Addis Ababa University

College of Health Science

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Department of Medical Laboratory Science

Bacterial etiology of bloodstream infections, prevalence of multidrug resistance and extended beta lactamase production among patients referred to Arsho advanced medical laboratory.

A thesis submitted to the Department of Medical Laboratory Science, College of Health Science, and school of Allied health science, Addis Ababa University in partial fulfillment of the requirements degree of masters of postgraduate program in clinical laboratory science (diagnostic and public health microbiology specialty track).

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This is to certify that the thesis prepared by **SebleSodere**, entitled: **Bacterial etiology of bloodstream infections, prevalence of multidrug resistance and extended beta lactamase production among patients referred to Arsho advanced medical laboratory**and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (diagnostic and public health microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of abbreviation

AAML	Arsho Advanced diagnostic laboratory
ATCC	American Type Culture Collection
AMR	Antimicrobial resistance
AST	Antibacterial susceptibility testing
BSI	Blood stream infection
CLSI	Clinical Laboratory standards Institute
CONS	Coagulase Negative Staphylococcus
ESBL	Extended spectrum beta-lactamase
MDR	Multidrug resistance
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
PI	Principal Investigator.
PLC	Private limited company
SOP	Standard operating procedure
Spp	species
V2C	Viteck 2 compact

Abstract

Background:*Bloodstream infection is one of the most important causes of morbidityand mortality globally. Staphylococcus aureus, coagulase negative staphylococci ,Streptococcus pneumonia are major Gram- positive bacteria in causing blood stream infectionEscherichia coli,Klebsiellapneumoniae, Pseudomonas aeruginosa,Acinetobacterbaumannii are major Gram-negative bacteria in causing blood stream infections. Development of multidrug resistance in major bacteria has increased the morbidity and mortality rate of blood stream infection.*
Objective*: To assess the distribution of bacteria implicated in causing blood stream infections and prevalence of their multidrug resistance profile and Extended spectrum betalactamase production.*

Method: *The present study was a laboratory based cross-sectional study conducted at Arsho Advanced Medical laboratory, Addis Ababa, Ethiopia from January to April 2019; A total of 422 blood samples was collected and inoculated on to primary isolation culture following standards protocols. Species identification and antimicrobial susceptibility testing of bacteria were determined by automated Vitek2 compact system (bio Merieux, France) using AST, GN72 card for gram negatives and AST,GP71 card for gram positive bacteria.*

Result :*Out of a total of 422 samples processed, bacterial pathogens were isolated from 89(21.1%) samples. Among the isolates, 52 were Gram- positive and 37 were Gram negative bacteria.Coagulasenegative staphylococci and Klebsiellaspp were the dominant isolates. Penicillin (92.3%) was the least effective antibiotic against Gram-positive bacteria while cephalotin (91.1%) and cephazolin (89.2%) were the least effective antibiotic against Gram-negative bacteria. Linzolid and Tigcyclne(98.1%) were the most effective antibiotic against Gram positive and piperacillin(86.1%) were the most effective antibiotic against Gram-negative bacteria, Out of 52 isolate of Gram-positive bacteria 30(57.7%) were MDR and out of 37 isolates of gram negative bacteria 20(54%) were MDR.*

Conclusion:*The magnitude of blood stream bacterial infection and the prevalence rate of multi-drug resistant bacterial strains causing blood stream infections were high. These findings warranted the need for continuous investigations bacterial blood stream infection.*

Keywords:*Antimicrobial resistance, Multidrug resistance and Antimicrobial susceptibility*

1. Introduction

1.1 Background

Bloodstream infections (BSI) refersto the condition in which pathogens have entered the bloodstream and are characterized by high morbidity and mortality all over the world.Around 200,000 cases of bacteremia occur annually with mortality rates ranging from 20 - 50% worldwide (1). In case of neonatal sepsis it contributes nearly 13-15 % of all deaths and in developing countries where it contributes between30-50 % in sub Saharan countries including Ethiopia septicemia is an important cause of illness and death in children, the mortality rate approaches 53% which makes it a significant health problem in developing countries(2).

A minor injury occurring during tooth brushing, tooth extraction, abscesses, infected wound or boils, insertion of intravenous of bladder catheter, surgery and existing infections like lung infection, Urinary tract infection (UTI), gastrointestinal tract (GTI), burns or bedsores or from areas of localized disease as in pneumococcal pneumonia, meningitis, pyelonephritis, osteomyelitis, cholangitis, peritonitis, enterocolitis and puerperal sepsis are the predisposing factor for bacteremia (3).

BSI can be categorized as either community acquired or hospital acquired and can be the result of a broad variety of microorganisms (1).*S.aureus*,*CONS*and *E. fecalis*are commonest bacterial causes of BSI from gram positive organisms and *E.coli*and *K.pneumonia* are from Enterobacteriaceae, *Pseudomonasspp* and*A.baumannii* are the commonest from the non-fermenter gram negative organisms (4).

Individuals with bacteremia may develop septicemia, a life threatening condition in which multiplying bacteria release toxins into the bloodstream and trigger the release of cytokines, causing fever, chills, malaise and lethargy, with difficulty in breathing especially in children.(5).BSIs ranging from mild infection to severe sepsis and septic shock causes high mortality, longer hospitalizations, and excessive cost to the patients and healthcare system(6).

Antimicrobial resistance in bacterial pathogens is a worldwide challenge leading high morbidity and mortality in clinical settings. Multidrug resistant patterns in gram-positive and gram negative bacteria have resulted in difficulttotreat or even untreatable infections with conventional antimicrobials .Since the early identification of causative microorganisms and their antimicrobial susceptibility patterns in patients with bacteremia and other serious infections is lacking in many health care institutions, broad spectrum antibiotics are liberally and mostly unnecessarily used. Such practice has, in turn, caused dramatic increases in emerging resistance and when coupled with poor practice of infection control, resistant bacteria can easily be disseminated to the other patients and the environment.(7)

Antibiotics resistance is also a growing problem in developing countries such as Ethiopia. In Ethiopia the unregulated over-the-counter sale of these antimicrobials, mainly for self-treatment of suspected infection in humans, and to a lesser extent for use in animals without prescription, would inevitably lead to emergence and rapid dissemination of resistance (8).

Antimicrobial resistance occurs as a result of the selective pressure of antimicrobial usage and Extended-spectrum beta-lactamases (ESBLs) is also as an important cause of resistance in gram-negative bacteria. Beta-lactam antibiotics are among the safest and most frequently prescribed antimicrobial agents all over the world in treating Gram positive and gram negative infections. Production of beta-lactamases is the most common mechanism of bacterial resistance to these antibiotics(9).

Extended spectrum beta lactamases(ESBL)areenzymesproduced by Enterobacteriaceae that hydrolyze and inactivate mostbeta-lactams. Theyarefrequently encoded by plasmids that carry genes conveyingresistancetootherantibioticgroups,suchasaminoglycosidesandfluoroquinolones.Accordi ngto other 2013 reportoftheCentersforDiseaseControlandPrevention,ESBLproducingEnterobacteriaceaewereclassi fiedasaserious threat andtheprevalenceofESBLinfectionskeepsrising (9,10) .

Recently,new techniques have become available for the rapid identification of pathogens from positive bloodcultures. The VITEK 2 compact (bioMérieux, France) is a machine capable of running bacterial identification and drug susceptibility simultaneously. With regard to identification, VITEK 2 compact system utilizes 64 biochemical and substrates tocover a total of 115 Gram-positive and 135 Gram-negative spp with an approximate turnaround time of seven hours. Identification of an isolate provides essential information on itspathogenic potential and is the most importance for the correct interpretation of antibiotic susceptibility testing Reduced turnaround times, better specimen management, enhanced quality control, reproducibility, precision, and the ability to track results are other benefits of the VITEK 2 compact system over conventional methods. The aim of this study is study prevalence of drug susceptibility profile of bacteria isolated blood samples collected from patients referred to Arsho Advanced Medical Laboratory by employing the fully automated VITEK 2 compact system (11).

1.2. Statement of the problem

BSI caused by multidrug resistant (MDR) bacteria cause high morbidity and mortality .This willlead to great economic loss encompassing use of more expensive antibiotics to treat infection as well as threat of resistance to them. The infections caused by MDR organisms are more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics .In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available by keeping in mind that high mortality andmorbidity are associated with septicemia and right choice of empiric therapy is of importance. The increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community acquired BSI infections is making different classes of antimicrobial agents less effective resulting in emergence of antimicrobial resistance (3).

In our country, Ethiopia, it is wide practice that antibiotics can be purchased without prescription and without real etiological agent identification. This leads to misuse of antibiotics by the public thus contributing to increase spread of antimicrobial resistance of bacteria isolated from blood samples. Hence, a study on the prevalence of blood stream infection, the distribution of etiological agents and their drug susceptibility patter by employing a fully automated VITEK 2 compact system for identification and AST simultaneously is of the highest priority

1.3 Significance of the study

The antimicrobial susceptibility patterns of common pathogenic bacteria are essential to guide empirical and pathogen specific therapy; therefore, empiric antibiotic treatment is not effective in elimination of these pathogens much time in clinical practice.Soidentifying the various bacteria isolated from blood and studying their antibiotic susceptibility patterns in our study area will be one indicator in the appropriate treatment of patients to guide empirical and pathogen specific therapy for clinicians.

The results of this study could help to provide information on the magnitude of MDR and ESBL production of BSI causing organisms, help the physician in the selection of better antibiotic for treatment and helps to initiate further large scale epidemiological study on MDR and ESBL production in BSI causing organisms

2. Literature Review

A study in Northern Vietnam on bacterial bloodstream infections etiology, drug resistance, and treatment outcome shows that from a total of 738 patients with BSI ,the predominant pathogens were *K.pneumonia*(17.5%),*E. coli*(17.3%),*S.aureus*(14.9%),*S.maltophilia*(9.6%) and*S.suis*(7.6%). The overall proportion of extended spectrum beta-lactamase (ESBL) production amongEnterobacteriaceaewas 25.1% and of methicillin-resistance in S. aureus (MRSA) 37%. This study shows that gram negative bacteria are the most common cause of both community and hospital acquired blood stream infection in adults presenting to their tertiary referral hospital, with high associated mortality. The extended spectrum beta-lactamase (ESBL).producingenterobacteriaceae were prevalent in both community and health care setting(12).

A study conducted in Nepal on Etiology of bloodstream infection and antibiotic susceptibility pattern of the isolates shows that out of 1,205 blood samples, 15.4 %were culture positive. The most common bacteria isolated were: *Salmonella spp*.*E.coli*, *K.pneumonia* and CONS. Gram-negative bacteria were the predominant causes of BSIs. *S.typhi* was isolated in 71 % cases of bloodstream infection followed by Salmonella Paratyphi A in 16 %, Escherichia coli in 5.3 % and *K.pneumonia* 0.5 %. The gram-positive organism responsible for causing BSI was coagulase-negative staphylococcus in 7 % cases. During ASTs, Gram-negative bacteria were sensitive to Chloramphenicol with only 0.5 % resistivity. *S.typhi* (85.6 % of isolates) showed resistance to Nalidixic acid. Gram-positive bacteria showed 100 % sensitivity towards Chloramphenicol and Gentamicin and were least sensitive to Amoxicillin. This study shows that Salmonella spp., was major cause of BSIs and recommend increase in antibiotic resistivity for BSI causing pathogens has necessitated continuous monitoring of the susceptibility of organisms towards antibiotics (13).

A study from Iran on antimicrobial profiles of bacterial strains isolated from patients with hospital acquired blood stream and urinary tract infections shows that the most prevalent blood stream infection(BSI) pathogen was CoNS 34.8% with highest resistance rate against penicillin (91.1%) followed by ampicillin (75.6%), and the lowest rate was against vancomycin (4.4%) whereas *S. aureus*is found to *be* (3.9%) with high resistance against penicillin, ampicillin and cotrimoxazole which is 100% and susceptible to vancomycin.finally they proposed that to reduce the incidence of nosocomial infections, the appropriate use of antibiotics according to the standard antimicrobial susceptibility tests has to be done (14).

A study from India carried out on prevalence & antimicrobial resistance pattern of extended spectrum b-lactamase producing *Klebsiellaspp* isolated from cases of neonatal septicemia shows that from the 100 *Klebsiella*isolates, 58 were positive for ESBL production, which was much lower than 86.6 per cent reported in 2003. Almost all the isolates were sensitive to imipenem and meropenem.In this study drug resistance was found to be significantly more common in ESBL producing isolates than in non-ESBL producers and this study shows that there is a need of carefully formulated therapeutic strategies to control infections in NICUs and the high percentage of drug resistance in ESBL producing *Klebsiella*spp suggests that routine detection of ESBL is required by reliable laboratory methods (15).

Another prospective study conducted in India on resistant patterns of bacteria isolated from bloodstream infections at a university hospital shows from 168 isolated bacterial strains , The most frequently identified Gram-positive bacteria were *CONS* 63.5%, *S.aureus* 23.1%, *enterococci* 5.8% and alpha haemolytic*streptococci* 5.8%. The most frequently Gram-negative bacteria identified were Acinetobacter*spp* 31%, *S.typhi* 24.1%, *E. coli* 23.3% and *P.aeruginosa* 13.8%. *CONS* showed maximum resistance to cefaclor 57.1% and ampicillin 46.9%. *S.aureus* showed maximum resistance to amoxicillin 100% and ampicillin 91.7%. Acinetobacter*spp* showed maximum resistance to amoxicillin 89.7%,amoxi-clavulic acid87.1% and ampicillin 85.7%. *S.typhi, E.coli, P.aeruginosa* and *K.pneumoniae* showed maximum resistance to ampicillin, 46.4%, 92%, 93.8% and 100%, respectively (16).

A hospital-based prospective cross-sectional study conducted in Tanzania on Bacteremia and resistant gram-negative pathogens among under-fives shows of the 1081 children admitted during the studyperiod,The prevalence of bacteremia was 6.6%.*E.coli* and *K. pneumonia* accounted for (33.3%)and(28.6%) of all the isolates respectively. Others gram negative bacteria *Citrobacterspp*(9.5%), *Enterobacterspp* (4.25%), *Pseudomonas spp*(9.5%),*Proteus spp*(4.25%)and*Salmonella spp1*(4.25%). These isolates were highly resistant to ampicillin (95%), cotrimoxazole(90%), tetracycline (90%), gentamicin (80%), augmentin (80%), chloramphenicol (65%), ceftriaxone (35%), cefotaxime(35%) ciprofloxacin (30%), amikacin (30%), ceftazidime (25%) and norfloxacine (10%)(17).

A study from Egypt carried out on an emerging antimicrobial resistance in early and late onset neonatal sepsis, among the isolates gram positive cocci showed highest resistance to ampicillin (amoxicillin-sulbactam 100% and amoxicillin-clavulanate75%), cephalosporins (Ceftazidime 94%, cefoperazone 100%,cefepime 86%, ceftriaxone 100%,cefuroxime 100%, cefoxitin 80%), carbapenems(Imipenem 84%, meropenem 86%),piperacillin-tazobactam (100%),and erythromycin (86%). Less resistance was evident to amino glycosides (Amikacin, 49%, gentamicin, 57%), quinolones (ciprofloxacin 77%, Levofloxacin 75%), clindamycin (53%), and rifampicin (49%). Least resistance among gram positive bacteria was found to vancomycin (18%) finally they concluded that there shall be global regulations to restrict the use of antimicrobials in the community as well as in the hospital setting (18).

A study on bacterial blood stream infections and antibiogram among febrile patients at Bahir Dar Regional Health Research Laboratory Center, Ethiopia shows of561 blood specimens requested for blood culture, 220 blood cultures had aerobic bacterial growth. Gram negative bacterial isolates constituted 115 (52.3%) of the isolated bacteria. *S. aureus*50 (22.7%),*CONS*35(15.9%), *K. pneumoniae35* (15.9%), *E. coli* 19 (8.6%), *P. aeruginosa*15 (6.8%) and *Acinetobacterspp* 13(5.9%) were the most dominant isolates. Overall, drug resistance for gram positive bacteria were 7 to 61% and for gram negatives 6.9 to 82.6%. Among the gram positive bacteria, high resistance levels were observed against penicillin (61%) and oxacillin (52.9%)(19).

A study on multi-drug resistance profile of bacteria isolated from blood stream infection at tikuranbessa specialized hospital, addisababa, Ethiopia,out of a total of 422 samples processed, bacterial pathogens were isolated from 64 (15.2%) samples. Among the isolates, 29 were Gram-positive and35were gram negativebacteria.Staphylococcusaureusand Klebsiella Pneumoniawerethe dominant isolates. Penicillin (86.7%) was the least effective antibiotic against Gram-positive bacteria while ampicillin (85.7%) and amoxicillin clavulanic acid (77.14%) were the least effective antibiotic against Gram-negative bacteria. Clindamycin (80%) and amikacin (97.1%) were the most effective antibiotic against Gram positive and Gram-negative bacteria, respectively. Out of 29 isolate of Gram-positive bacteria, 16 (55.2%) were multidrug resistant of which 11 (35.9.3%) were extensively drug resistant and 2 (6.9%) were pan drug resistant. Out of 35 isolates of Gram-negative bacteria, 26 (74.3%) were multidrug resistant of which 18 (54.4%) were extensively drug resistant(20).

A laboratory based prospective cross sectional study performed byAbera K. and his friends on Bacterial Profile of Adult Sepsis and their Antimicrobial Susceptibility Pattern at Jimma university specialized hospital, south west Ethiopia shows from a total of 95 adult septic cases involved in this research, 15 (15.8 %) were positive to eight different types of bacteria. Gram positive organisms were isolated in 53.3 % of these episodes with Staphylococcus aureusbeing the most frequent, while Gram negative accounted for the remaining 46.7 % with Escherichia coli being the commonest isolate among gram negative bacteria. The isolates shows high rates of resistance to most antibiotics tested in-vitro. The ranges of resistance to Gram positive bacteria were 0 % to 100 %, and to gram negative from 14.3 % to 85.7 %. In this study multi-drug resistance (resistance to three or more drugs) was observed in 80 % of isolates. Of this 87.5% and 71.4 % accounted for gram positive and gram negative bacteria respectivelyand ciprofloxacin is the effective drug against the tested gram positive and gram negative bacteria isolates(21).

A study onassessment of bacterial profile and antimicrobial resistance pattern of bacterial isolates from blood culture in Addis Ababa regional laboratory,Addis Ababa, Ethiopia showsOut of 500 blood culture results reviewed, the frequency of blood culture positive was 164(32.8%). Out of a total 164 isolates,127 (77.4%) were gram-positive bacteria and 37 (22.6%) were gram-negativebacteria.Thepredominant bacteria species isolated comprise Staphylococcus aureus 82 (50.0%), Coagulasenegative staphylococci (CONS) 43 (26.21%), *K.pneumoniae* 23 (14.02%), *E. coli* 6 (3.6%),*A.baumannii* 4 (2.4%), *Streptococcus spp* 3 (1.8%), *P.aeruginosa*2 (1.2%) and *N. meningitis* 1 (0.6%). Generally, in this study majority of gram-positive isolates showed high resistance to commonly used antimicrobials to Penicillin(83.5%),Trimethoprim sulphamethoxazole(83.5%),Erythromycin (77.3%),Doxycycline (76.5%),Tetracycline (76.5%), Gentamycin (75.0%), and least resistant to Clindamycin (5.4%)and Chloramphenicol (46.1%) and high resistant gram-negative isolates was seen to Ampcillin (88.5%),Amoxicillinclavulanic acid (80%), Trimethoprim-sulphamethoxazole (80%), Ceftriaxone (77.1%) and least resistant toCeftriaxone (42.8%) and Cefepime (51.5%). In this study it was also revealed that isolated bacteria speciesdeveloped multi drug resistance to most of the antibiotics commonly tested(22).

A study done at Mekelle hospital Northern Ethiopianbacteriological profile and antimicrobial susceptibility patterns of blood culture isolates among febrile patients shows out of the total 514 febrile patients, 144 (28%) culture positive were isolated. *S.aureus*54 (37.5%),*CONS*44 (30.6%), *E. coli*16 (3.1%), *Citrobacterspp*. 9 (1.7%) and *S.typhi* 8(1.6%) were the most dominant isolates, collectively accounting for >90% of the isolates. Antimicrobial resistance pattern for gram positive and gram negative bacteria was 0–83.3% and 0–100%, respectively. High resistance was seen to Trimethoprim/sulphamethoxazole101 (70.1%), Oxacillin 65 (62.5%), Ceftriaxone 79 (58.9%) and Doxycycline 71 (49.3%). Fifty-nine percent of the isolated bacteria in this study were multi drug resistant. Most bacterial isolates were sensitive to Gentamicin,Ciprofloxacin and Amoxicillin clavulanic acid. All gram positive isolates in this current study were sensitive to vancomycin. Prevalence of bacterial isolates in blood was high.It also reveals isolated bacteria species developed multi drug resistance to most of theantibioticstested, whichhighlightsforperiodicsurveillanceofetiologicagent,antibioticsusceptibility to prevent further emergence and spread of resistant bacterial pathogens(23).

3. Objectives

3.1. General :

- To determine species distribution, the prevalence of multidrug resistance and extended spectrum beta lactamase production of bacteria isolated from blood sample of patients referredto Arsho advanced medical laboratory.

3.2. Specific objectives:

- To determine the distribution of bacteria implicated in causing blood stream infection
- To determine the overall antimicrobial susceptibility profile of the bacteria
- To determine the extended spectrum beta lactamase production of the bacteria

4. Hypothesis

There is no difference in the Prevalence of BSI causing,multi drug resistant and ESBL production of bacteria isolated with the previous studies conducted in Ethiopia

5. Materials and Method

5.1. Study Area

The study was conducted on patients referred to Arsho Advanced Medical laboratory (AAML) from different health facilities (hospitals, health centers, clinics, institution) and Arsho branch laboratories. AAML is a private diagnostic laboratory found in Addis Ababa Ethiopia with nine branches located different part of the city. It is one of Accredited Laboratory by Ethiopian National Accreditation office with specific scopes since 2015.The laboratory is found at Kirkos Sub city, Woreda 02,House No 612.Curently the microbiology laboratory runs around 45-50 microbiological samples on average per day.

5.2. Study design and period

A descriptive cross sectional study was conducted from January to April 2019

5.3. Population

5.3.1. Source population

All patients referred to Arsho Advanced medical laboratory for blood culture test was the study subjects.

5.3.2. Study population

All patients that are volunteer to the study was the study Population.

5.4. Inclusion and Exclusion criteria

5.4.1. Inclusion criteria

All age and sex groups referred for blood culture test and that are volunteer were included.

5.4.2. Exclusion criteria

Patients who took antibiotics currently within the last 14 days were excluded

5.5. Study variables

5.5.1. Dependent variable

- Prevalence of isolated bacteria from blood culture
- Antimicrobial susceptibility pattern of bacteria

5.5.2. Independent variable

- Age
- Sex

5.6. Measurement and Data collection

5.6.1. Sample size calculation

The sample size was calculated based on single population proportion. Since there is Limited published data available on prevalence of antimicrobial susceptibility pattern and extended beta lactamase production from blood samplesin Ethiopia The value of p was taken as 50% (0.50).Considering 95% confidence interval, 5% margin of error, and the sample size is calculated using the following standard formula.

The sample size $n = z\ (\alpha/2)^2\ p\ (1-p)/d^2$

Where

n = Sample size

α = level of significance

z = at 95% confidence interval Z value (a = 0.05) =>Z α/2=1.96

p = prevalence

d = Margin of error at (5%) (0.05)

$n = (1.96)^2 0.5(1-0.5)/\ (0.05)^2$

n=384

To minimize errors arising from the likelihood of noncompliance, ten percent of the sample size will be added to the normal sample. Accordingly the required sample size was 422.

5.6.2. Sampling method

Convenient sampling technique were used and all volunteer patients referred for blood culture test during the study period were included in the sampling procedure.

5.6.3. Data collection procedure

Data was collected using structured data collection form to obtain information on Socio demographic status and previous antibiotic usage. Informed consent was taken from each patient and verbal informed consent was taken on behalf of children from their parents or guardians (see more on annex 1-8).

5.7. Laboratory analysis

5.7.1Specimen collection and culturing

Prior to blood collection, the skin of each study participant was disinfected with 70% alcohol and subsequently with Povidone-iodine.About 10 ml and 5 ml of venous blood in duplicates were collected aseptically from adults and children, respectively. Blood collected from each patient was inoculated into two blood culture bottles containing 50 ml and 25 ml of sterile brain heart infusion broth aseptically. All blood culture broths were then incubated at 37^0C; checked for sign of bacterial growth daily up to 7 days. Blood culture bottles that showed signs of growth were sub-cultured onto Blood agar base to which 10% sheep blood is added, Chocholate agar and,MacConkey agar in safety cabinet. Blood culture broth with no bacterial growth after 7 days were sub-cultured before being reported as a negative. Pure isolates of bacterial pathogen were preliminary characterized by colony morphology and Gram-stain. Identification and antimicrobial susceptibility testing of bacterial isolates were determined with automated VITEK 2 compact system following the instruction of the manufacturer.

5.7.2 Principle of VITEK 2 compact system

The VITEK 2 compact system is an automated microbiology bacterial identification and antimicrobial susceptibility system. Uses advanced colorimetry technology to determine individual biochemical reactions contained in a variety of microbe identification cards. After inoculation with a standardized suspension of the unknown organism, each self-contained cards is incubated and

read by the instrument's internal optics. Comparison of results to known species specific reactions in the VITEK 2 database yields organism identifications. A transmittance optical system allows interpretation of test reactions using different wavelengths in the visiblespectrum. During incubation, each test reaction is read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present (11).

5.7.3 Suspension Preparation for ID card and AST card

Was done by suspending in 3 ml of sterile saline (Aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube to achieve a turbidity equivalent to that of a McFarland 0.50 standard (range, 0.50 to 0.63), as measured by the DensiChek (bioMe´rieux) turbidity meter. These suspensions were used for the inoculation of identification cards, while AST cards were inoculated after the bacterial suspensions will be further diluted following the instruction of the manufacture.Thenplacethe ID card into the test tube and thentransfer the test tube into the Cassette(11).

5.7.4 Inoculation

Identification cards were inoculated or filled with microorganism suspensions using anintegrated vacuum apparatus. Each card has a pre-inserted transfer tube used for inoculation andhas bar codes that contain information on product type, lot number, expiration date, and unique identifier that can be linked to the sample. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card were placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassettes canaccommodateup to 10 tests (VITEK 2 Compact). The filled cassette were placed manually (VITEK 2 compact) into a vacuum chamber station. After the vacuum applied and air was re-introduced into thestation, the organism suspension was forced through the transfer tube into micro-channels thatfill all the test wells (12).

5.7.5. Card sealing, Loading and Incubation

Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30cards. All card types were incubated on-line at 35.5 + 1.0ºC. Each card removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings based on their wave length, and then returned to the incubator until the next read time. Then data collected at 15-minuteintervals during the entire incubation period (12).

5.7.6. Bacterial Identification

Identification and antimicrobial susceptibility testing of isolated bacteria were determined with automated VITEK 2 compact system using bacterial isolation and identification cards based on manufacturer's instruction.

The VITEK 2 compact system is an integrated modular system that consists of a filling, sealer unit, a reader-incubator, a computer control module, a data terminal, and a multi copy printer. The system detects bacterial growth and metabolic changes in the micro-wells of thin plastic cards by using a fluorescence-based technology.

The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. (24)

Substrates and biochemical tests used for identification of gram negative bacteria were Ala-Phe-Pro-Arylamidase, Adonitol, L-Pyrrolydonyl-Arylamidase, L-Arabitol, D-Cellobiose, Beta-Galactosidase, H2S production, Beta-N-Acetyl-Glucosaminidase, GlutamylArylamidasepNA, D-Glucose,Gamma-Glutamyl-Transferase,Fermentation/Glucose, Beta-Glucosidase, D-Maltose, D-Mannitol, D-Mannose, Beta-Xylosidase, Beta-Alanine arylamidaseetc.. and Substrates and biochemical tests used for identification of gram positive bacteria were D-Amygdalin,phaspatidiylinstolphospholipase,D-xylose,Urease,Ala-Phe-Pro-Arylamidase,B-galactosidase,Alphaglucosidase,cyclodextrine,Optochinresistance,Bacitracinresisistance,L-lactatealkalization etc..(24).

5.7.7. Drug susceptibility testing

5.7.7. Drug susceptibility testing

Antimicrobial Susceptibility testing with the VITEK-2 compact system was performed using AST cards known as GN AST and GPAST cards. The cards were filled with inoculums in filling chambers .The VITEK-2 System automatically processes the antimicrobial susceptibility cards until MIC's are obtained. The VITEK-2 compact system subsequently corrects, where necessary for MIC's or clinical category in accordance with the internal database of possible phenotypes for microorganism antimicrobial agent combinations .Preparation of inoculums was done by transferring 280µL of culture suspension from the 0.5 McFarland culture suspension for gram positives and145 micro liter for gram negative for filling the identification cards(11,24).

Antibiotics with their different concentration used for determination of drug susceptibility profile in this investigation were Quinopristin/Dalfopristin,Cefoxitin,Benzylpenicillin,Oxacillin,Gentamicin,Ciprofloxacin,Levofloxacin,InducibleClindamycinResistance,Erythromycin,Clindamycine,Vancomycin and Tetracycline for gram positives. Trimethoprim/Sulfamethoxaole,Ampicillin,Amoxicillin/ClavulanicAcid,piperacillin/Tazobactam, Cefalotin,Cefazolin,Cefuroxime,Cefoxitin,Cefpodoxime,Ceftazidime,Ceftriaxone,Cefepime,Gentamycine,Toberamycin,Ciprofloxacin,Levofloxacin and Teteracycline for gram negatives.(11,25)

5.7.8Quality control

The quality control strains like E .coliATCC25922for gram negative and*S.* auresATCC 25923for gram positives run simultaneously with the test organisms .In addition CLSI guide line, 2019 were strictly followed.

5.8. Data quality assurance

To maintain the quality of data the data collection form was pre-tested and collected data was checkedcarefully on spot and daily basis for their completeness, accuracy, and clarity. To assure the quality of laboratory results Standard operating procedures (SOPs) of the microbiology laboratory of AAML were strictly followedin all steps of thePre-analytical, analytical and post-analytical

5.8.1.Pre analytical phase

Socio-demographic characteristics of patients were collected using structured data collection sheets after getting informed consent. All blood culture specimens were collected by well trained laboratory personnel by following standard operational Procedure. After specimens reach the laboratory, it was checked to ensure that the correct specimen had been sent and the name on the specimen is the same as that on data collection form. To avoid sample contamination leak proof and sterile sample container was used.

5.8.2.Analytical phase

All materials, equipment and Procedures was adequately controlled. All stains and reagents was clearly labeled, dated, and stored correctly. The preparation, fixation, staining and reporting of smears as detailed in the SOPs of the microbiology laboratory of AAML was strictly followed. At regular intervals and whenever a new batch of gram stain is prepared, control smears of appropriate organisms were stained to ensure correct staining reactions. For each item of equipment there is clear operating and cleaning instructions, and service sheets. The temperature of a refrigerator, incubator, and water-bath will be monitored and documented Culture media will be tested for Performance and sterility

5.8.3Post Analytical Phase

Post-analytical phase the results were recorded with the patients' identification number. The terminology and format used in reporting was standardized. All reports were concise and clearly presented. Before leaving the microbiology laboratory, all reports were double checkedforcorrectness. Purified bacterial cultures will be stored in nutrient broth with 20%

glycerol at –81℃ by sub culturing every month .These cultures may be stocked in this condition for 5-10 years.

5.9. Data analysis and interpretation

Data entry and analysis was done using SPSS(Statistical Package for social sciences statistical software version 20).The descriptive statistics was used to calculate and to see the relation between dependent variable and independent variablesusing frequencies and crosstabs. Finally, the results were presented on words, charts.

5.10. Ethical consideration

All ethical considerations and obligations were fully addressed, and the study was conducted after the approval of the Internal Review Board (IRB) of Arsho Advanced Medical Laboratory private limited company and Departmental Ethics and Research committee of the Department of Medical Laboratory Sciences, Collage Health Science, and School of Allied Health Science of Addis Ababa University. Written informed consent was obtained from the participants before data collection. Each respondent was given the right to refuse to take part in the study and to withdraw at any time during the study period. All the information obtained from the study subjects were coded to maintain confidentially. When the participant was found to be positive for bacterial pathogen, they were informed and receive proper treatment. An assent form was completed and signed by a family member and/or adult guardian for participants under the age of 18 years.

5.11. Dissemination of results

The findings of this study will be presented to the Department of Medical Laboratory Sciences, School of Allied Health Sciences, and Addis Ababa University. And an attempt will be made to present the findings in different scientific conferences and will be sent for peer-review journals for publication

5.12. Operational Definition

Blood stream infections (BSIs) -are defined as the condition in which pathogens have entered the bloodstream(1)

Multi Drug Resistance: MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories (26).

Antimicrobial resistance: Antimicrobial resistance is the ability of microbes to resist the effects of drugs that is, the germs are not killed, and their growth is not stopped. It happens when microorganisms such as bacteria change when they are exposed to antimicrobial drugs (27).

PanDrugResistance(PDR):isdefined as non Susceptibility toallagents in allantimicrobialcategories (i.e.noagentstestedassusceptiblefor that organism)(26).

Minimal Inhibitory Concentration (MIC): is the lowest concentration of antimicrobial agent that prevents visible growth of microorganisms in agar or broth dilution susceptibility test(28).

Non-susceptible: A bacterial isolate was considered non-susceptible to an antimicrobial agent when it tested resistant or intermediate when using clinical breakpoints as interpretive criteria provided by the Clinical and Laboratory Standards Institute (CLSI) (28).

Susceptible: A bacterial isolate was considered susceptible to an antimicrobial agent when inhibited by usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used (28,29).

Intermediate: A category that implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used (28,29).

Resistant: A bacterial Isolate was considered susceptible to an antimicrobial agent when not inhibited by the usually achievable concentrations of the agent with normal dosage (28,29).

6. Results

6.1. Socio demographic characteristics

Four hundred twenty-two (n=422) eligible study participants were investigated during the study period.Of these participant 58% (n=243/422) of them were females and 42 % (n=149/422) were males.The majority of patients 41% (n=41/100) and 22% (n=22/100) were between 25-44 and 45-64 years of age respectively as shown below in Table 1,and the mean (std. deviation) ages of patients was 36.5(21.7).Among the total blood samples processed 78.9% (n=333/422) showed no growth for blood culture and 21% (89/422) show growth for gram positive and gram negative bacteria. From this 58.4% (52/89) were gram positive and 41.6% (37/89) were gram negative bacteria. Females had a higher isolation rate than males 56.8% (n=21/37) versus 43.2 % (n=16/37) among the isolated gram negative bacteria and males had higher isolation rate than females 51.9%(n=27/52) versus 48.1%(n=25/52) among isolated gram positive bacteria.There was no association between sex of patient and blood culture result (P=0.647). Rate of isolation of gram positive bacteria was highest in 25-44 years 36.5% (n=19/52) and in <1year 56.8%(21/37).as shown in Table 1.However, there is no association between bacterial isolates and age categories (P=0.999).

Table 1: Frequency of gram-positive and gram negative isolates in relation to sex and age group at AAML from January to April 2019, Addis Ababa, Ethiopia (N=422).

Variables	Category	Total blood samples	No growth for bacteria N (%)	Growth of Gram positive N (%)	Growth of gram negative N(%)
Sex	Female	243(57.6)	197(59.2)	25 (48.1)	21(56.8)
	Male	179 (42.4)	136(40.8)	27(51.9)	16(43.2)
	Total	422(100)	333(78.9)	52(12.3)	37(8.7)
Age group[1]	<1	23(5.5)	8(2.4)	3(5.8)	12(32.4)
	1-14	43(10.2)	31(9.3)	6(11.5)	5(13.5)
	15-24	38(9.0)	34(10.2)	4(7.7)	0
	25-44	172(40.8)	147(44.1)	19(36.5)	7(18.9)
	45-64	93(22.0)	72(21.6)	11(21.2)	10(27)
	>65	53(12.6)	41(12.3)	9(17.3)	3(8.1)
	Total	422(100)	333(78.9)	52(12.3)	37(8.7)

[1]WHO age classification for health [38]

6.2. Prevalence of gram positive, gram negative bacteria and ESBL production

From the total bacterial isolates gram positive bacteria shown high prevalence than gram negative bacteria. As shown in table 2 below CONS are the predominant isolated bacteria 41(78.8%) among gram positives followed by *S.aures* 7 (13.5%),*E.fecalis* 3(5.8%),*S.lugdinesis* 1(1.9%) respectively.

Table 2: Frequency distribution of Gram positive bacteria isolated from blood specimens at AAML from January to April 2019 (N=52)

species	Frequency (N=52)	percentage
CONS		
S.warneri	8	15.4%
S.epidermidis	12	23.1%
S.haemoliyticus	6	11.5%
S.intermedius	5	9.6%
S.hominis	4	7.7%
S.simulans	2	3.8%
S.sapropyticus	1	1.9%
S. xylosus	3	5.8%
Others		
S.aures	7	13.5%
E.fecalis	3	5.8%
S.lugdinesis	1	1.9%
Total	52	100

As shown in table3 below *Klebsilla*spp 14(37.8%)are the most predominant one among the isolated gram negative bacteria followed by *Acinetobacterspp* 8(21.6%),*E.coli*6(16.2%),*E.cloacae* 4(10.8%) *P.aeruginosa* 2(5.4%),*Morexella*spp1(2.7%),*L.adecarboxylica* 1(2.7%) and*s.marcines* 1(2.7%) respectively. A total of 14 isolates of *Klebsiella*spp and 2 isolates of *E.coli* were isolated from blood. Of these isolates, 7 (58.3%) were ESBL positive for *K.pneumonae* and 2(33.3%) for *E.coli*

Table 3: Frequency distribution of Gram negative bacteria and ESBL producing strains isolated from blood specimens at AAML from January to April 2019 (N=37)

Species	Frequency n(%)	ESBL N(%)
Klebsillaspp		
K.pneumonae	12 (32.4%)	7(58.3%)
K.oxytoca	2 (5.4%)	
Acinetobacterspp		
A.baumani	3 (8.1%)	
A.woffii	4 (10.8%)	
A.xylosidans	1 (2.7%)	
Others		
E.coli	6 (16.2%)	2(33.3%)
E.cloacae	4 (10.8%)	
***morexella*spp**	1 (2.7%)	
P.aeruginosa	2 (5.4%)	
L.adecarboxylica	1 (2.7%)	
S.marcines	1 (2.7%)	
Total	37(100)	

6.3. Antimicrobial susceptibility pattern of gram positive and gram negative isolates.

The overall drug susceptibility profile of Gram positive and gram negative bacteria against seventeen antibacterial drugs tested is summarized under Table 4 and Table5 for both gram positive and negative bacteria. Gram positive bacteria showed highest sensitivity towards Daptomycin(100%) followed by Linezolid and Tigicycline (98.1%) respectively. Penicillin showed the highest resistance rate (92.3%) followed by oxacillin(71.2%) and Sulphamethazole /trimethoprim (61.5%).

The first commonly isolated gram positive organisms *CoNs* exhibited high resistance for Penicillin (92.7%), oxacillin (78%) and Tetracycline (63.4%).The least resistance was observed in Quinopristin /Dalfopristin, Linezolid, Minocycline and Tigicycline (2.4%) each .

*S.aureus*showed high resistance rates for penicillin (85.5%), trimethoprim/sulfamethoxazole (71.4%), Erythromycine (57.1%) and oxacillin (28.6%) which is MRSA. Whereas gentamycin,Quinopristin /Dalfopristin, Linezolid,minocycline, vancommycin,Daptomycin and Tigecycline showed no resistance. The least resistance was seen for tetracycline, clindamycin, ciprofloxacin and Rifampicin (14.3 %) respectively.

Similarly, three of the isolated *E.faecalis*showed 100% resistance for penicillin followed byMinocycline,trimethoprim/sulfamethoxazole. The least resistance was observed in the case of gentamycin followed by ciprofloxacin and levofloxacin respectively. No isolate of *E.faecalis* were found vancomycin,daptomycin and linezolid resistant.

*S.lugdinesis*which the least isolated gram positive organism was susceptible to most of the drugs but it showed100% resistance toPenicillin,Oxacillin,Levofloxacin, Moxifloxacin,TetracyclineandErythromycin.

Table4: Percentage of antibacterial susceptibility pattern of gram positive bacteria isolated from blood at AAML from January to April 2019 (N=52).

species	P	PEN	OXA	GEN	CIP	LEV	MXF	ERY	CL	QDA	LNZ	DAP	VAN	MNO	TET	TGC	RIF	TMP
S.aureus (n=7)	I	0	0	0	0	14.3	0	0	0	0	0	0	0	0	0	0	0	0
	R	85.7	28.6	0	14.3	0	0	57.1	14.3	0	0	0	0	0	14.3	0	14.3	71.4
	S	14.3	71.4	100	85.7	85.7	100	42.9	85.7	100	100	100	100	100	85.7	100	85.7	28.6
E.faecalis (n=3)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	100	66.7	33.3	33.3	33.3	33.3	66.7	66.7	100	0	0	0	100	66.7	33.3	33.3	100
	S	0	33.3	66.7	66.7	66.7	66.7	33.3	33.3	0	100	100	100	0	33.3	66.7	66.7	0
CoNS (n=41)	I	0	0	4.9	0	7.3	4.9	2.4	0	0	0	0	4.9	4.9	9.8	0	0	0
	R	92.7	78	12.2	26.8	19.5	4.9	63.4	34.1	2.4	2.4	4.9	4.9	2.4	43.9	2.4	24.4	34.1
	S	7.3	22	82.9	73.2	73.2	90.2	34.2	65.9	97.6	97.6	95.1	90.2	92.7	46.3	97.6	75.6	65.9
S.lugdinesis(n=1)=	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	100	100	0	0	100	100	100	0	0	0	0	0	0	100	0	0	0
	S	0	0	100	100	0	0	0	100	100	100	100	100	100	0	100	100	100
Total (n=52)	I	0	0	3.8	0	7.7	3.8	3.8	0	0	0	0	7.7	0	11.5	0	0	0
	R	92.3	71.2	11.5	25	19.2	7.7	61.5	32.7	7.7	1.9	0	3.8	7.7	42.3	1.9	23.1	50
	S	7.7	28.8	84.6	75	73.1	88.5	34.6	67.3	92.3	98.1	100	88.5	92.3	46.2	98.1	76.9	50

Among gram negatives Cefalotin shows high resistance (91.1%) followed by Cefazolin (89.2%) and ampicillin(81.1%) respectively. High level of sensitivity is shown by Piperacilline /Tazobactam (86.5%),Levofloxacin(75.7%) followed by Gentamycin and Tobramycin (64.9%).

The first commonly isolated gram negative organisms*klebsilla*spp show high resistance for ampicillin, cefalotin,cefazolinand cefuroxime (92.9%).The least resistance was observed in gentamycine (50%) and ciprofloxacin (42.9%) respectively

The second commonly isolated gram negative organisms*acinetobacterspp* show high resistance for cefalotin and cefazolin (87.5%) followed by cefoxtine (75%) .Ceftazidime, levofloxacin, piperacillin/tazobactam and tobramycin show least resistant (12.5%).

Among the isolated gram negative bacteria *E. coli* were the third predominant isolate with highly resistance for Ampicillin ,trimethoprim/sulfamethoxazole ,cefuroxime and ciprofloxacin (100%) for each The most active agent for *E.coli* were cefoxtine (66. 6%), and piperacillin / tazobactam (66.7%).

The fourth and fifth isolated bacteria *E.cloacae* and *pseudomonas*spp showed high resistance 100% for ampicillin,amoxicilline/Clavulinicacid,cefoxitine, cefalotin,cefazolin and cefpodoxime, cefteriaxone,cefepime and high sensitivity 100%were seen on cefepime,ciprofloxacin, gentamycin, levofloxacin piperacilline /tazobactam and trimethoprim/sulfamethoxazole.

*L.adecarboxylica ,S.marcescens*and *Morexella*spp are the least isolated gram negative bacteria. *L.adecarboxylica*show high susceptibility rate (100%) for all drugs and*S.marcescens*and *Morexella*spp showed (100%) resistance to cefalotin,cefazolin,cefuroxime,cefoxtine and ciprofloxacin and high senstivity (100%) for ceftazidime ,ceftriaxone, cefepime, gentamycin, levofloxacin,piperacillin /tazobuctum,tetracycline and trimethoprim/sulfamethoxazole

Table 5: Percentage of antibacterial susceptibility pattern of gram negative bacteria isolated from blood at AAML from January to April2019(N=37)

SPP	P	AMP	AMC	CEP HA	CEF AZ	CEF UR	CEF OX	CEFP O	CEFT A	CEFT R	CEFEP	CIP	GEN	LEV	PIP	TOB	TET	TMP
Klebsilla Spp(N=14)	I	0	7.1	0	0	0	0	7.1	0	0	0	7.1	0	0	0	42.9	0	0
	R	92.9	64.3	92.9	92.9	92.9	78.6	85.7	85.7	85.7	85.7	42.9	50	85.7	85.7	0	64.3	85.7
	S	7.1	28.6	7.1	7.1	7.1	21.4	7.1	14.3	14.3	14.3	50	50	14.3	14.3	57.1	35.7	14.3
E. coli(N=6)	I	0	0	0	0	0	16.7	0	0	0	0	0	0	0	0	33.3	0	0
	R	100	83.3	83.3	83.3	100	16.7	83.3	83.3	83.3	83.3	100	66.7	83.3	33.3	50	83.3	100
	S	0	16.7	16.7	16.7	0	66.6	16.7	16.7	16.7	16.7	0	33.3	16.7	66.7	16.7	16.7	0
Acinetobacter Spp(N=8)	I	0	0	12.5	0	0	0	25	0	25	0	0	0	12.5	0	0	0	0
	R	50	50	87.5	87.5	62.5	75	62.5	12.5	25	37.5	37.5	25	12.5	12.5	12.5	25	50
	S	50	50	0	1	37.5	25	12.5	87.5	50	62.5	62.5	75	75	87.5	87.5	75	50
Pseudomonas spp (N=2)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	100	100	100	100	100	100	100	0	100	0	0	0	0	0	0	50	100
	S	0	0	0	0	0	0	0	100	0	100	100	100	100	100	100	50	0
E.clocae (N=4)	I	0	0	0	0	0	0	0	25	0	0	0	0	0	0	25	0	0
	R	100	100	100	100	75	100	100	0	25	0	0	0	0	0	0	25	0
	S	0	0	0	0	25	0	0	75	75	100	100	100	100	100	75	75	100
Morexellaspp (N=1)	I	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
	R	0	0	100	100	100	100	0	0	0	0	100	0	0	0	0	0	100
	S	100	100	0	0	0	0	0	100	100	100	0	100	100	100	100	100	0
L.adecarboxylica (N=1)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
S.marcescens (N=1)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	100	100	100	100	100	100	100	0	0	0	100	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	100	100	100	0	100	100	100	100	100	100
Total(N=37)	I	0	2.7	2.7	0	2.7	2.7	10.8	2.7	5.4	0	2.7	0	2.7	0	24.3	2.7	0
	R	81.1	67.6	91.9	89.2	81.1	48.6	78.4	48.6	59.5	54.1	45.9	35.5	21.6	13.5	10.8	48.6	67.6
	S	18.9	29.7	5.4	10.8	16.2	48.6	10.8	48.6	35.1	45.9	51.4	64.9	75.7	86.5	64.9	48.6	32.4

6.4.Multidrug resistance patterns for the isolated gram positive and gram negative bacteria

Based on the finding of the present study as shown that in Table 6 below, the overall prevalence of multi drug resistance (resistant for≥ three different classes of antibiotics) rate of gram positive bacteria was (57.7%).Of the 37 isolates of gram negative bacteria20(54%) were MDR. Among the gram positive *CoNS* and *Enterococcus spp* isolates were highly multi-drug resistant, while in gram negatives, MDR were observed in *E. coli* and *klebsilla spp.*

Table 6: Multidrug resistance pattern of gram positive and negative bacterial isolates from blood samples at Arsho Advanced medical laboratory from JanuarytoApril2019.

Isolated Gram Positive Bacteria	**Resistant antibiotics N (%) in Gram positive bactreria**			
	R0	R1	R2	MDR(≥R3)
CONS(n=41)	3(7.3)	4(9.8)	9(21.9)	25(61%)
S.aureus(n=7)	1(14.3)	3(42.9)	1(14.3)	2(28.6)
E.faecalis(n=3)	0	0	1(33.3)	2(66.6%)
S.lugdinesis(n=1)	0	0	0	1(100%)
Total	4(7.7)	7(13.5)	11(21.2)	30(57.7%)
	Resistant antibiotics N (%) in Gram negative bacteria			
Klebsillaspp(n=14)	1(7.1)	1(7.1)	1(7.1)	11(78.6%)
Acinetobacterspp(n=8)	3(37.5)	2(25	1(12.5)	2(25%)
E.coli (n=6)	0	0	0	6(100%)
E.clocae(n=4)	0	3(75)	1(25)	0
Pseudomonasspp(n=2)	0	0	1(50)	1(50%)
Morexellaspp(n=1)	0	1(100)	0	0
L.adecarboxylica(n=1	1(100	0	0	0
S.marcescens(n=1)	0	1(100)	0	0
Total	5(13.5)	8(21.6)	4(10.8)	20(54%)

R0- no resistant for any antibiotic, R1-resistant for 1 class of antibiotic, R2- resistant for 2 class of antibiotics, R3- resistant for 3 classes of antibiotics ,>R3- resistant for more than3 class of antibiotics.**NB**: Class of antibiotics is made based on CLSI 2019 category (25).

7. Discussion

This study found that 89 (21 %) out of 422total blood sample screened from suspected BSIcases were positive for the presence of bacteria **(Table 1).**This bacterial isolation rate is comparable with the previous studies done in Gondar 24.2%by Ali and Kebede and Addis ababa21.4% by Asrat and Amanuel [30,31].Even ifit was lower than reports fromMekelle Ethiopia 28% and Gambia 34% but it was higher than studies conducted InGondar Ethiopia 18.2% , Nigeria 18.2% .[23,27,32,33].Possible explanation for this variation could bethe difference in blood culture system, the study design,geographical location, nature of patient population, epidemiological difference of the etiological agents, seasonal variations, difference in infection control policies between nations.

Gram-positive bacterial strains have been the most prevalent causative pathogens of BSI in the present findings. This result is similar to the study conducted in Jimma, where Gram-positive microorganisms were the 53.3% and gram-negative microorganisms were 46.7% and Mekelle Ethiopia 72.2% of infections were caused by gram-positive and 27.8% by gram-negative bacteria.Contrarly cause of BSIs in other studies conducted in India, shows 69% gram negative and 31% gram positives,Tanzania (69.7, 30.3%)and Nepal (89.19 and 10.81%) .This variation couldbe due to epidemiological difference of the etiological agents.[16,21,23, 34,35].

In this study among gram positive bacterial isolates the most common one is *CONS* 78.8% (n=41/52) and which is similar to the finding of previous works conducted in Gondar Ethiopia 31.6%, India 63.5%, and 33.3%[36, 16, 32].Even if there is variation in their prevalence and dissimilar to the other studies that report *S.aures*as major isolate. This variation is due to the difference in study settings .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 [23].

Gram negative organisms were accounted for 41.5% in the present finding. *K.pneumonae* was the leading Gram negative species with frequency of 32.4 % and other Gram negative bacteria such as *Acenetobacterspp* and *E,coli*each accounts 21.6 % and 16.2% respectivelySimilar studies also reported *K.pneumonae* as the most frequently isolated organism with the study conducted at Tikuranbessa hospital at Addis Ababa ,Gondar, Addis Ababa byAsrat and Amanuel[20,31,32].In contrary other studies in Mekelle by Wasihun,E. coli(10.4%) was the predominant gram negative bacteria followed by S. typhi and Citrobacter spp.(5.6% each).[23].

In this study the overall drug resistant rate of Gram positive ranges from 0% for daptomycine and 92.3% for penicillin and of Gram-negative bacteria ranged from 10.8% for tobramicin to 91.8% cephalotin .Similarly the overall antibiotic resistant rate of gram positive bacteria showed in India0-100% [16].and gram negatives inGondar20-100%[32].About 92% strains of *CONS,* the most commonly isolated gram positive bacteria was resistant to penicillin. This result was comparable with the result conducted in Gondar,Jimma and addisababa[16,21,22].

The overall multidrug resistance level of gram positive and negative bacterial isolates was 57.7% (n=30/52) and54%(n=20/37) respectively.MDR level of the most frequently isolated pathogens, *CONS* and*klebsillaspp*showed 61% (n=25/41) and 78.6%(n=11/14)lower than the previous studies done byAdaneBitew.etal75%(n=6/8) and 100%(n=13/13) respectively[20].This difference might be due to the difference in study setting, previous antibiotic usage.

8. Strength and limitation

8.1. Strength

- Used the advanced VITEK 2 Compact system which is an automated microbial identification and antimicrobial susceptibility system that provides highly accurate and reproducible results.

8.2. Limitation of the study

- Lack of patient's clinical history in their request paper which could have been a good variable for this study.
- Being the study as a single laboratory based it may lack representativeness

9. Conclusion

In this study both Gram negative and Gram positive bacteria were responsible for BSI. CONS and klebsillaspp were among the most common Gram positive and Gram negativeorganisms identified causing BSI, respectively. Multidrug resistance was detected in 56.2 % of isolates. To prevent further emergence and spread of MDR bacterial pathogens, rational use of antibiotics and regular monitoring of antimicrobial resistance patterns are essential and mandatory in the management of BSI.

10. Recommendations

- A periodically surveillance of antimicrobial resistance pattern record and report isessential to develop treatment guideline.
- Establish antibiogram based on the susceptibility pattern for empiric therapy at national level is very crucial.
- Establishing health laboratories with modern methods such as VITEK 2 compact system for accurate identification of bacteria pathogens to the species level and determining drug susceptibility pattern of the etiologic agents for efficient management of bacterial infections should be considered for routine laboratory diagnosis.

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Annexes

Annex I: participants information sheet [English version].

Principal Investigator: SebleSodere, Addis Ababa University school of Allied Health Sciences.

Purpose: The purpose of this study is to assess the prevalence of multi drug resistance and extended beta lactamase production of isolated bacteria from blood culture.

Procedures to be carried on: you are invited to participate in the study after giving your consent and by giving the requested sample for investigation.

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study and there is no additional time required from you to stay during study.

Benefits of the study: There will be no financial benefit to you. But the result of the study will be used for to develop antibiogram that helps the patients avoiding empirical treatment.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you later on change your mind 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.

Annex 2. Informed Consent [English version]

I, the undersigned individual, am oriented about the objective of the study. I have informed that all of my information will be kept confidential and used only for this study. Your signature below indicates that you have read /or listened, and understand the information provided for you about the study.

Before you sign, please understand purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have any question.

I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study.

Based on the above information I agree to participate in the research

Signature: _____ Date: _____

Name of Data collector _____ Signature _____ if you have any question you can ask the principal investigator

Principal investigatorMrsSebleSodere [Msc candidate]

Mobile 0933187312

E-mail.seblisod@gmail.com

Annex 3: Participant's information sheet [Amharic version]

ጥናቱንየምታጠናው፤ሰብለ :ሶደሬበአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየህክምናላቦራቶሪሳይንስዲፓርትመንት

የጥናቱአላማ፤

የጥናቱዓላማመድሃኒትየተለማመዱበደም ውስጥየሚገኙ ባክቴሪያዎችንስርጭትበአርሾአድቫንስድሚዲካልላቦራቶሪለብለድ ካልቸርምርመራከተላኩናሙናውስጥበመለየትየፀረባክቴሪያመድሃኒትየመቋቋምአቅማቸውንማወቅ፤
አሁንያሉበትንደረጃማሳየትእናየመፍትሄአቅጥጫማስቀመጥ::

ለጥናቱተሳታፊዎችያለውልዩጥቅም፤
በጥናቱለሚሳተፉፍቃደኛተሳታፊዎችምንምአይነትየገንዘብክፍያየለውምነገርግንከጥናቱየሚገኘውውጤትለርስዎህክምናተጨማ ሪመረጃለማግኘትበተመሰሳይለመድሃኒትልምምድያደረጉትንካላደረጉትበመለየትውጤታማየሆኑትንመድሃኒቶችይጠቁማል::

በጥናቱተሳታፊዎችላይያለውጉዳት፤
በጥናቱመጀመሪያምይሁንመጨረሻበዚህጥናትላይበመሳተፍዎሊደርስብዎየሚችልአንድምጉዳትአይኖርም::
በጥናቱምክንያትየሚያባክኑትተጨማሪጊዜምአይኖርም::

የመረጃሚስጥራዊአጠባበቅ፣
የሚሰጡትመረጃበጥናቱወቅትምሆነከዚያበኋላባሉትጊዜያትሙሉበሙሉሚስጥራዊነቱየሚጠበቅናመረጃውምየሚያዘዉበስም ሳይሆንበመለያቁጥርይሆናል::በጥናቱላይያለመሳተፍመብትአለዎት::ይህመረጃበጥንቃቄየሚያዝይሆናል::
በመጨረሻምየጥናቱውጤትለሚመለከተዉአካልለጥናቱአላማናለህክምናባለሙያዎችብቻየሚገለፅይሆናል።

ስለዚህጥናትማንኛውምጥያቄካለዎትበማንኛውምጊዜከዚህበታችበተጠቀሱትአድራሻዎችመጠየቅይችላሉ::

ፊርማ ----------

መረጃውንየሰበሰበውግለሰብስም---------- ፊርማ ----------

የዋናተመራማሪዋአድራሻ

ሰብለ ሶደሬ

አዲስአበባዩኒቨርሲቲ፣የጤናሳይንስኮሌጅ፣የሕክምናላቦራቶሪቴክኖሎጂዲፓርትመንት

አዲስአበባ፣ኢትዮጵያ

ኢ-ሜይል፣seblisod@gmail.com

ስ.ቁ +251-933-187312

Annex 4. Informed Consent [Amharic version]

በዚህ ጥናት ለሚሳተፉ ሃሳባችውን መግለጥ ለሚችሉ የስምምነት መጠየቅያ **ቅጽ**

እኔፊርማዬከዚህበታችየተቀመጠውግለሰብከእኔየሚወሰደዉናሙናለጥናቱአላማብቻእንደሚዉልተረድቻለሁ፡፡
ሁሉምመረጃዎችእናየናሙናዉጤቱምስጢራዊመሆኑንተገንዝቤአለሁ፡፡
በጥናቱላይበመሳተፌምንምየገንዘብክፍያእንደማላገኝተረድቻለሁ፡፡
ከምርመሩመሳተፍወይምአለመሳተፍመብቴየተጠበቀመሆኑንእናላለመሳተፍብወስንበላቦራቶሪውበሚደረግበል
ኝምርመራላይምንምተፅዕኖእንደማይኖረዉተረድቻለሁ፡፡
ስለዚህየጥናቱንጠቃሚነትአምኜበትየስምምነትቃሌንየሰጠሁትበፍፁምፈቃደኝነትነዉ፡፡

ስለዚህጥናትማንኛውምጥያቄካለዎትበማንኛውምጊዜከዚህበታችበተጠቀሱትአድራሻዎችመጠየቅይችላሉ፡፡

እኔምየጥናቱተሳታፊይህንንበመገንዘብጥናቱላይለመሳተፍተስማምቼያለሁ፡፡

የጥናቱተሳታፊፊርማ ----------ቀን----------

መረጃውንየሰበሰበውግለሰብስም----------

ፊርማ ----------

የዋናተመራማሪዋአድራሻ

ሰብለ ሶደሬ

አዲስአበባዩኒቨርሲቲ፣የጤናሳይንስኮሌጅ፣የሕክምናላቦራቶሪቴክኖሎጂዲፓርትመንት

አዲስአበባ፣ኢትዮጵያ

ኢ-ሜይል፣seblisod@gmail.com

ስ.ቁ +251-933-187312

Annex 5. Parental/Guardian consent form in English

I, the undersigned, have been told about this research. My child has to say to choose if I want to be in the study. 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.

Parent/guardian Signature: _____ Date: _____

Name of Data collector _____ Signature _____ if you have any question you can ask the principal investigator

Principal investigatorMrsSebleSodere [Msc candidate]

Mobile 0933187312

E-mail.seblisod@gmail.com

Annex 6.Guardian /parental consent form in Amharic

የወላጅ/የአሳዳጊ/የሞግዚትየስስምምነትመጠየቂያቅጽ

እኔፊርማዬከዚህበታችየተቀመጠው -
የታማሚውወላጅ/አሳዳጊ/ሞግዚትስሆንየዚህንጥናትአላማበዉልተረድቻለሁ፡፡

በጥናቱወቅትምታማሚውመረጀዎችበሚስጥርስለሚያዝበሌላሰዉዘንድእንደማይታወቅተረድቻለሁ፡፡
በውጤቱከሚገኘዉየህክምናአገልግሎትበቀርሌላታማሚውበግሉየሚያገኘዉጥቅምእንደሌለተረድቻለ
ሁ፡፡ጥያቄእንድጠይቅዕድልተሰጥቶኝለጥያቄዎቼምበቂምላሽአግኝቻለሁ፡፡
የልጀበጥናቱመሳተፍበእኔፍላጎትብቻእንደሆነእናበጥናቱምአለመሳተፍምንምአይነትተፅዕኖታማሚ
ውላይእንደማያስከትልተረድቻለሁ፡፡
በከዚህባሻገርታማሚውበጥናቱውስጥለመካተትየእኔወላጅአሳዳጊ/ሞግዚትፈቃድእንደሚያስፈልግተረ
ድቻለሁ፡፡ በእኔፍቃደኝነትታማሚውበጥናቱእንደሚሳተፍከዚህበታችበፊርማዬአረጋግጣለሁ፡፡

የጥናቱተሳታፊወላጅ/አሳዳጊ/ሞግዚትፊርማ _____

መረጃውንየሰበሰበውግለሰብስም----------

ፊርማ ----------

የዋናተመራማሪዋአድራሻ

ሰብለ ሶደሬ

አዲስአበባዩኒቨርሲቲ፣የጤናሳይንስኮሌጅ፣የሕክምናላቦራቶሪቴክኖሎጂዲፓርትመንት

አዲስአበባ፣ኢትዮጵያ

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ስ.ቁ +251-933-187312

Annex7: Assent form for adolescent (12 -17 years old) study participants (English version)

I, the undersigned, have been told about this research. My parents or guardian have to say to choose if I want to be in the study. I have been informed that other people will not know my results as it coded with number rather than writing my name if I am in this study. 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 parents/guardian. By signing below I agree to participate in this research study.

Study participant Signature: _____ Date: _____

Name of Data collector _____ Signature _____ if you have any question you can ask the principal investigator

Principal investigatorMrsSebleSodere [Msc candidate]

Mobile 0933187312

E-mail.seblisod@gmail.com

Annex 8: Assent form for adolescent (12-17 years old) study participants (Amharic version)

በአማርኛየተዘጋጀዕድሜያቸዉ ከ12 እስከ17ዓመት ለሆኑታዳጊወጣትየጥናት-ተሳታፊዎችየተሳትፎማራጋጋጫቅጽ

ከዚህበታችስሜየተገለጠውበዚህጥናትውስጥእንድሳተፍፍቃደኝነቴንተጠይቂያለሁ።።
ወላጆቼም/አሳዳጊዎቼምበጥናቱእንድሳተፍወይምእንዳልሳተፍምርጫውየእኔመሆኑንነግረውኛልበጥናቱወቅትምየእኔመረጀዎችበሚስጥርስለሚያዝበሌላሰዉዘንድእንደማይታወቅተረድቻለሁበውጤቱከሚገኘዉየህክምናአገልግሎትበቀርሌላበግሌየማገኘዉጥቅምእንደሌለተረድቻለሁ።።
ጥያቄእንድጠይቅዕድልተሰጥቶኝለጥያቄዎቼምበቂምላሽአግኝቻለሁ።።
በጥናቱመሳተፍበእኔፍላጎት-ብቻእንደሆነእናበጥናቱምአለመሳተፍምንምአይነት-ተፅዕኖበእኔላይእንደማያስከትልተረድቻለሁ።።
በከዚህባሻገርየኔበጥናቱውስጥለመካተትየወላጆችምወይምየአሳዳጊዎቸፈቃድእንደሚያስፈልግተረድቻለሁ።። በፍቃደኝነቴበጥናቱእንደምሳተፍምከዚህበታችበፊርማዬአረጋግጣለሁ።።

የጥናቱተሳታፊፊርማ ----------ቀን----------

መረጃውንየሰበሰበውግለሰብስም----------

ፊርማ ----------

የዋናተመራማሪዋአድራሻ

ሰብለ ሶደሬ

አዲስአበባዩኒቨርሲቲ፣የጤናሳይንስኮሌጅ፣የሕክምናላቦራቶሪቴክኖሎጂዲፓርትመንት

አዲስአበባ፣ኢትዮጵያ

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Annex 9: Laboratory data collection form

1. Patient identification

Sample ID._____

Age (years) _____

Gender Male ☐ Female ☐

Antibiotic intake before 7 days yes ☐ No ☐

II. Laboratory Data

1. Date of specimen collection_____

2. Specimen type: _____

3. Media used _____

4. Gram stains result _____

5. Biochemical test_____

6. Organism isolated _____

7. Drug susceptibility pattern

7.1. Sensitive to _____

7.2. Intermediate to _____

7.3 Resistance to _____

III. Comments_____

Name of principal investigator _____

Signature _____ Date _____

Annex 10. Procedure for blood collection

1. Palpate and identify appropriate vein
2. Disinfect the puncture site with iodine or alcohol (70%) beginning in the center and rubbing vigorously outward in concentric circles (approximately 50mm diameter).
3. Iodine should remain in contact with skin for about 1 minute or until dry to ensure disinfection.
4. Blood is obtained by inserting a needle into a vein in the arm.
5. **1-3 ml** of blood from a child is added to **25 ml** of blood culture broth and **5-7 ml** of blood from an adult is added to **50 ml** of blood culture broth. It is important to use appropriate ratios of blood to culture broth for optimal bacterial growth.
6. Put the first collected blood in one bottle and repeat collecting of another blood from different site (example; left and right hand) with the same volume for the second bottle. These two bottles constitute one blood culture set.
7. Gently rotate the bottles to mix the blood & the broth (do not shake vigorously).
8. Label both bottles with, Patient name, unique identifier, Time of collection, and Initial of collector as well as site of collection.
9. Safely dispose of all contaminated materials.

Annex 11. Laboratory procedure for Gram staining technique

1. Labeling the slides clearly with patient code number.

2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.

3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.

4. Fix the dried smear by using heat or chemicals (methanol).

5. Cover the fixed smear with crystal violet stain for 30-60 seconds.

6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.

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

8. Wash off the iodine with clean water.

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

10. Cover the smear with neutral red or safranine stain for 2 minutes.

11. Wash off the stain with clean water.

12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.

13. Examine the smear microscopically, first with the 40 X objective to check the staining and to

see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

• Gram positive bacteria ----------dark purple

• Gram -negative bacteria ----------pale to dark red

Annex 12. Laboratory procedure for Media Preparation

A.SOP for preparation of Blood agar plate (BAP)

AIM of Blood agar plate: A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms. The medium is often used to investigate the forms of haemolysis from pathogenic microorganisms from clinical specimen. Blood Agar Base formulation has been used as a base for preparation of blood agar and to support good growth of a wide variety of fastidious microorganisms. Because it is a highly nutritious medium it can also be used as a general purpose growth media without adding blood. Blood Agar Base is suitable to isolate and cultivate a wide range of microorganisms with difficult growth.

Procedure for Preparation to make about 30-35agar plates

- Measure 500ml of distilled water using a measuring cylinder.
- Transfer the distilled water into a 1litre capacity conical flask.
- Weigh 20g of Blood Agar Base II powder using a weighing balance.
- And then add into the 500ml of distilled water and mix thoroughly.
- Boil until completely dissolved
- Autoclave at 121°C for 15 minutes.
- Allow to cool to 45-50°C in a water bath.
- Once the medium has been melted and cooled to 45-50 ºC
- Add 5-10% of defibrinated sterile sheep blood, in this case you can recuperate Haemophylus. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution.
- Aseptically add 25 ml of sterile defibrinated sheep blood with constant shaking.
- When mixing, avoiding froth formation.
- Gently pour 15-20 ml of the ready media on to the plates by using dispenser and allow setting.
- If air bubbles occurred, using a Bunsen burner gently invert and pass the flame over the poured blood agar in the plate to remove air bubbles. Leave standing for thirty minutes to solidify.
- Label on the bottom top of the blood agar plates the batch number & date prepared.
- Store the culture media plates upside down at 2-8^{0}C sealed in plastic bags to reduce chances of contamination. Shelf life: up to sixteen weeks provided there is no change in the appearance of the medium to suggest contamination, haemolysis, or deterioration.

B. Sop for preparation of Chocolate (Heated Blood) Agar

AIM of Chocolate (Heated Blood) Agar: Chocolate agar is a non selective media which supplies the factors X and V required for the proper growth of Haemophilus influenza. It is also used to culture nutritionally demanding pathogens such as Neisseria meningitis and Streptococcus pneumoniae. When Blood agar is heated, the red cells are lysed and the medium becomes brown in colour; it is referred to as chocolate agar. It is appropriate for isolating pathogenic bacteria in sputum, throat swabs, eye swabs, ear swabs, urogenital swabs, cerebrospinal fluid.

Procedure for preparation:

- Prepare as described for Blood agar except after adding blood, heat the medium in a 70^{o} C water bath until it becomes brown in color. This takes about 10-15 minutes during which time the medium should be mixed gently several times.
- Allow the medium to cool to about 45^{o}C,
- Remix and dispense in sterile petri dishes using a dispenser as described for blood agar.
- Leave standing for thirty minutes to solidify.
- Perform sterility testing as described for blood agar plate.
- Label the bottom of each plate with date of preparation and batch number.
- Store the culture media plates upside down at 2-8^{o} C sealed in plastic bags to reduce chances of contamination.

Important: Care must be taken not to overheat or prolong the heating of the medium because this will cause it to become granular and unfit for use. Up to sixteen weeks provided there is no change in the appearance of the medium to suggest contamination or deterioration.

C. Sop for preparation of Mac Conkey Agar

AIM of MacconkeyAgar;It is preferable for the isolation and differentiation of clinically important gram negative rods by inhibiting gram positive cocci.

Principle

MacConkey agar is selective and differential medium to distinguish gram negative entrobactericae and lactose fermenting bacteria from non lactose fermenters. 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.

Procedure for preparation:

- Prepare as instructed by the manufacturer.
- Suspend 51.1g of powder in 1 liter of distilled or deionized water.
- Heat and boil until completely dissolved with frequent agitation.
- Sterilize in autoclave at 1210C for 15 minutes
- Cool to 45-50 0C
- Mix well and dispense by dispenser (15-20 ml) aseptically into sterile petri dishes.
- Leave standing for thirty minutes to solidify.
- Perform sterility testing as described before.
- Label the bottom of each plate with date of preparation and batch number.
- Store the culture media plates upside down at 2-80C sealed in plastic bags to reduce chances of contamination.
- Test Samples for performance, using stable, typical control cultures.

Annex 13. SOP of Vitek 2 compact analyzer

Purpose

To describe the procedures for the preparation and identification of test microorganisms (test microbes and Quality Control Organisms) using the VITEK 2 Compact Instrument.

Procedure and Analysis

Follow the operational instructions below strictly for the proper use and required quality control activities on VITEK 2 Compact analyzer.

1. Initiation of the V2C System

- The V2C Instrument is always "on"; the instrument will say "Ready" or "Not Ready" on the digital screen. Once the computer is initialized, the instrument will say "Ready."The V2C will not run if it is not on ready mode.

- Select VITEK 2 Compact to initiate the system from the upper left side of the screen.

- After the system is initiated, log onto the system using the appropriate user name and password.

- The system is now initialized and ready for data entry.

2. Preparation of Organisms

A. QC organisms

- If starting from a frozen stock culture, remove the 0.5 mL cryovials from the -80°C freezer. Avoid repeated thawing and freezing of the frozen culture by aseptically removing a small portion (or loopful) of the frozen inoculum, then immediately return cryovials to -80°C freezer.
- Streak isolates the inoculum from a frozen stock culture or other source onto agar plate appropriate for the QC organism.
- Following this streak isolation, a second streak isolation on the appropriate media is recommended.

B. Non-QC organisms

- Use growth on tubes or plates to perform streak isolation on BAP or NA warmed to room temperature. A second streak isolation step is not required unless there is evidence of a mixed culture.

C. For cultures used on BCL and GN cards, incubate cultures for 18-24 h at 36±1°C. For cultures used on GP cards, incubate cultures for 12-48 h at 36±1°C. For cultures used on ANC cards, incubate cultures under anaerobic conditions for 18-24 h (or until sufficient growth is obtained) at 36±1°C. All organisms to be identified must be pure cultures.

3. Perform Gram stain using an isolated colony from a pure culture plate anddocument the Gram stain reaction.

4. Preparation of Inoculums

Select the appropriate card based on the Gram stain reaction and the organism's microscopic appearance. Allow the card(s) to come to room temperature before opening the package liner.

Aseptically transfer at least 3 mL of sterile saline into a clear polystyrene 12×75 mm testtube. Using sterile cotton swabs, prepare a homogenous organism suspension by transferring several isolated colonies from the plates to the saline tube. Adjust the suspension to the McFarland standard required by the ID reagent using a calibrated V2C DensiCHEK plus Meter, see below table.

Table 5: Suspension Turbidities Used for Card Inoculation.

Card	McF Range
GN	0.5-0.63
GP	0.5-0.63
ANC	2.7-3.3
BCL	1.8-2.2

Place the prepared suspensions in the cassette (see section 15, Instrument User Manual).

To use the DensiCHEKplus Meter to read samples:

i. Ensure the instrument is ON and set to the PLASTIC tube setting.
ii. Blank the Densi CHEK Plus by filling a test tube with sterile saline and inserting the tube into the instrument. Press the "0" key and slowly rotate the test tube. Ensure one full rotation is completed before the reading is displayed. The instrument will display a series of dashes followed by 0.00.
iii. To measure a sample, place a well-mixed organism suspension into the instrument and slowlyrotate the test tube. Ensure one full rotation has completed before the reading isdisplayed. The instrument will display a series of dashes followed by a reading.

iv. Remove the test tube after completion of a reading. The instrument will automatically shut off when test tubes are not inserted after one minute.

NOTE: If the instrument flashes 0.00 or 4.00, the suspension is either below 0.0 McF or above4.0 McF and is not within the reading range. Ensure suspensions are within the appropriate reading range to avoid compromised card results. If necessary, re-calibrate the DensiCHEK Plus instrument after processing each cassette.

5. Insert the straw (in the V2C card) into the inoculated suspension tube in the cassette.
NOTE: The age of the suspension must not exceed 30 minutes before inoculating the cards.

6. Proceed to data entry.

7. Filling the Cards

- Place the cassette in the Filler box on the left side of the V2C unit and hit Start Fill button on the instrument. Filling the cards takes approximately 70 seconds for a cassette regardless of thenumber of cards in the cassette holder. The V2C instrument will beep when the filling cycle is complete.
- Discard individual cards that may have been exposed to multiple fill cycles.

NOTE: The cassette must be placed inside the Loader Door within 10 minutes from the end of the filling cycle to avoid the cards being rejected.

- When the cards are finished filling, the Load Door is automatically unlocked. Place thecassette in the Load Door. The V2C Instrument will verify the scanned barcodes against theVirtual Cassette (the information scanned in by the analyst). Cards are sealed; straws arecut and the cards are loaded automatically into the carousel. The V2C will beep once all cardsare loaded into the cassette.

- When the cards are loaded, remove the cassette and dispose of the tubes and straws in biohazard container.

- The V2C automatically processes the cards once all the cards are loaded.

NOTE: Review the Navigation Tree. If the cassette status description in the Navigation Tree is red, the cassette needs more information to completely process the tests cards. Open up the red colored file and make sure all fields are defined.

8. Results

The VITEK system analyses the data results and determines the identity of the test microbes/QC organism based on colorimetric tests (biochemical reactions).Results are concurrently printed and the data sent to the Results View folder on the left side of the screen also called the Navigation Tree where the information is archived. A red cassette in the Navigation Tree is indicative of an error. If an error occurs during processing, refer to the Software User Manual.

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.

SebleSodere [Msc candidate]

Signature_____ Date of submission_____

This thesis has been submitted with our approval as advisors

Advisor:Dr. AdaneBitew [PhD. Associate Professor]

Signature_____

date _____

Place: Addis Ababa, Ethiopia