለመሳተፍና የማቋረጥ መብት ጉዳይ

እርሶ በጥናቱ ላይ የሚሳተፉት ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው፡፡ ስለዚህ እርሶ በማንኛውም ሰዓት በጥናቱ ላይ መሳተፍ ማቆም ይችላሉ፡፡ ያለመሳተፍ መብትዎ ነው፣ አንዳንድ ጥያቄዎችን ካልመለሱ ያለመመለስ መብት አለዎት፡፡ ይህን በማድረግዎ ምንም አይነት ቅጣት አያስከትልብዎትም፣ ማግኘት የሚገባዎትን ሁሉ ነገር አያሳጣዎትም፡፡

ከጥናቱ ጋር የተያያዘ ተጓዳኝ አደጋ ጉዳይ

ይህጥናትበሆስፒታልዉስጥየሚሩብዘምርመራዎችንያካትታል፡፡ሆኖምየተለያዩናሙናዎችስወሰዱከሚመነሣመነሻ ስተቀርምንምአይነ ትየ ከፋጉዳትበጥናቱምክንያትአይከሰትም፡፡

የተመራመሪመሚጋገጫ

እኔከስርፊርማየንያስቀመጥኩትሳይንሳዊጥናቱንበግብረገ ብበተሟሟልኩእንደማከሀደናየ ጥናቱንርፖርትለሚመለ ከታቸዉለድርሻአካላትእንደማቅርብበፊርማየአረጋግጣለሁ፡፡

ዉበትተስፋዬ(ዋናተመራማሪ)፡ ፊርማ_____ቀን_____

ማስታወሻ፡ ስለጥናቱማንኛዉምክያቄካለዎትበማዕከላዉኤድረሻነ ፃሆነ ወደጠይቁን፡

የዋናተመራማሪአድራሻ፣ ዉበትተስፋዬበአዲስአበባዩኒቨርሲቲ፣ የጤፍሳይንስኮሌጅ፣ የህክምናላቦራቶሪትምህርት ክፍል፤ አዲስአበባኢትዮጲያ፡፡ ኢሜል: wubet2004@gmail.com: ስልክ: +251929012503

Annex III. Consent form for hospital (English version)

I have been informed about the study which plans to isolate Bacterial profile and antimicrobial Sensitivity pattern of blood stream isolates among febrile patients admitted in zewditu hospital, Addis Ababa, Ethiopia. The objective and the application of the study were briefly explained to me. I have been well informed of my right to refuse information, decline to cooperate and dropout of the study if I want and none of my actions will have any bearing at all on the hospital and overall health care .

It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give blood sample to mentioned study. I agreed that the specimen would be tested for bacterial pathogens. I have had the opportunity to ask questions about the project and received clarifications to my satisfaction in a language I understand. I was also informed that the findings of the sample will be given to the health facility and that I may ask the information if I want .

If literate

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy) _____

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy) _____

Annex IV. Consent form for Hospital (Amharic version)

በዘወዲቱሆስፒታልሜዲካልዋርዶውስጥየተኝፍተኛመጮትየላቸዉታካሚዎችየሚጋልጡዋቸዉንመኖራቸውናለበሽ
ታዉየሚጋልጡዋቸዉንለመድህኒትያላቸዉማሽ፡ ስለሜረገ ማሞጎሞርበቂሚጀአግንቻለሁ፡ ፡ የጥናቱአላማኹአ
ተግ ባበርበማቱተግ ልፆልናል፡ ፡ በጥናቱላይያለመሳባበር፤ ያለመሳተፍናከተሳተፍከማቋረጥመብትእንዳ
ለኝተረድቻለሁ፡ ፡ እኔባለመሳባበረፃከንያትንምንምአይነ ትበደልበጤተቹማይእንደሜርስብኝተግ ልፆልናል፡ ፡
ስለዚህያለዉሀኔታመሉበመሉተረድቸበመሉፈቃደኝነ ትስለዚህይህጥናትበዘወዲቱሆስፒታልአዲስአበባከተማይያ
ለዉዋናዋናየ ተዋህሲያንአይነ ትለይየለመቅናናተዋስያንለመድህኒትያላቸዉማሽላይበማከተየ ተለያዩናሙ
ናፆችንበተጠየ ቅህጊዚእንዲወስደምሚምሯእንዲከናወንስልፈቃደኛመሆን ንአረጋግጣለሁ፡ ፡ የ ተወሰዱትናሙፆችም
በሽታአምጯዋስያንንለመሞሞናተዋስያኑለመድሃንትያላቸዉንማሽለማወቅእንደሆነ አወቄተስማምቻለሁ፡ ፡ ጥ
ያቄዎችንየ ማቅረብእዲልአግኝቻለሁጥያቄየ ምኣኔበሚዳወጀንቋባግባቡተመልሶልናል፡ ፡ ከዚህማበተጨማሪየ ናሙ
ፆቹዉጤትለህክምናተቋሙእንደሚጥተ ግሮናልስለዚህመጀመሯባስፈለገ ኝጊዜማገናትእንደሚቸልአወቅያለሁ፡ ፡
መፃፍየ ሚችለከሆነ ስምናቀንፅፈዉይፈርሙ

ስም_____ቀን____ፊረማ_____

የ ተመሯመዉስምፍርማ

ስም_____ቀን_____ፊረማ_____

Annex V. Questionnaire

Addis Ababa University Collage of Health Sciences, Department of Medical Laboratory Science. Questionnaire for the demographic characteristics and assessment Morbidity, Mortality among

febrile patients who admitted Zewuditu Memorial Hospital. This information is very valuable to us. The information you provide is completely voluntary and anonymous. We value any information you will be able to provide for us.

Patient Identification

Facility name _____ Year _____ Participant code _____

Participants address (Sub city) _____ Telephone _____ signature_____

Data collector name_____ date_____ signature_____

I. Socio- Demographic Characteristics of the Study participants.

1. Age _____
2. Sex A. Male B. female
3. Marital status married □ single □ window□
4. Monthly income per individual (in Ethiopian birr) _____

   A.0-600 B.601-1605 C.1651-3200 D. 3201-5250 E. 5251-7801 F.7802-10900 G. >10900
5. Educational status A. No formal education □ B. Write and read only □ C. 1-7 grade □ D. 8-12 grade □ E.12+ □ F. First degree & above
6. Occupation Farmer □ Government employed □ Student□ Unemployed□ House wife □ Private business □

II.Clinical and Septicemia associated questions

7. Date of admission (in the hospital)(dd/mm/yy)_____
8. Ward of the patient A. OPD Emergency B.G –Ward C. M- Ward D. S-Ward E .P-Ward F. MICU G. NICU H. Other (specify) _____
9. SIRS [systemic inflammatory response syndrome

   Temperature in °C, _____Heart rate in beat/min_____Respiratory rate in breath/min, _____WBC in cells/ml, _____SBP in mmHg, mean (SD) DBP in mmHg, mean_____
10. When did the patient manifest the clinical future of septicemia?

    A 48 hr. before admission B. 48 hr. after admission

12 Site of infection

A. Respiratory Tract B. Gastro intestinal tract C. Urinary tract D. Nervous system

E. Soft tissue/skin, Bone F. Cardiovascular system G. Multiple site infection H. Unknown

13 Comorbidities

A. Chronic renal disease B. Heart failure C. Stroke D. Diabetes mellitus E Hematological malignancy F .Wound infection, G.HIV/AIDS H. Pneumonia I. Other_____

14 . Clinical features of the patient

A. Fever E. Lethargy I. Jaundice

B. Vomiting F .Abdominal distension J. Shock

C. Chills G. Refusal of feeds

D. Tachycardia H. Difficulty in breathing

15. Is there any indwelling medical device? 1. Yes 2.No

16. If you say yes for no.15 what kind of device?

A, Intravenous devices B. Endotracheal C. Urinary catheters D. Enteral feeding tube E. wound drains F. Oxygen or ventilation support I. Other_____

17. Antibiotics given before sampling_____18. Antibiotics given after sampling _____

III. Laboratory Data.

19. Date of specimen collection_____

20.WBC result_____ 21. Platelet count _____

22. Neutrophil count_____ 23. Hemoglobin r_____

24. Final culture result A. Positive B. Negative

25. Gram stains result_____ 26. Biochemical test_____

27. Organism isolated _____

28. Drug susceptibility pattern

A. Sensitive to _____

B. Intermediate to _____

C. Resistance to _____

29 Hospitals. Length of stay _____day

30 Hospital outcomes

A Death B. Discharge

Comments_____

Name of principal investigator _____

Signature _____ Date _____

V.ማጣቀሻ:

በአዲስአበባዩኒቨርሲቲየጤናሳይንስኮሌጅየሕክምናላቦራቶሪዲፓርትመንትይህመጠይቅበዘወዲቱመታሰቢያሆስፒታ
ልተኝተዉየሚከሰትናመቅትመጠንያላቸውታካሚዎችየስነ ህዝብአወቃቀር፤ የበሽታህዴታእንዲሁምየሞትመጠንለ
ማወቅየተዘጋጀነዉ ።.
ይህመጀመርያበጣምጋአለዉ ፡የምትሰጡትመጀመሪያደረጃነነ ትላይየ ተመረተነ ወሳምትሰጡትማናቸዉመጀምም
ዋጋእንሰጠዋለን፡፡

የ ታካሚዉመላያመጀ

የ ጤተቃመክም----------አመት ----------የ ጥናቱተሳታፊመላያቁጥር----------

አድራሻ፡ ክ/ከ----------ስልክ----------ፊርማ----------

የ ደምመፍየ ወሰደወገለሰብስም----------ቀን----------ፊርማ----------

I .**የ ጥናቱተሳታፊዎችየ ስነ ህዝብወቃቀርመጀ**

1. ዕድሜ----------

2. ጾታ 1)ወንድ 2)ሴት

3 የ ጋብቻህዴታ

1የ ገ ባ 2ያላገ ባ 3 የ ተፋታ

4 ወርሃዊ ቢ(በኢትዮፕያብር)

1.0-600 2.601-1605 3.1651-3200 4. 3201-5250

5. 5251-7801 6. 7802-10900 7. >10900

5የ ትምህርትህዴታ

1.ያልተማረ2. መ ፍናማ በብB. 1-7 ክፍል

4. 8-12 ክፍል5.12በላይ 6. የ መጀመሪያዲግሪናከዛብላይ

6.የ ስራህዴታ

1.ገበሬ 2.የማግስት ሰራተኛ 3.ተማሪ

4.ያልተቀጠረ 5. የቤት እመቤት 6.የግል ስራ

II . ከበሽታው ጋር ግንኙነት ያላቸው ጥያቄዎች

7.አልጋ የያዘበት ቀን-------------------

8 ታካሚው የተኛበት ክፍል

1. ድንገተኛ 2.ማዋለጃ 3. የጠና 4. የቀዶ ጥገና 5 የህፃናት

6. ፅኑ ህሙማን 7. የህፃናት ፅኑ ህሙማን 8.ሌላ__________

9 ሰውነታችን በሽታውን ሲከላከል የሚያሳየው ሁኔታ

የሙቀት መጠን----------------የልብ ምት-------------------

የትንፋሽ መጠን--------------የነጭ ደም ሴል መጠን---------

10. የበሽታው ህመምና ስሜት መታየት የጀመረው መቼ ነው

1. አልጋ ከመያዙ 48 በፊት ሰዓት 2. አልጋ ከያዘ 48 ሰዓት በኋላ

11.ለዚህ በሽታ መነሻ ሊሆኑ የሚችሉ በሽታዎች

1 የመተንፈሻ አካል ችግር 2 የአንጀት ክፍል ችግር 3 የሽንት ቱቦ 4 የነርቭ ችግር

5 የቆዳ፤ የአጥንት ችግሮች 6 የልብና አካባቢው ችግሮች 7 ከአንድ በላይ ቦታዎች 8 ያልታወቀ

12 ተያያዥ በሽታች

1 የኩላሊት በሽታ 2 የልብ በሽታ 3 የአንጎል ጠቃት 4 የስኳር ህመም 5 የጉበት በሽታ

6 ደምና የደም አምራች ክፍሎች በሽታ 6 የኤችአይቪ ኤድስ 7 መተንፈሻ አካል ችግር 8.ሌላ ካለ----

13. የበሽታው ገጽታ

1.ትኩሳት 5.ማዝለፍለፍ 9.ሚፈስፈስ/ማዝለፍለፍ

2. ማስመላስ 6. የሆድመነፋት 10.እረስንመሳት

3.ብርድብርድምላት7.አለመመጣብ

4.የልብማመፍጠን 8.የአተፋፈስችግር

14. ወደሰውነት የገባ/የተሰካ/የተለጠፈየህክምናመሳሪያአለ

1.አዎ 2.የለም

15. ለ 14ኛውጥያቄመልሰአዎከሆነ ምንአይነት ትመላሪያ

1. የደምቧንቧፊኛ 2. የአየርቱቦ3. የሽንትማሸናቱቦ4.የምግቦምከተላለፊያቱቦ

5 የቁስልፈሳሽማከማቻ 6 የጫንቅላትፈሳሽማከማቻ7 ኦክስጅን

8 የመተንፈሻደግፍመሳጫ.ለለ

16.የደምመፍከመጠኑዳበፊትየተሰጠጸረተህዋሲያን----------

17 የደምመፍከተጠኗደ በጎላየተሰጠጸረተህዋሲያን----------

III የላቦራቶሪመጀመሻ

18 ናሙናወየተሰበሰበበትቀን----------

19 የጠቅላላየደምሞርሙዉጤት----------20. ፕላትሌትመጠን----------

21 ኒዉትሮፊልመጠን---------- 22. ሄሞግለቢንመጠን----------

23 የካልቸርዉጤት----------

24 ግሪዎስቴንዉጤት----------25 ባዮኬሚልዉጤት----------

26 የተለየ ዉህዋስያን----------

27. ለመዳኒቱያለዉቦርነት

1 _ብዮር----------

2. መካከለኛ----------

3 የ ሜዐበገ ር----------

28የ ሆስፒታልቆይታ----------ቀን

39የ ሆስፒታልዉዝ

1 ሞት

2 ከሆስፒታልመቀናበት

አስተያየት_____

የ ተመራመዉም_____

ፊርማ_____ ቀን _____

-

Annex VI: Standard operating procedures (SOPs)

SOPs that are required for media preparation, reagent preparation, specimen collection and laboratory investigation are described below. But all procedures that should be followed for media preparation are not included. In general manufacturers' instructions will be followed to prepare, to store, to perform quality control for each regents and medium used in this study.

SOP for MacConkey agar media preparation

Purpose: This procedure provides instructions how to prepare MacConkey agar media

Principle: MacConkey Agar is a selective and differential medium. It is only slightly selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci. Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

Abbreviation:

MA=macConkyy agar

Materials, Supplies and eqipment

1. MA agar powder(oxoid)
2. weighing paper
3. distilled water
4. spatula
1. Balance
2. Autoclave
3. Hot plate
4. Bunsen burner
5. distiller
6. dispenser
7. graduated cylinder
8. flask
9. test tube
10. Ph meter
11. Agar dispenser

Clinical utility: For the isolation and differentiation of clinically important gram negative rods by inhibiting gram positive cocci

SOP for blood agar media preparation

Purpose: This procedure provides instructions how to prepare Blood agar media.

Principle: example it was used for studying irradiated *Escherichia coli* , phages of *Clostridium perfringens*. Meat extract and Peptone provide nitrogenous compounds, vitamins, carbon,

sulphur and amino acids in Blood Agar Base. The medium contains sodium chloride for the osmotic balance. Blood Agar Bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic streptococci. Sheep blood gives best results for Group A Streptococci. When horse blood is used, Haemophilushaemolyticus colonies produce haemolysis and mimic Streptococcus. Haemolytic patterns may vary with the source of animal blood or type of base medium used. Norton found that slight acidic pH (6.8 ± 0.2) favours distinct haemalytic reaction and is advantageous for cultivation of Streptococci and Pneumococci. The low pH helps in stabilization of red blood corpuscles and favours the formation of clear haemolysis zone.

Abbreviation:

BA= blood agar

Materials, Supplies and equipment

- Blood agar base powder
- weighing paper
- distilled water
- spatula
- Sterile sheep blood
- Refrigerator
- Balance
- Autoclave
- Hot plate
- Bunsen burner
- distiller
- dispenser
- graduated cylinder
- flask
- test tube
- PH meter

Procedure:

1. Weigh and Suspend 40grams of powder in1littr distilled water
2. Mix thoroughly and heat to boiling to dissolve the medium completely with frequent agitation
3. When cool adjust the ph to 7.3
4. Autoclave at 15 lbs pressure at (121oc)for 15 minute
5. Cool the medium at 50 oc
6. Add 50ml of difibrnated and mix with gentle rotation sheep blood
7. Dispense 20ml of the solution in to sterile Petri dish
8. Allow the medium to solidify label with date and store at 4 oc

Limitations: Blood agar is not a selective media so we couldn't differentiate microorganisms from the agar. **Clinical utility**: A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms like *Neisseria*, *Streptococci* etc. The medium is often used to observate the forms of haemolysis from pathogenic microorganisms.

SOP for Motility Test

Purpose: This procedure provides instructions for performing the detection of motility of gram-negative enteric bacilli.

Principle: Bacterial motility can be observed directly from examination of the following incubation. Growth spreads out from the line of inoculation if the organism is motile. Highly motile organisms provide growth throughout the tube. Growth of non -motile organisms only occurs along the stab line

Materials, reagent, supplies and equipment required:

•Nutrient Broth
• Semi solid agar
• Pasteur pipette
• Rubber tit
• Wire loop
• Incubator 37ºC
• Bunsen burner

Sample retention: Samples are discarded after 24 hrs Quality control: ATCC strain

Control preparation: the same as indole test

Shigella strain (ATCC) = Non motile

Note: If the colony is not pure re-culture from the stock

Procedure

1. Take nutrient Broth tube
2. Label the tube
3. Take pure colony on MacConkey Agar Plate near Bunsen burner
4. Suspend in Nutrient Broth
5. Vortex the suspension
6. Incubate at 37ºC incubator
7. When the suspension become turbid take drop of suspension aseptically and stab the medium not drop the broth
8. Incubate the inoculated media at 37ºC incubator for overnight
9. Observe change of color (diffusion of bacteria) on the media

Result interpretation: Motility is observed visually by diffuse growth spreading from the line of inoculation. Certain strains of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points only, appearing as nodular growths along the stab line. Non-motile organisms grow only along the line of inoculation.

Limitation:

1. Many organisms fail to grow deep in semisolid media; inoculating pour plates may be advantageous.

2. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Clinical utility: This test is used to determine if an organism is motile or non-motile. Motile organisms are generally bacilli although a few motile cocci do exist.

SOP for gram staining

Purpose: The gram staining reaction is used to help identify pathogens in specimens and cultures by their gram reaction and morphology. Abbreviations: ATCC=American type culture collection.

Principle: Difference in gram reaction between bacteria is thought to be due to differences in th permeability of the cell wall of gram positive and gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violate 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 violate by gram positive organisms may also be due in part to the more acidic protoplasm of these organism binding to the basic dye(helped by the iodine).

Materials,Reagents, supplies and equipment

1. Crystal violate (or gentian violate)
2. Lugol's iodine
3. Aceton-alcohol decolorizer (or ethanol-iodine)

Reagent Stability and storage: Store at room temperature. When kept with tightly stopper, the stain is stable for several weeks.

- 3" X 1" single frosted, pre-cleaned glass slides.
- Wooden applicator sticks
- Immersion oil
- Disposable glove
- Light Microscope
- Staining rack/jar
- Timer with a second hand
- Staining jars
- Racks for drying slides

Sample: Sample type: smear can be prepared from broth cultures and colonies

Amount of sample required: One drop Storage: Fresh colonies at room temperature

Sample retention: Samples are discarded after the test has been done.

Safety precaution

1. Wear protective clothing such as lab coats and disposable gloves during staining and the entire testing process
2. Do not use month pipetting (use pipette filler). Do not eat or smoke while handling specimens

3. Clean and disinfect all spills of specimens or reagents using 10% bleach or other suitable disinfectant. The bleach solution should be made fresh each day.

4. Decontaminate and dispose all specimens and other potentially contaminated materials as if they were infectious wastes.

5. Slides should be broken and buried to prevent their re-use.

6. Acetone-alcohol is highly flammable; therefore use it well away from an open flame.

7. Grease, lint and dust-free clean slides should be used

Maintenance:

Daily Cleaning

Weekly cleaning

Periodic cleaning

Quality control:

S.aureus (ATCC 25923), Gram negative: E. coli (ATCC 25922 before doing any gram staining test.Always check new batches of stain and reagents for correct staining reactions using a smear containing known gram positive and gram negative organisms.

Procedure

1. Fix the dry smear. (When the smear is for the detection of gonococci, it should be fixed with methanol for 2 minute).

2. Cover the fixed smear with crystal violates stain for 30 -60 seconds.

3. Rapidly wash off the stain with clean water

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

5. Wash off the iodine with clean water.

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

7. Cover the smear with neutral red stain for 2 minutes.

8. Wash off the stain with clean water.

9. Wipe the back of the slide clean; place it in a draining rack for the smear to air-dry.

10. Examine the smear microscopically, first with 10X objective to check the staining and to see the distribution of material, and then with oil immersion objective to report the bacteria and cells.

Result interpretation:

Gram positive bacteria ……. Dark purple

Gram negative bacteria ……. Pale to dark red

Yeast Cells……. Dark purple

The reporting should include

1. Number of bacteria present, whether many, moderate, few or scanty.
2. Gram reaction of the bacteria, whether gram positive or negative.
3. Morphology of the bacteria, whether cocci, diplo cocci, streptococci, rods, or Cocco bacilli.
4. Presence and number of pus cells.
5. Presence of yeast cells and epithelial cells.

Limitation

1. Gram positive organisms may lose their ability to retain crystal violate and stain gram negatively because of:

   1.1 Cell wall damage due to antibiotic therapy or excessive heat-fixation of the smear.

   1.2 Over-decolonization of the smear.

   1.3 Use of an iodine solution which is too old, i.e. yellow instead of brown in color (always store in a brown bottle or other light opaque container)

   1.4. Smear has prepared from an old culture.
2. Gram negative organisms may not be fully decolorized and appear as gram positive when a smear is too thick.

Clinical utility: The Gram's stain is used to classify bacteria on the basis of their forms, sizes, cellular-morphologies, and gram reaction. It is a critical test for rapid presumptive diagnosis.

SOP for indole test

Purpose: This procedure provides instructions to detect the production of indole by bacteria growing on media containing tryptophan.

Principle: The indole test determines the ability of an organism to produce indole from the degradation of the amino acid tryptophan. Tryptophan is hydrolyzed by tryptophanase to produce three possible end products –one of which is indole. A coloured product is produced when the indole is combined with certain aldehydes (kovacs Reagent).

Reagents, supplies and equipment

• Nutrient Broth

• Pasteur pipette

• Rubber tit

• Wire loop

• Incubator 37ºC

• Bunsen burner

Sample type; The colony which is suspended in nutrient broth

Limitations: Mixed colony

Sample retention: Samples are discarded after 24 hrs Quality control: ATCC strain

Control preparation:

● Reconstitute the lyophilized sample by TSY broth or Normal saline ● Open the seal and aseptically add 1ml of

broth or Saline ● Inoculate on medias (BAP and MAP) ● incubate at 37ºC incubator ● After overnight incubation(18-24hr) observe the colony and perform Biochemical tests

Escherichia coli ATCC 25922 -dark pink color develops

Enterobacteraerogenes ATCC13048 -fair to good growth blue

Note: If the colony is not pure re-culture from the stock

Procedure:

1. Take nutrient Broth tube

2. Label the tube

3. Take pure colony on MacConkey Agar Plate near Bunsen burner

4. Suspend in Nutrient Broth

5. Vortex the suspension

6. Incubate at 37ºC incubator

7. When the suspension become turbid take drop of suspension aseptically and add drops into broth

8. Incubate the inoculated media at 37ºC incubator for overnight

9. Add drops of kovacs reagent

10. Observe the production of red Ring or not

Result interpretation: Indole positive bacteria such as *Escherichia coli* produce tryptophanase, an enzyme that cleaves tryptophan, producing indole and other products. When Kovac's reagent

(p-dimethyl-amino-benzaldehyde) is added to a broth with indole in it, a dark pink color develops.

Limitations: The indole test must be read by 48 hours of incubation because the indole can be further degraded if prolonged incubation occurs. The acidic pH produced by *Escherichia coli* limits its growth.

Clinical utility: A test used to identify members of the Entero-bacteriaceae family and other Gram-negative

bacilli, based on the ability of the organisms to produce indole from tryptophan

SOP for Urea hydrolysis test

Purpose: This procedure provides instructions for the differentiation of bacteria on the basis of urea hydrolysis.

Principle: The urea medium of Rustigian and Stuart3 is particularly suited for the differentiation of Proteus speciesfrom other gramnegative enteric bacilli capable of utilizing urea; The complete Urea Agar contains 15.0 g/L of agarin addition to the ingredients in the base medium. When organisms utilize urea, ammonia is formed duringincubation which makes the reaction of these media alkaline, producing a red-pink color. consequently, urease production may be detected by the change in the phenol red indicator.

Materials: Reagents:

Nutrient Broth

• 40% urea solution

Material, Supplies and equipment

• Urea Agar

• Pasteur pipette

• Rubber tit

• Wire loop

• Incubator 37ºC

● Bunsen burner Sample type and retention: the same as indole test Quality control: ATCC strain Control preparation:

Refer procedure for indoletest(Similar) *Escherichia coli* ATCC 25922 yellow slant Proteus mirabilis *ATCC* 49565 pink red slant and butt

Note: If the colony is not pure re-culture from the stock Procedure:1. Take nutrient Broth tube

2. Label the tube

3. Sterilize wire loop using the Bunsen burner

4. Using wire loop take a heavy inoculum of growth from an 18-24hours pure culture

5. Suspend in Nutrient Broth

6. Vortex the suspension

7. Incubate at 37ºC incubator

8. When the suspension become turbid take drop of suspension aseptically and add drops into urea slant

9. Incubate the inoculated media at 37ºC incubator for overnight

10. Observe change of color on the media

Interpretation of results: The production of urease is indicated by an intense pink-red (red-violet) color on the slant. The color may penetrate into the agar (butt); the extent of the color indicates the rate of urea hydrolysis. A negative reaction is no color change. The agar medium remains pale yellow.

Limitations:

1. Pseudomonas aeruginosa, for example) or other proteins which raise the pH due to protein hydrolysis and the release of excessive amino acid residues. To eliminate possible protein hydrolysis, perform a control test with the same test medium without urea.

2. Do not heat or reheat the medium because urea decomposes very easily.

3. Urea Agar detects rapid urease activity of only the urease positive

4. Proteus species. For results to be valid for the detection of Proteus, the results must be read within the first 2-6 hours after incubation. Urease-positive *Enterobacter*, *Citrobacter* or *Klebsiella*, in contrast, hydrolyze urea much more slowly, showing only slight penetration of the alkaline reaction into the butt of the medium in 6 hours and requiring 3-5 days to change the reaction of the entire butt.

Clinical utility: Urea Agar and Urease Test Broth are used for the differentiation of organisms, especially the *Entero-bacteriaceae*, on the basis of urease production.

SOP forMuler Hinton Agar

Purpose: This procedure provides instructions to prepare Mueller Hinton agar.

Principle: Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim

Abbreviation:

MHA= Mueller hinton agar

RT= Room temperature

PABA =para-aminobenzoic acid

Materials, Supplies and equipment

- MHA powder
- Distilled water
- Flask
- Petri dish
- Graduated cylinder
- Balance
- Distiller
- Bunsen burner
- Autoclave
- Hot plate
- PH meter

Procedure:

1. Suspend 38 gm of MHA powder & transfer in to a flask containing 1000 ml of distilled water.
2. Boil until the powder completely dissolved
3. Autoclave at 121^{0}C for 15 minute.

4. Final PH at 25oc is 7.3 +/- 0.2.

5. Mix well and dispense aseptically in to sterile Petri dish.

Clinical Utility: MHA is used in antimicrobial susceptibility testing by the disk diffusion method.

SOP forAntibiotic sensitivity testing procedure

Purpose: This procedure provides instructions to determine the drug sensitivity pattern of bacteria using Kirby Bauer disk diffusion method.

Principle: The antibiotic will diffuse in a radial manner from the disc and will inhibit bacterial growth around it

Abbreviation:

ATCC= American Type Culture Collection

GcAp =Gonococci agar plate

CLSI =Clinical and laboratory standards institute

Materials Reagents: 0.5 McFarland standards

Reagent preparation:

Turbidity standard number	**Barium chloride dihydrate (1.175%)**	**Sulfuric acid (1%)**	**Corresponding approximate density of bacteria**
0.5ml	0.5ml	99.5ml	**1x108**

Reagent stability: **f**or six months at +2-+8 oc

Material, Supplies, reagent and equipment

• Muller hinton agar

• Mulleur Hinton agar with 5% sheep blood

• Gc agar and Haemophlus testing media

• Normal saline

• Test tube

• Wooden applicator sticks with cotton

• Antimicrobial disks

• Safety cabinet

• Bunsen burner

• Incubator

• Measuring caliper

• Vortex

• Candle jar

	Sample type	Amount required	Transport and storage	Stability
Sample	Pure colony equivalent to 0.5 McFarland	2ml	The test should be done immediately after the suspension has been made	Stable up to 24 hours

Limitations: Comparing the inoculums turbidity with the standard McFarland is subjective.

Sample retention: Samples are discarded after the test has been done.

Quality Control

Control	Stability	Frequency	Preparation Y/N

ATCC	6 weeks at room temperature	Weekly sub-cultured	Y

Control Preparation

6. Reconstitute the lyophilized standard strain in to 1ml TSY broth or Normal Saline
7. Inoculate in to BAP & MAP
8. Incubate for 16 – 24 hrs at 35 – 37 o
9. Perform sensitivity test
10. Compare the sensitivity result with CLSI guideline.

Note: If the results are out of expected value, repeat the test and take corrective action.

Procedure:

1. Prepare pure colony suspension in to normal saline equivalent to 0.5 Mcfarland standards.

2. Streak on appropriate media the entire surface.

3. Select antimicrobial agents according to the CLSI guideline & Put the disc on the plate aseptically

4. Incubate for 16 – 24 hrs at 35 +/- 2oC

5. Measure zone of inhibition and interpret the result based on CLSI break point.

Result interpretation:

1. Susceptible (S)

The 'susceptible' category implies that isolates are inhibited by the usual achievable concentration of antimicrobial agent when the recommended dosage is used for the site of infection.

2. Intermediate (I)

The 'intermediate' category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug can be used. This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretation, especially for drugs with narrow pharmacotoxicity margin. **3. Resistant (R):** The

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

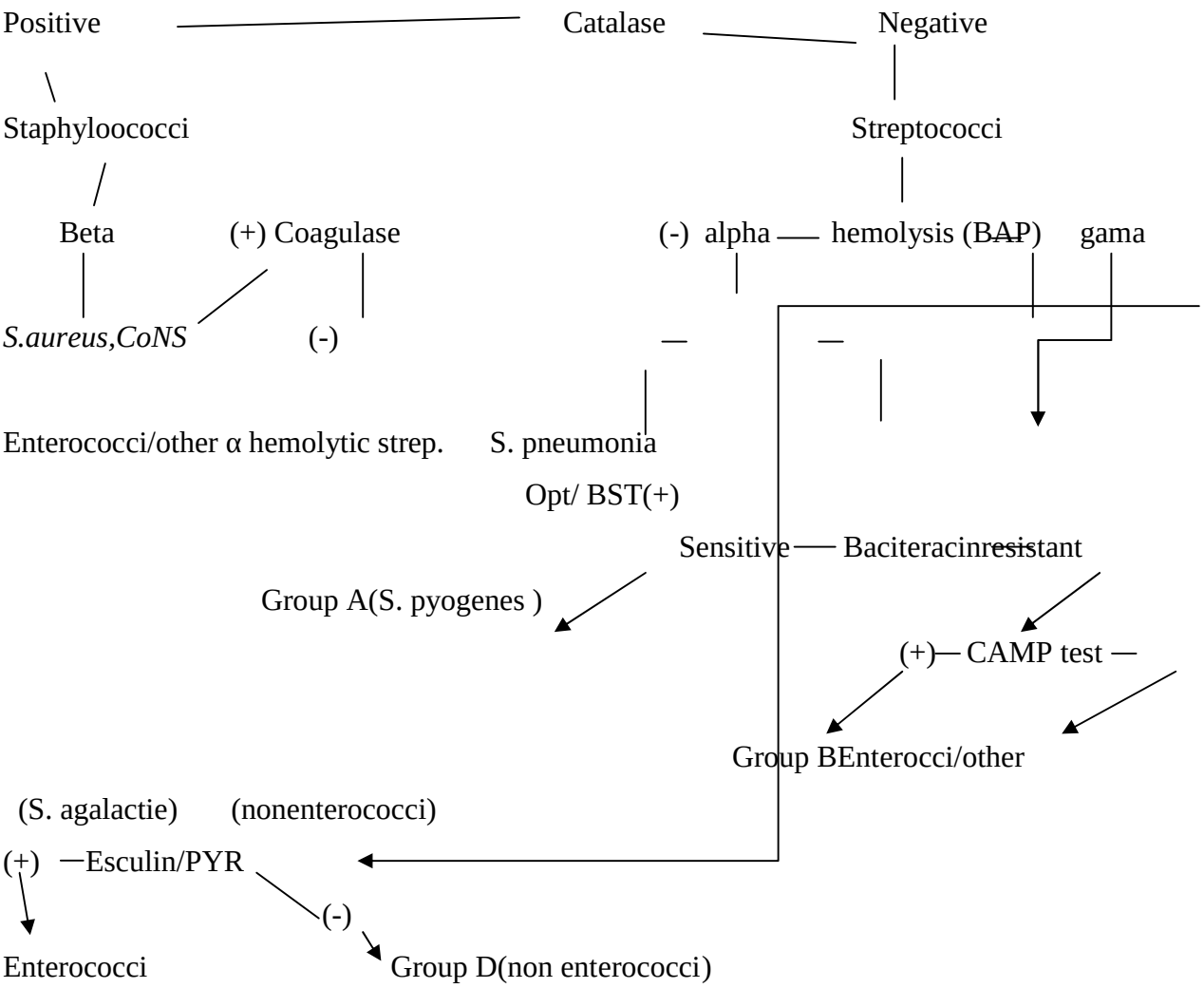
Limitation: The response to antimicrobial therapy in vivo may not always reflect results in vitro.

Clinical utility: To detect in vitro the relationship between an organism and an antibiotic to predict the failure or success of therapy in vivo (in patient).

Annex VII. Flow chart for identification of Gram positive bacteria

Figure 3 flow chart for identification of gram positive bacteria

Gram(+) cocci


Positive
Catalase
Negative
Staphyloococci
Streptococci
Beta
(+) Coagulase
(-) alpha
hemolysis (BAP)
gama
S.aureus,CoNS
(-)
Enterococci/other α hemolytic strep.
S. pneumonia
Opt/ BST(+)
Sensitive
Baciteracinresistant
Group A(S. pyogenes)
(+)
CAMP test
Group BEnterocci/other
(S. agalactie)
(nonenterococci)
(+)
Esculin/PYR
(-)
Enterococci
Group D(non enterococci)

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 duly acknowledged.

M.Sc. candidate: **Wubet Tesfaye(B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor:

KassuDesta(Bsc,Msc,Assocciate professor PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.