BACTERIAL PROFILE AND ANTIBIOTICS RESISTANCE PATTERN AMONG ADULT PATEINTS SUSPECTED FOR BLOODSTREAM INFECTION AT JIMMA UNIVERSITY MEDICAL CENTER, JIMMA, SOUTHWEST ETHIOPIA, 2019.

By

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Bacterial profile and antibiotics resistance pattern among adult patients suspected of having bloodstream infection at Jimma university medical center, Jimma, Southwest Ethiopia.

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Abstract

***Background**: Bacterial bloodstream infections are a major public health problem, which cause high morbidity and mortality. A wide range of gram positive and gram negative bacteria have been isolated from patients with bloodstream infection. Bloodstream infection may have life-threatening outcome if not diagnosed and treated early.*

***Objective**: To determine bacterial profile and their antibiotics resistance pattern among adult patients suspected for bloodstream infection at Jimma University Medical Center, Ethiopia, 2019.*

***Materials and Methods**: hospital-based cross sectional study was conducted at Jimma University Medical Center from March 15, 2019 to September 30, 2019. Consecutives sampling technique was used. Ten ml (two 5ml from two different site) blood samples was collected aseptically from the study participant and inoculated into Tryptic Soya Broth and incubated at 37^0c for 7 days. Pure colonies from culture plates that showed growth were further identified with a panel of biochemical tests to identify isolates. Antibiotic susceptibility test was then done for isolates. Data was entered into Epidata version 3.1 and analyzed by SPSS version 23. Logistic regression was used to determine relationship between dependent and independent variable with significance level at $p<0.05$.*

***Result**: A blood culture of 171 respondents was done of which, 30 (17.54%) were positive. The common bacteria isolated from blood culture were S. aureus 8 (26.67%), Coagulase negative Staphylococci 6 (20%) and E. coli 6 (20%). Less frequently isolates were Citrobacter spp. 2(6.67%) and P.auroginosa, Salmonella spp., S. pyogenes and S. pneumoniae which all account the same 1(3.33%). Gram positive and gram negative bacteria constituted 16 (53.33%) and 14 (46.67%) respectively. The range of resistance of Gram positive and Gram negative were from 0% (ciprofloxacin) – 93.7% (ampicillin), and 0% (Meropenem) – 100% (ampicillin) respectively. Educational level (no formal education p= 0.04) and having comorbid (p=0.003) were statistically significant factors for the occurrence of bloodstream infection.*

***Conclusion:** The overall culture confirmed prevalence rate of blood isolate was high. S. aureus and E. coli were the most common Gram-positive and Gram-negative bacteria causing bloodstream infection respectively in study area. More than $3/4^{th}$ of the isolated bacteria were multidrug resistant. Therefore physicians should have to consider bloodstream infection and manage patient as early as possible based on blood culture and antimicrobial sensitivity test.*

***Keywords**: Bloodstream infection, Adult, Bacterial profile, antibiotics resistance pattern, Jimma, Ethiopia*

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Abbreviations and Acronym

AOR	Adjusted Odd Ratio
ATCC	American Type Culture Collection
BSI	Bloodstream Infection
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
COR	Crude Odd Ratio
ESKAPE	*Enterococcus, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa* and *E. coli*
ESBL	Extended-spectrum beta-lactamase
GNB	Gram Negative Bacteria
GPB	Gram Positive Bacteria
KIA	Klingler Iron Agar
JUMC	Jimma University Medical Center
ICU	Intensive Care Unit
LDC	Lysine Decarboxylase
MRSA	Methicillin Resistance *Staphylococcus aureus*
MRCoNS	Methicillin Resistance *Coagulase Negative Staphylococci*
MHA	Muller Hinton Agar
Spp.	Species
SPSS	Statistical Package for Social Science
TSB	Tryptic Soya Broth
TTC	Triphenyl Tetrazolium Chloride

1. Background

1.1 Introduction

Bloodstream infection (BSI) is a potential life-threatening infection with mortality rate ranging from 20 to 50% and is one of the major causes of death throughout the world [1]. Bloodstream infection is caused by different microorganism such as fungi, virus, parasite and bacterial [2] of which bacteria is the most common cause of bloodstream infection . It can be as a primary infection if no identifiable extravascular focus of infection or secondary if occurred from dissemination of primary infections such as respiratory system, urinary tract, Endocarditis and intra-abdominal infections which are common source of BSIs [3]

Normally, bloodstream is sterile environment; however several types of bacteria live on/in different body parts of human body as a normal flora. When bacterial niches are disturbed by different factors and the immunity of individuals is compromised they may enter into the circulating blood from their normal residency and causing bloodstream infection [4]. Bloodstream infection may also be related to compromise of immune system that unable to hold infection elsewhere on/in the body from which bacteria disseminate to blood circulation. Bacteria from elsewhere of these infections enter the blood circulation and multiply rapidly, causing bloodstream infection [5].

A wide range of bacteria have been isolated from blood of patients who have bloodstream infection. Both gram negative and gram positive bacteria were responsible to cause bloodstream infection [6]. The most common bacteria have been isolated from blood are: gram positive bacteria such as *Staphylococcus aureus (S.aureus), coagulase negative Staphylococci* (*CoNS*), *Streptococcus pneumoniae (S. Pneumoniae), Streptococcus pyogenes (S. pyogenes), Enterococcus species (Enterococcus spp.*) and gram negative bacteria such *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Salmonella species (Salmonella spp.), Citrobacter species (Citrobacter spp.), Pseudomonas species (Pseudomonas spp.), Acinetobacter species* and others [6-9].

The pathogenesis of bloodstream infection involves complex interactions between the invading bacteria and the defense mechanisms of the host [10]. If not diagnosed early, BSIs continue to be a severe, often life-threatening complication such as severe sepsis, septic shock, and multisystem organ dysfunction will occur. These complications are associated with increased hospital stay, health care expenditures and mortality [11]. After onset, a

bacterial cell component /product like bacterial endotoxin (lipopolysaccharide) of gram negative and lipoteichoic acid, peptidoglycan, and extracellular products (toxins, enzyme and likes) of Gram-positive bacteria triggers the host immune response that may handle infection (elimination of the pathogen) or result further complication to different body parts (excess tissue damage) [2]. Paradoxically, the host immune and inflammatory responses, essential for the control of infection, also contribute to the deleterious sequelae on host cells [10]. In cases of impaired immunity due to underlying diseases or therapy frequent in the hospital environment, patients may die of infection while an immunocompetent host may hold the progress of bloodstream infection [12].

Currently, antibiotic resistance is recognized as a global health problem that has been escalated by world health organizations to one of the top health challenges facing the 21st century [13]. Different reports have shown antibiotics resistance arises as a consequence of mutations in the genomes of microbes and improper selection of antibiotic used for treatment which provides a competitive advantage for mutated strains [14]. Monitoring and controlling AMR is challenging especially in developing countries, due to lack of surveillance systems, limited resources, poor adherence to infection control measures, use of antibiotics without physician prescription and limited antimicrobial formularies [15]. Early diagnosis and adequate antibiotic treatment of BSI has been shown to be associated with a substantial reduction in mortality as well as in treatment costs [16]. [17].

Early identification of the causative pathogen facilitates the improvement and shortening of antibiotic treatment and results in lower case fatality rates and reduced development of antibiotic resistance [18]. Blood culture remains the most practical and reliable method for diagnosis and management of blood stream infection as it allow the detection of causative pathogens with its drug susceptibility for optimization of antibiotic therapy [18]. Blood cultures should be obtained along with clinical investigations to search for a focus of infection and therefore provide specific antimicrobial therapy [19].

In Ethiopia BSI is one of the most cause of morbidity and mortality in all age group [20]. Different reports indicate that there are wide practices of misuse of antimicrobials by health care providers, unskilled practitioners, animal husbandry operations, and drug users [21]. These has led to an increase in the multidrug-resistant and thus worsened the condition. Early diagnosis and treatment of bacterial bloodstream infection is the best approach to reduce morbidities and morbidities related to BSI [22]

1.2 Statement of problem

Bloodstream infections (BSI) are potentially life-threatening condition which result in a serious problem and is a leading cause of mortality worldwide in all age group, being recently listed as a global health priority by World Health Organization [23]. BSI remains one of the most common causes of morbidity and mortality globally with an annual incidence of 100/100 000 patient days and a case fatality rate of 20–50% and was the third most common cause of death in Germany [24]. More than half of Million episodes and 79 000–94 000 deaths of BSI per year in North America and greater than one million episodes of BSI and 157 000 deaths per year in Europe [25].

Most of complicated case related with bloodstream infection caused by drug resistant bacteria [26]. This may result a considerable economical and human cost especially infection caused by ESKAPE [27]. In United States, at least 2 million people acquire serious infections per year with bacteria that are resistant to at least one antibiotics designed to treat those infections. Among this more than 23,000 people die as a direct result of these antibiotic-resistant infections per year. The economic cost of antibiotic resistance to the U.S. was estimated to $20 billion in excess direct healthcare costs, with additional costs to society for lost productivity as high as $35 billion a year [28]. When looking only at a part of the impact of AMR, the continued rise in resistance by 2050 would lead to 10 million people dying every year and a reduction of 2% to 3.5% in Gross Domestic Product. It costs the world up to 100 trillion United State Dollar [26].

However, the problem is still common in developed nations, sub Saharan countries is in the highest burden [29]. Blood stream infection accounts for 10-20% of all nosocomial infections and is the eighth leading cause of mortality [30]. Antibiotic resistant bacteria are getting increased day by day alarmingly, hence results common infections either more difficult to treat or untreatable [31]. It is one of the most devastating complications resulting in prolonged length of hospital-stay, high costs and loss of life with mortality rate of 19 % [32]. Many bacterial pathogens have developed resistance to most of the antibiotics and it has become a serious health problem with many economic and social inferences [33].

Numerous classes of antimicrobial agents have become less effective as a result of the emergence of antimicrobial resistance, often as a result of the selective pressure of antimicrobial usage. Among the most important emerging antimicrobial resistance problems in recent years are Methicillin resistance in staphylococci, resistance to extended spectrum

cephalosporin, fluoroquinolones and carbapenem of *Enterobacteriaceae* [34]. ESBL-producing *Enterobacteriaceae* are often also possessing resistance determinants to other important antibiotic group results in an extremely limited range of effective agents. Knowing prevalence of ESBL producers is therefore crucial in order to prevent their dissemination and to guide the treatments of infected patients.

In our country few studies were reported regarding bacterial profile and their antimicrobial resistance pattern on the blood culture isolates from adult patients. However bacterial blood stream infection is common and bacterial antibiotic resistance is a dynamic that vary from region to region even from time to time in the same area. Therefore routine investigation may play important role to provide updated status about bacterial profile and its drug resistance pattern. Most of the previous studies in the area did not show prevalence of ESBL producing bacteria even though rapidly emerging of such bacteria. In addition, most of the studies on blood stream infection excluded those patients who have been recently started treatment with empirical antibiotics before blood sample collection however growing challenge of drug resistant bacteria have been continue. So our study tries to include them accordingly.

1.3 Significance of study

Prevailing data on bacterial species causing BSI and their antibiotics sensitivity are essential for proper management of patients. Therefore, this study share updated information on profiles of bacteria that commonly cause bloodstream infection. It also provides current status of antibiotics resistance pattern of common bacteria that causing bloodstream infections among adult patients. It may alarm health sector of the area to implement preventive activities like expansion and strengthening prevention and monitoring of bloodstream infection and spread of drug resistant bacteria. It can be used as a baseline for further studies in the area.

2. Literature Review

Bloodstream infection (BSI) due to bacterial pathogens is a global concern and it remains a growing public health challenge despite the great advances in medical science in the past century. Pathogen frequency and it's susceptibility to specific drug varied somewhat over time and by region [6].

2.1. Prevalence and Epidemiology of etiological agents

A prospective observational study among all adult patients with clinical signs of sepsis was conducted in an inner-city hospital in New York City between May, 2010 and May, 2011. A total of 722 adult patients were suspected for sepsis of which 12.6% were culture positive. Among positive isolates male accounts 47.2% (43/91). Gram-positive bacteria 72 (59%) was common than Gram-negative 38 (31.1%). Among the Gram-positive isolates, the most common organism identified was CNS 28 (38.8%), followed by *Staphylococcus aureus* 15 (20.8%), *Enterococcus Spp.* 12 (16.6%), and *Streptococcus pneumoniae* 8 (11.1%) [8]. The other study conducted in United State commonly tested agents among the most prevalent species identified show that CoNS (42.0%), *S.aureus* (16.5%), *E.faecalis* (8.3%), *E. coli* (7.2%), *K. pneumoniae* (3.6%), and *E. faecium* (3.5%) were the most frequently isolated bacteria from blood cultures [30].

In India, a cross-sectional study to determine the prevalent organisms causing bloodstream infection was conducted on 170 blood sample by BACTEC BD 9050 system. Positive rate was 53 (31.2%). Age groups of 31 to 40 years were the most affected (11/53). Of the 53 culture-positive patients, 30 were adults admitted to medical ICUs. CoNS was the most commonly isolated organism (34%) followed by *Staphylococcus aureus (30%)* [17]. Other study in Bangladesh showed that *S.typhi* was the most frequently blood isolated bacterial pathogen (36.9%) followed by *CoNS* (21.5%), *Pseudomonas Spp. (*12.5%), *S. paratyph (*8.9%) *and Acinetobacter Spp.* (5.1%) [35].

Again, other study in the same country showed similar positivity rate which was 32.75%. Study enrolled 400 patients suspected of blood stream infection. Among culture positive 44.61% and 55.72% were males and females respectively. Gram positive organism (57.69%) were more common than gram negative organism. Most frequent pathogen identified among gram negative bacteria were *Klebsiella Spp.* 42.8%, followed by *E. coli* 32.14%, *Acinetobacter* 17.85%, *Pseudomonas* 3.57% and *Salmonella* 3.57% respectively [36]. A

retrospective study conducted in Dhahira Region, India reveal that of the 360 bacterial pathogens isolated 57.8% were gram-positive and 42.2% were gram-negative. The common isolates were: *Streptococcus* spp. (21.1%), *CoNS* (20.8%), *E. coli* (11.9%), *S. aureus* (11.4%) [37].

In Tanzania there is one study on isolation of bacteria from blood stream and their antimicrobial susceptibility. A total of 13833 blood cultures were enrolled. Over all prevalence was 13.4% (1855). Among this 82.1% were gram positive bacteria and the rest were 17.9% gram negative bacteria. Among these 51.2% were from adults. The most common bacterial pathogens isolated were CoNS (67.4%), *S. aureus* (13.2%), *E. coli* (7%) and *Klebsiella* spp. (7.0%) and Other were *Proteus* spp. (1.8%), *Pseudomonas* spp. (1.2%), *Streptococcus* spp. (1.5%), *Salmonella spp.* (0.6%), *Enterobacter* spp. (0.2%) and *Acinetobacter* spp. (0.1%) [31].

In Ethiopia, only very few published studies and review were conducted on BSI. A retrospective study was conducted from January, 2015 to December, 2016 on 500 blood culture result from Addis Ababa Regional Laboratory. Among these overall prevalence of blood culture positive was 164 (32.8%). Out of a total 164 isolates, 77.4% were gram positive bacteria and 22.6% were gram negative bacteria. The isolated bacteria were *S. aureus* 50.0%, CoNS 26.21%, *K. pneumoniae* 14.02%, E. coli 3.6%, *A. baumannii* 2.4%, Streptococcus spp.1.8%, *P. aeruginosa* 1.2% and *N. meningitidis* 0.6% [22]. In other part of the country, Mekelle, cross sectional study conducted from March to October 2014 showed Isolation rate of 28%. Most commonly isolated were *S. aureus* 37.5%, CoNS 30.6% and *E. coli* 3.1%. *Citrobacter* spp. 1.7% and *S.typhi* 1.6% were the least isolates. More bacteria were isolated from females than males. Age group of 15–29 years was more affected 48.6%) [33].

Laboratory based retrospective study was conducted in University of Gondar. Blood cultures of 390 patients were enrolled. 71 (18.2%) were culture positive. Prevalence is high in females (41/71). Isolates of bacteria were CoNS 42.3%, *S. aureus* 23.9%, *Klebsiella spp.* 12.9%, E. coli 7.0%, *P.auroginosa* 5.6% and *Salmonella spp.* 4.2%. Gram positive and gram negative bacteria constituted 69% and 31% respectively [38]. Other study in the same region, among a total of 856 blood samples analyzed, 169 (19.7%) were positive of which 58% and 42% were male and female respectively. In their study, CoNS (31.6%) were the most common causative agent for BSI followed by *S.aureus* (27.6%), *E. coli* (8.6%) and *Citrobacter* spp.(4.6%). *Salmonella spp.* (3.4%), *P.auroginosa* (3.4%), *S. pyogenes* (3.4%), *K. pneumoniae* (3.4%)

Enterobacter spp. (3.4%), *Salmonella* spp. (2.3%), *Providential* spp. (1.7%), *Proteus* spp.(1.7%), *S. viridians* (1.1%), and *Enterococcus* spp. (0.6%) [39].

Retrospective study in Bahirdar reveals that over all isolation rate was 39.2%. GNB isolates constituted 52.3%. *S. aureus* 50 (22.7%), CoNS 35(15.9%), *K. pneumoniae* 35 (15.9%), *E. coli* 19 (8.6%), *P. aeruginosa* 15 (6.8%) and *Acinetobacter* spp. 13(5.9%) were the most dominant isolates. 104 (38.2%) male and 116 (40.1%) females had blood culture positive. Highest percentage 115 (70.1%) of microbial isolates were reported in the age group of patients less than one year [20].

In Jimma, A cross sectional study was conducted on 260 adult febrile patients in Jimma University Medical Center from 27 October 2009 to 26 March2010. From the total of 260 blood specimens only 23(8.8%) were positive to seven different types of bacteria. The isolated bacteria were: *Coagulase negative staphylococci* (26.1%), *S. aureus* (21.7%), *S. pyogenes* (13.0%), *E. coli* (17.4%), *K. pneumoniae* (13.0%), *Salmonella spp*. (4.3%), and *Citrobacter spp*. (4.3%) [40].

Other laboratory based prospective cross sectional study was performed in 95 adult septic cases in the same hospital during the period of March to June 2013. From a total of 95 suspected 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 *S. aureus* being the most frequent, while Gram negative accounted for the remaining 46.7 % with E. coli being the commonest isolate among Gram negative bacteria [32]

2.2. Drug Susceptibility Pattern

Drug susceptibility result of a prospective observational study conducted in New York City for 91 bacterial isolates show that most isolated *CoNS* 21 (75%) and *S.aureus* 6(40%) were resistant to methicillin. Resistance rates for *MRSA* were as follows: erythromycin, 6 (100%); clindamycin, 4(66.6%); fluoroquinolones, 5 (83.3%); and no sulphamethoxazole resistance. resistance to gentamicin and streptomycin *Streptococcus* spp. synergy was found in 4 (50%) and 5 (62.5%) isolates, respectively [8].

A retrospective study conducted in India determined susceptibility of the 360 isolated bacterial pathogens that *Staphylococcus aureus* and *CoNS* were more commonly resistant to trimethoprim/ Sulphamethoxazole (35.3%) and penicillin (25.9%). About 9.8% of isolated *S.*

aureus and (18.7% and 1.7%) *CoNS* were resistance to gentamycin and amoxicillin Clavulanic acid respectively. *Streptococcus* spp. was resistant to trimethoprim Sulphamethoxazole (39.1%), erythromycin (19.6%) and cefuroxime (9.8%). All isolated *Streptococcus* spp. were susceptible to Cefotaxime. Ampicillin resistance was frequently shown in *Acinetobacter* (85.7%), *E. coli* (54.3), *Enterobacter* (9.1%) and *K. pneumoniae* (71.4%). All *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *P.auroginosa were susceptible to amikacin.* Five point seven %, 5.7%, 11.4% and 17.4% of *E. coli* were resistance to cefotaxime, ceftazidem, ceftriaxone and ciprofloxacin respectively [37].

Antimicrobial susceptibility for 1855 bacterial isolates from study in Tanzania report all bacterial isolated showed high resistance to penicillin G (70.6%), tetracycline (63.8%), cefotaxime (62.5%) and ampicillin (62.3%). Moderate to high resistance was seen against chloramphenicol (45.2%), erythromycin (35.0%), ciprofloxacin (29.3%), trimethoprim Sulphamethoxazole (25.0%) and gentamicin (23.5%). Of *S. aureus* isolates, 23.3% were resistant to methicillin [31].

A retrospective study conducted in 2015/6 on 500 blood culture results from Clinical microbiology laboratory unit of Addis Ababa Regional Laboratory Provide drug susceptibility character of 164 isolates. *S. aureus* show resistance to Penicillin (82.90%), erythromycin (75.60%), trimethoprim sulphamethoxazole (85.40%), Doxycycline (74.30%), tetracycline (74.30%), ciprofloxacin (59.70%), chloramphenicol (46.30%) and gentamycin (73.10%). *CoNS*: high resistance to penicillin 90.50%, erythromycin (83.30%), trimethoprim sulphamethoxazole (85.70%), doxycycline (85.70%), tetracycline (85.70%) and gentamycin (83.30%) and low resistance to Ciprofloxacin (47.60%) and chloramphenicol (47.60%). All isolated *S. pyogenes* were susceptible to all used antimicrobial except ampicillin. *K. pneumoniae*: high resistance to ampicillin (100%), amoxicillin clavulanic acid (91.3%), ceftriaxone (86.9%), trimethoprim sulphamethoxazole (86.9%), ceftazidem (78.3%) and tetracycline (73.9%) and high resistance of *E. coli* to ampicillin and trimethoprim sulphamethoxazole which accounts 83.3% for both [22].

The other Cross sectional study carried out in Mekelle hospital reveal that antimicrobial resistance pattern for gram positive bacteria was 0–83.3%. Most of their isolated *S. aureus* were resistant to trimethoprim sulphamethoxazole (66.7%), ceftriaxone (57.4%) and doxycycline (53.7%). Near to 82% and 62% resistance was seen by *CoNS* to trimethoprim sulphamethoxazole and doxycycline, respectively. From isolated bacteria in their study 59%

show multi drug resistant. Antimicrobial resistance level of GNB was from 0 to 100%. *E. coli* were resistant to ceftriaxone (60%). Three fourth of *S.typhi* were resistant to doxycycline and trimethoprim sulphamethoxazole. GPB were sensitive to amoxicillin clavulanic acid and ciprofloxacin. This study shows that *S. aureus* (63%), CoNS (61.2%), *S. pyogenes* (50%) and *E. coli* 8 (53.3%) were multiple drug resistance (MDR). In general, 59% of the isolates were MDR [33].

Drug susceptibility pattern of 71 isolates of a total sample of 390 of study in Gondar showed high rates of resistance that 23.5% – 58.8%, and 20%– 100% for gram positive and gram negative bacteria respectively. *Klebsiella spps:* resistant to ampicillin (75%), trimethoprim sulphamethoxazole (50%), tetracycline (75%), chloramphenicol (62.5%), amoxicillin (62.5%) and ceftriaxone (62.5%). *E. coli* were resistant to ampicillin (100%), tetracycline (60%), and chloramphenicol (40%). *Salmonella spp.* were resistant to chloramphenicol (100%), gentamycin /ampicillin/ ceftriaxone/ trimethoprim sulphamethoxazole (66.7% each). Two third of their isolated *S. aureus* were resistant to erythromycin, trimethoprim sulphamethoxazole and penicillin-G. Ciprofloxacin was relatively effective drug against both gram positive and gram negative bacteria [38].

Other study in the same region, describe drug susceptibility pattern of 169 isolated bacteria. The overall drug resistance of gram-positive isolates was an intermediate level of resistance (60%–80%) with resistance pattern in response to: penicillin was 72%, ampicillin was 63.4%, and erythromycin was 60.3% with a low level of resistance (<60%) in ceftriaxone, amoxicillin, gentamycin and chloramphenicol. Gram-negative bacteria showed a high level of resistance (>80%) against ampicillin and amoxicillin, an intermediate level of resistance to tetracycline (74.2%) and trimethoprim sulphamethoxazole (62.3%), and a low level of resistance (<60%) to ceftriaxone, cefotaxime, ciprofloxacin, gentamycin and chloramphenicol. MDR was observed in 84% of Gram-positive and 92% of Gram-negative isolates [39].

Other retrospective study in Bahirdar describes drug susceptibility pattern of their 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%) while lower resistance to Ciprofloxacin (14.4 %), trimethoprim sulphamethoxazole (26.1%), Tetracycline (7%), Chloramphenicol (37.5%), Clindamycin (12.8%) and Gentamycin (16.6%). Gram negative bacteria showed 82.6%, 68%

and 66% resistance to ampicillin, ceftriaxone and trimethoprim sulphamethoxazole respectively [20].

Antibiotic test for 23 isolated bacteria from cross sectional study in Jimma showed that high rates of resistance to most antibiotics tested. The range of resistance for gram positive bacteria was 0% to 85.7% with high resistance to Penicillin (85.7%), ampicillin (71.4%) and trimethoprim sulphamethoxazole (78.6%). All gram positive and gram negative bacteria were susceptible to ciprofloxacin and ceftriaxone. Gram negative bacteria show high resistance to ampicillin (100%), tetracycline (88.9%), trimethoprim sulphamethoxazole (88.9%) and chloramphenicol (77.9%). All the *S. aureus* and 83.3 % of *CoNS* isolates were multidrug resistant. Whereas two and one S. *pyogenes* isolates were resistant to two and three drugs, respectively. All the gram negative bacteria isolates were multidrug resistant [40].

Cross sectional study performed from 95 adult septic cases in Jimma University specialized hospital determine drug susceptibility characteristics for 15 bacterial isolated. They showed high rates of resistance to most antibiotics tested in-vitro. The ranges of resistance to Gram positive bacteria were 0 % to 100 %. All isolates of gram positives showed resistance against penicillin-G (100 %); but high susceptibility to most of the other antimicrobials tested: ceftriaxone (87.5 %), chloramphenicol (87.5%), ciprofloxacin (87.5%), amoxicillin-clavulanic acid (75 %) and erythromycin (62.5 %). Around 33.3 % *S. aureus*, were *MRSA*. The range of resistance to Gram negative bacteria was from 14.3 % to 85.7 %. In their study multidrug resistance was observed in 80 % of isolates. Of this 87.5% and 71.4 % accounted for gram positive and gram negative bacteria respectively. ciprofloxacin was the effective drugs against the tested Gram positive and Gram negative bacteria [32].

3. Objective

3.1. General objective

- To determine bacterial profile and antibiotic resistance pattern among adult patients suspected of having bloodstream infection at JUMC from March15-September 30, 2019.

3.2. Specific objective

- To determine the bacterial profile among adult patients suspected of having bloodstream infections at JUMC.
- To determine antibiotic resistance pattern of bacterial isolates from adult patients suspected of having bloodstream infections at JUMC.
- To assess factors associated with blood culture positivity among adult patients suspected of having bloodstream infections at JUMC.

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4. Methods and materials

4.1. Study area and period

Study was conducted at Jimma university medical center from March to September 2019. Jimma university medical center is located in Jimma town, which is 352 km to south west from the Addis Ababa which is a capital city of Ethiopia. It is a teaching and referral hospital in south western part of the country. It has been provide service for about 12.5 million population of south west. It has been under the administration of federal government. This medical center contain total of 659 active beds among which 120 beds are dedicated for internal medicine ward, 72 beds in adult surgical ward and 16 beds in adult ICU.

4.2. Study design

Hospital based cross sectional study was conducted

4.3. Participants

4.2. 1. Source of participants

All adult patients clinically diagnosed with blood stream infection at Jimma University Medical Center.

4.2.2. Study participants

All adult patients clinically diagnosed with blood stream infection at Jimma University Medical Center during the study period.

4.2.3. Study unit

Adult patient clinically diagnosed with blood stream infection at Jimma University Medical Center during the study period and who fulfill the eligibility criteria.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

All adult patients who are equal or greater than 18 years old and clinically diagnosed with blood stream infection were included.

4.4.2. Exclusion criteria

Patients who did not give consent and those with life threatening medical condition however the Results of their blood cultures were reported to their respective physician for management of their infection.

4.5. Sample size

No sample size calculation. All adult patients clinically diagnosed with blood stream infection from March 15 to September 30 were added and became 171 respondents.

4.6. Sampling Technique

Consecutive sampling technique was used

4.7. Study variables

4.7.1 Dependent Variables

- Bacterial isolates
- Antibiotic resistance

4.7.2 Independent Variables (Background characteristics of patients with bloodstream infection)

- Socio demographic factors
  - ✓ Sex
  - ✓ Marital status
  - ✓ Residence area
  - ✓ Age
  - ✓ Educational level
- Clinical data
  - ✓ Admission unit/department
  - ✓ Suspected focus of infection
  - ✓ antibiotics Use before blood sample collection
  - ✓ Comorbidity

4.8. Operational Definition and definition of term

Multidrug resistance: resistance of isolated bacteria to more than two drugs of different classes [41]

Adult: Patients who are greater or equal to 18 years old (EDHS 2016).

Bloodstream infection: Patient with any of the following signs and symptoms: fever (>38 °C) / hypothermia (<36^0C), Chills/rigours or hypotension and at least one positive blood culture not related to contamination [42]

Antibiotic resistance: growth of bacteria in the presence of one or more of the antibiotics designed to treat them.

Admission unit/ Department: location where patient has been in the hospital at a time of blood sample collection.

4.9. Data collection procedures

4.9.1. Sociodemographic and clinical data

After physicians identify those patients who have fulfilled the criteria of bloodstream infection according to medical record: - data on sociodemographic characteristics and other clinical data were collected using interview administered questionnaire. All necessary information was collected by data collector by interviewing the patients or care giver.

4.9.2. Blood Sample collection, transportation and processing

4.9.2.1. Blood sample collection and transportation

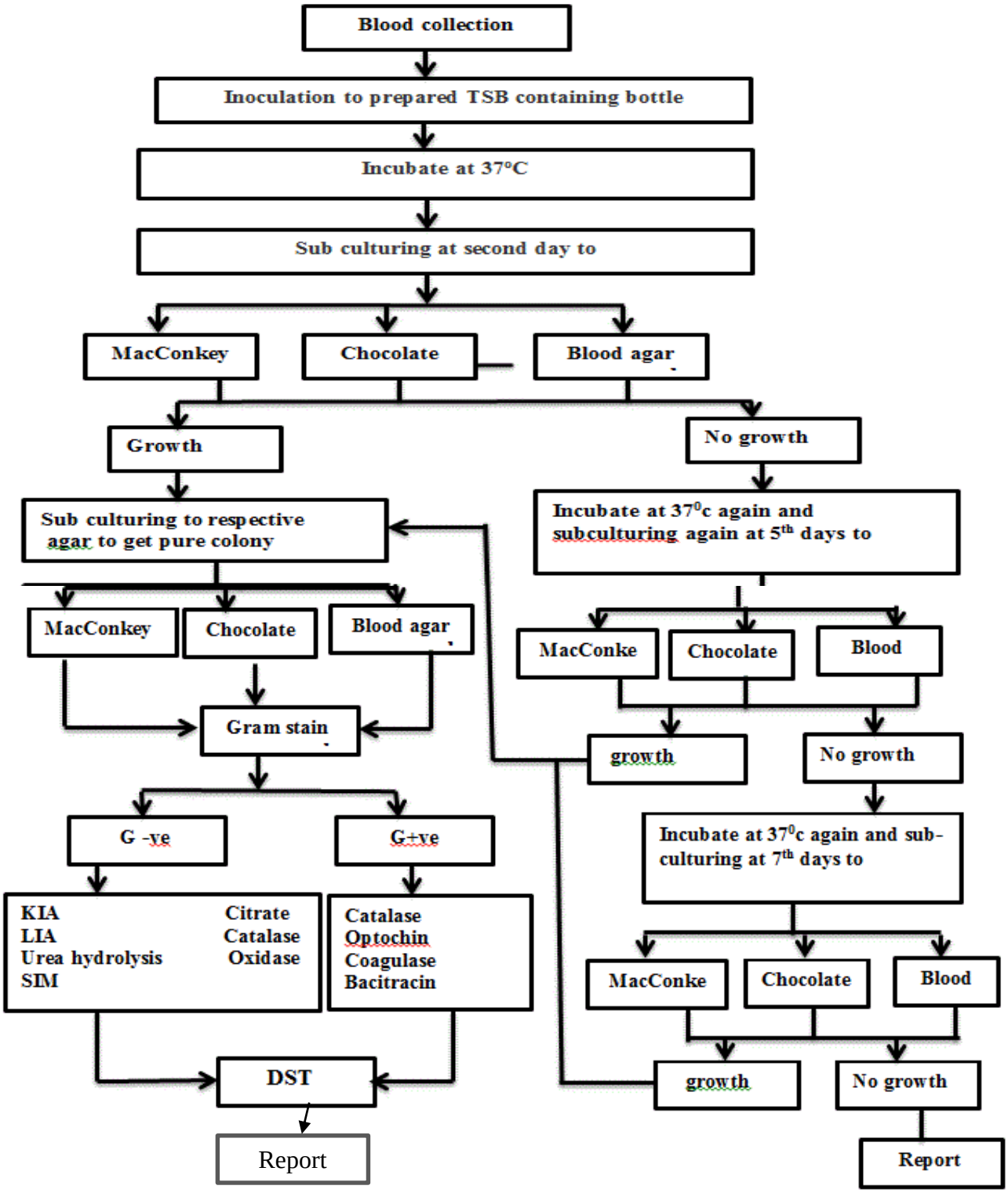
About 10 ml of venous blood was collected from two different sites of vein aseptically by disinfecting with 70% alcohol and 2% tincture of iodine. Blood was collected by experienced nurses. The collected blood samples (5ml) were inoculated into each bottle containing 45ml of sterile Tryptic soya broth (Oxoid Ltd). Inoculated bottle was then labeled with patient name, patient's identification number, date and time of collection. Labeled blood culture bottles were transported within 30 minutes to core research laboratory microbiology unit for culture and antibiotics susceptibility testing. Blood sample collection was done using standard protocols attached in the annex.

4.9.2.2. Sample processing

Isolation and identification: Manual blood culture system was used to grow microorganism from blood. Blood culture bottles were incubated at 35-37°C with daily inspection for visible microbial growth for 7 days by observing visually for any of the following: turbidity, gas production, hemolysis and/or coagulation of broth. For blood cultures that show signs of microbial growth, subcultures were made onto MacConkey agar (Oxoid Ltd, UK), Blood agar and chocolate agar plates (Difco TM and Accumix). MacConkey agar and blood agar plates were incubated in aerobic whereas the chocolate agar plates were in a candle jar at 35-37°C for 24 to 48 hrs. For blood culture that did not show sign of microbial growth, blindly sub culturing was also performed at the 2^{nd}, 5^{th} and 7^{th} day of an inoculation. Blood culture result with no microbial growth after 7 days were recorded as culture negative. For positive blood culture the isolates were identified with macroscopic colony characteristics, gram staining result and biochemical test. Identification panels including Bacitracin and Optochin sensitivity, catalase and Coagulase test for gram positive bacteria and kligler iron agar (carbohydrate fermentation test and gas production), Simon citrate agar (citrate utilization test), oxidase test, urea agar (urease test), LIA agar (Lysine decarboxylase test) and

SIM+TTC (sulfur production, Indole and motility test) were done for gram negative bacteria following standard procedures.

Antibiotic susceptibility test: Antibiotic susceptibility testing was performed using Kirby-Bauer agar disc diffusion method for the isolated organisms according to Clinical Laboratory Standards Institute (CLSI, 2017) guidelines. Pure colonies from subculture plate were picked and transferred to a tube containing 3 ml sterile normal saline and mixed thoroughly to make the suspension homogenous until its turbidity is equivalent to turbidity of 0.5 McFarland. Then suspension was swabbed onto Mueller Hinton agar (for *S. pneumoniae* Muller-Hinton agar with 5% sheep blood was used) and then incubated at 37^0C for 18-24 hours. The zone of inhibition was measured and interpreted according to the standardized table supplied by CLSI. Based on CLSI recommendation, antibiotic discs (Oxoid Ltd and Liofilchem): Penicillin G (P, 10 IU), Amoxicillin Clavulanic Acid (AMC, 30µg), Ampicillin (AMP, 10µg), Ciprofloxacin (CIP, 5µg), Trimethoprim Sulphamethoxazole (SXT, 25µg), Gentamicin(CN, 10µg), Ceftriaxone (CRO, 30µg), Erythromycin (E, 10µg), Cefotaxime (CXT, 30µg), Cefoxitin (FOX, 30µg), Ceftazidime (CAZ, 30µg), Clindamycin (CLN, 2µg), Chloramphenicol (CAF, 30µg), Cefepime (CFP, 30µg), Tetracycline (TE, 30µg), Doxycycline (DO, 30µg) and Meropenem (M, 10µg) was used.



Note: In addition to days indicated in chart flow, subculturing is performed when sign of microbial growth is observed during macroscopically daily inspection of microbial growth.

Figure1: Flow chart that shows work process of blood specimen for identification bacteria among adult patients suspected of having bloodstream infection at JUMC from March15, 2019-September 30, 2019.

ESBL and/or AmpC detection: Isolated pure colonies were used to screen ESBL and/or AmpC using a standard disk diffusion assay. Ceftriaxone (30 μg), Cefotaxime (30 μg), Ceftazidime (30 μg) and/or Cefepime (30 μg) containing antibiotic discs was used to phenotypic screening of ESBL and/or AmpC. Isolates that were resistant to Ceftriaxone, Cefotaxime, Ceftazidime and/or Cefepime was considered as a presumptive ESBL producer. Then disc diffusion phenotypic confirmation test was performed with disc A (Cefpodoxime), disc B (Cefpodoxime + ESBL inhibitor), disc C (Cefpodoxime + AmpC inhibitor), disc D (Cefpodoxime + ESBL inhibitor + AmpC inhibitor) to determine presence of an ESBL and/or AmpC. Five minute after inoculating plates with direct suspension of colony equivalent to turbidity of 0.5 McFarland, four discs (A, B, C and D) were placed at a distance of 24 mm apart to each other from center to center, and then incubated at 35-37 °C for 18–24 h aerobically. Then zones of inhibition were measured and then recorded on excel sheet. Finally, The recorded data from the excel sheet was transported to Mast group ESBL/AmpC and CARBA plus calculator spreadsheet (Mast group, UK). Result was displayed as negative or positive for ESBL, AmpC or both ESBL and AmpC.

4.10. Quality control

Training was provided for data collectors. The study was opened for audit during data collection. Susceptible strains of *E. coli* (ATCC 25922), *S.aureus* (ATCC 25923) and *P.auroginosa* (ATCC 27853) were used as a reference strains for identifications and drug susceptibility testing. In addition ESBL negative *E.coli* (ATCC 25922) and ESBL positive *K.pneumoniae* (ATCC 700603) was also used as positive and negative control for detection of ESBL producer bacteria. Media was checked for its performance and 5%-10% of the prepared culture media were randomly selected and checked for its sterility by incubating over night to see any growth. The whole procedure of sample processing and result interpretation was cross checked by trained professionals. In general reliability and validity of the study was guaranteed by implementing quality control measures throughout the whole process.

4.11. Data processing and analysis

Data collected on socio demographic factors and others clinical data were entered into Epidata version 3.1 and analyzed using SPSS version 23 statistical software. Binary logistic regressions were used to see association between explanatory and outcome variable. Results

were expressed using frequency and percentages. The results were summarized in table, charts and text. P-value of less than 0.05 was considered as statistically significant

4.12. Ethical considerations

Ethical clearance was obtained from Jimma university institute of health research ethics review board prior to the commencement of the study. Permission letter was obtained from Jimma university medical center administration office. Those all study participants who were volunteered to be part of the study were given written consent/ascent forms for full permission to enroll them. The result was reported to their respective Physician for appropriate treatment and all information was kept in well manner to keep their confidentiality.

4.13. Dissemination plan

A copy of the result will be given to Jimma university school of medical laboratory science. Summary of these results also given to JUMC office and Jimma town health administration office to give them updated information about the bacteria that cause BSI and their drug resistance. The result will also be disseminated through scientific journals. Finally it will be disseminated to all available audience during presentation of this document.

5. Results

5.1. Background characteristics of patients with bloodstream infection

A total of 171 study participants suspected for bloodstream infection were enrolled in the study. Among these, 87 (50.9%) were females and 84 (49.1%) were males with the female to male ratio 1.04. The mean age of the study participants was 40.25 years. The majority 61 (35.7%) of the study participants were found to be in the 18-33 years age group while the minority 12 (7%) were older than 60 years. In our study, most of the study participants 91 (53.2%) were married. Sixty eight (39.77%) of study participants had Primary school while 40 (23.39%) were had 9^{th} and above grade. Nearly 58% of study participants were rural dwellers.

Majority of our respondent 76 (44.5%) were from medical ward while least 24 (14%) were from surgical ward. One hundred twenty three (71.9%) of respondents had not taken antibiotics while 37 (21.7%) of respondents had recently been treated with antibiotics before blood sample collection. The most common suspected focus of infection were gastrointestinal tract 41 (24%) followed by respiratory tract 35 (20.5%). Among a total of respondents 95 (55.6%) had known comorbidity (HIV/AIDS (18), Stroke (8), Diabetes Mellitus (16), Heart failure (14), chronic Renal diseases (11), Hematological problem (11) and others (7)) (table 1

Table 1: Sociodemographic characteristics of adult patients suspected of having blood stream infection at Jimma University Medical Center from March15, 2019- September 30, 2019.

		Frequency	Percent
Sex	Female	87	50.9
	Male	84	49.1
Age	18-33 years	61	35.7
	34-48 years	57	33.3
	49-60 years	41	24
	60 + years	12	7
Marital status	Married	91	53.2
	Single	45	26.3
	Divorced	21	12.3
	Widowed	14	8.2
Educational status	No formal education	63	36.84
	Primary school	68	39.77
	Secondary and above	40	23.39

Residence area	Rural	99	57.9
	Urban	72	42.1
Admission unit/ Dep't	Medical ward	76	44.5
	Emergency	39	22.8
	Intensive care unit	32	18.7
	Surgical ward	24	14
Antibiotics Use before blood sample collection	No	123	71.9
	Yes	37	21.7
	No information	11	6.4
Focus of infection	Gastro intestinal tract	41	24
	Respiratory tract	35	20.5
	Urinary tract	31	18.1
	Skin/elsewhere Wound	18	10.5
	Nervous system	12	7
	Cardiovascular system	9	5.2
	Unknown origin	25	14.6
Comorbidity	Yes	95	55.6
	No	76	44.4

5. 2. Culture result

Among blood culture of 171 patients suspected of having blood stream infection 30 (17.54%) were found to be culture positive for 9 different bacteria and 141 (82.46%) were culture negative.

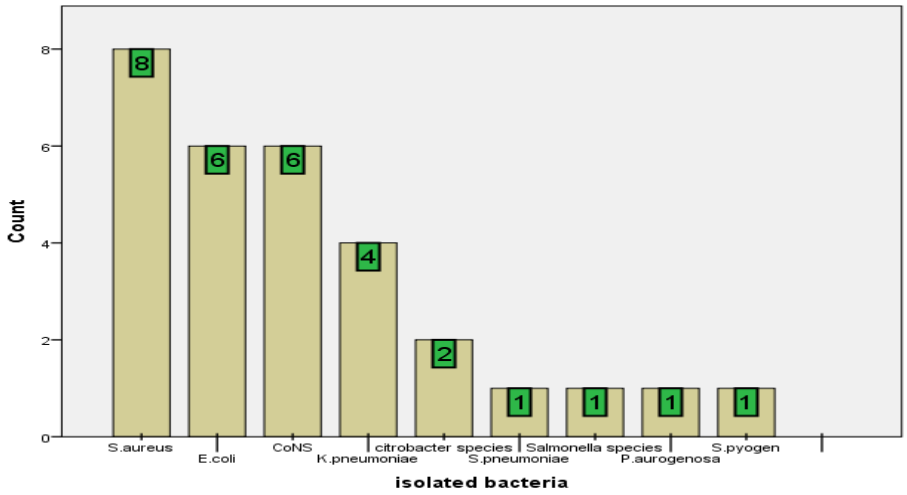


Figure 2: Types and frequency of bacterial isolates from blood culture of adult patients suspected of having bloodstream infection at Jimma University Medical Center from March15, 2019- September 30, 2019

Among the culture positive cases, 16 (53.33%) bacterial isolates were Gram positive and 14(46.67%) were gram negative bacteria. Among a total of 30 isolates of bacteria, *S.aureus* 8 (26.67%) were the predominant isolates followed by *CONS* 6 (20%) and *E. coli 6* (20%). *S.aureus and E. coli* were the predominant isolates of gram positive and gram negative bacteria respectively (figure 2)

5. 3. Types and frequency of bacterial isolate across patients admission unit/ department in hospital

Among 30 of blood culture isolates 22 (73.33%) of isolates from inpatients and the rest 8(26.67%) was from outpatient department. Out of the 22 of isolates from inpatients, most 11 (50%) of them were from medical ward and least 4 (18.2%) of them were from surgical ward (table 3).

Table 2: Frequency of bacterial isolate of adult patients suspected of blood stream infection across patient location in hospital at Jimma University Medical Center from March15, 2019-September 30, 2019

	Admission unit/ Department				
	Inpatient			Outpatient	
isolated bacteria	medical ward	surgical ward	intensive care unit	outpatient department	Total
S.aureus	4	0	2	2	8
CoNS	2	2	2	0	6
E. coli	1	2	1	2	6
K. pneumoniae	2	0	1	1	4
citrobacter spp.	1	0	0	1	2
S. pyogenes	0	0	0	1	1
S. pneumoniae	0	0	1	0	1
Salmonella spp.	0	0	0	1	1
P. auroginosa	1	0	0	0	1
Total	11	4	7	8	30

5. 4. Distribution of bacterial isolate in relation to taken antibiotics before taken blood sample

Our study found that 4 (13.33%) of the isolates were from patients who had recently been treated with antibiotics. The rest 23 (76.67%) and 3 (10%) were from patient who had not taken antibiotics and no information on taking antibiotics respectively. One *S.aureus, CoNS, K.pneumoniae* and *P.auroginosa* were isolated from patients who had antibiotics for at least three days.

5.5. Bivariate analysis for Background characteristics of respondents

Suspected focus of infection were not included in bivariate analysis because of low expected number in categories of cells. Among background characteristics of respondents: educational level (No formal education, p=0.007 and Primary school, p=0.188), age (49-60 age group, p= 0.245), having comorbidity, patients admission unit/ dep't in hospital and Antibiotics use before blood sample collection of the respondent have selected for multivariate analysis with blood culture positivity ($p < 0.25$) while Sex, marital Status and residence area of the respondent have not selected for multivariate analysis with blood culture positivity ($p > 0.25$). Among others clinical data variables: (table 4).

Table 3 Bivariate analysis of independent variables among adult patients suspected of bloodstream infection in relation to blood culture results at Jimma University Medical Center from March15, 2019-September 30, 2019

		Culture result			COR(95% CI)	P-value
		Positive	Negative	Total		
Sex	Male	13	71	84	R	
	Female	17	70	87	1.326 (0.600-2.934)	.486
Age	18-33 years	8	53	61	R	
	34-48 years	11	46	57	1.584 (0.587-4.275)	0.364
	49-60 years	9	32	41	1.863 (0.653-5.317)	**0.245***
	60 + years	2	10	12	1.325 (0.244-7.184)	0.744
Marital status	Married	19	72	91	R	
	Single	5	40	45	0.632 (0.168-2371)	0.666
	Divorced	3	18	21	1.033 (0.262-4.080)	0.496
	Widowed	3	11	14	0.582	0.962
Residence area	Rural	19	80	99	R	
	Urban	11	61	72	1.317 (0.584- 2.672)	0.507

Educational level	No formal Education	19	44	63	8.205 (1.794-37.524)	**0.007***
	Primary school	9	59	68	2.898 (0.594-14.149)	**0.188***
	Secondary & above	2	38	40	R	
Admission unit/ Dep't	Medical ward	11	65	76	R	
	Surgical ward	4	20	24	1.182 (0.339-4.122)	0.793
	Intensive care unit	7	25	32	2.127 (0.787-5.750)	**0.137***
	Outpatient department	8	31	39	1.144 (0.387-3.377)	0.808
Antibiotics use before blood sample collection	Yes	4	33	37	R	
	No	23	100	123	1.897 (0.612-5.888)	**0.238***
Comorbidity	NO	6	70	76	R	
	YES	24	71	95	3.944 (1.520-10.234)	**0.005***

Key: * = variables that are candidate for multivariable logistic regression

5. 6. Multivariate analysis for Background characteristics of respondents

To control simultaneously possible confounding effects of explanatory variables, the possible factors were further evaluated by multivariate binary logistic regression analysis with stepwise variable selection. All bivariate results that had p-value < 0.25 (age, patient admission unit/dep't in the hospital, educational status, used antibiotics before blood sample collection and having comorbid) were subjected to multivariate binary logistic regression model.

With multivariate logistic regression analysis: age, used antibiotics before blood sample collection and patient admission unit/dep't in hospital (p>0.05) was not significantly associated with blood culture positivity while educational level (no formal education) and having comorbid were independent associated factors for the occurrence of positive blood culture due to bacteria. Majority of isolated bacteria 11 (36.67%) were from 34-48 age group; however, there was no association between age of patient and having bloodstream infection (P = 0.492). Again, there was no association between patient admission unit/dep't and occurrence of bloodstream infection however most of isolated bacteria 11 (36.67%) were from medical ward.

After adjustment to multivariate logistic regression model, the odds of positive blood culture among respondent who had no formal education was 9.63 times more likely as compared to

those who had secondary school and above (AOR= 9.63, 95% CI = 2.046-45.32, P =0.04). Having comorbidity was 4.580 times more risk to develop blood stream infection than those who had no Comorbidity (AOR=4.580, 95% CI=1.701-12.332, P=0.003) (table 4).

Table 4: Multivariable analyses for socio demographic and others clinical variables among adult patients of suspected blood stream patients at Jimma University Medical Center from March15, 2019- September 30, 2019

		Culture result			AOR(95% CI)	P value
		Positive	Negative	Total		
Educational level	No formal education	19	44	63	9.630 (2.046-45.32)	**0.04****
	Primary school	9	59	68	3.055 (0.612-15.193)	0.172
	Secondary & above	2	38	40	R	
Age	18-33 years	8	53	61	R	
	34-48 years	11	46	57	1.504 (0.470-4.810)	0.492
	49-60 years	9	32	41	1.671 (0.501-5.597)	0.403
	60 + years	2	10	12	0.674 (0.093-4.904)	0.697
Used antimicrobial	Yes	4	33	37	R	
	No	23	100	123	3.133 (0.937-10.482)	0.064
Patient admission unit/dep't	Medical ward	11	65	76	R	
	Surgical ward	4	20	24	0. 939 (0.230-3.827)	0.930
	Intensive care unit	7	25	32	2.443 (0. 770-7.750)	0.129
	Outpatient department	8	31	39	1.083 (0.324-3.622)	0.897
Comorbidity	NO	6	70	76	R	
	YES	24	71	95	4.580 (1.701-12.332)	**0.003****

Key: **= variables that are statistically significant

5.7. Antibiotics resistance pattern of gram positive bacteria

Among the gram positive bacteria high resistance was observed to ampicillin 15 (94%) and penicillin G 13 (81%) and low resistance to clindamycin 5 (32%), chloramphenicol 3 (18.75%) and doxycycline 2 (13%). No gram positive isolates were resistance to ciprofloxacin. All isolated *S. aureus* were resistant against ampicillin and all isolated *CoNS* were resistant against both penicillin and ampicillin. Conversely, only 1(17%) of CoNS isolates were resistant against both chloramphenicol and doxycycline. Only *S. pneumoniae* was susceptible to all classes of used antibiotics (table 5)

Table 5: Antibiotics resistance pattern of gram positive bacteria among adult patients suspected of having blood stream infection at Jimma University Medical Center from March15, 2019- September 30, 2019.

Species of Bacteria	Number /Percent of strains resistance to:												
	P	AMP	AMC	CRO	CLN	CIP	CN	FOX	CAF	SXT	TE	E	DO
S.aureus n=8	7/88	8/100	6/75	3/38	2/25	0/0	1/13	2/25	2/25	6/75	5/63	3/38	1/13
CoNS n=6	6/100	6/100	4/67	3/50	2/33	0/0	0/0	4/67	1/17	2/33	3/50	4/67	1/17
S.pyogene n=1	0/0	1/100	0/0	0/0	1/0	0/0	0/0	0/0	0/0	1/100	0/0	1/0	0/0
S.pneumo-niae, n=1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Total=16	13/81	15/94	10/63	6/38	5/32	0/0	1/6	6/38	3/19	9/56	8/50	8/50	2/13

Key: CRO: Ceftriaxone, P: Penicillin G, AMP: Ampicillin, E: Erythromycin, SXT: Trimethoprim Sulphamethoxazole, CN: Gentamycin, CAF: Chloramphenicol, CIP: Ciprofloxacin, TE: Tetracycline, DO: Doxycycline, AMC: amoxillin Clavulanic acid, FOX: Cefoxitin, CLN; Clindamycin.

5.8. Antibiotic resistance pattern of gram negative bacteria

From isolated gram negative bacteria high resistance was seen to ampicillin (100%) followed by tetracycline and trimethoprim sulphamethoxazole which account 86% for both. Low resistance to ciprofloxacin 1 (7%) and gentamycin 1 (7%). No gram negative isolates were resistance to Meropenem. Overall the range of resistance for gram negative was from 0% to 100%. All isolated *E. coli* were resistant against ampicillin, ceftriaxone and erythromycin. No gentamycin and Meropenem resistant isolates of *E.coli* (table 6)

Table 6: Antibiotics resistance pattern of gram negative bacterial isolates of adult patients suspected of having blood stream infection at Jimma University Medical Center from March15, 2019-September 30, 2019.

Species of Bacteria	Number /Percent of strains resistance to												
	AMP	AMC	CRO	CAZ	CXT	CAF	CIP	E	CN	TE	CFP	M	SXT
E. coli. n=6	6/100	3/50	6/100	5/83	5/83	2/33	1/17	6/100	0/0	6/100	5/83	0/0	5/83
K. pneumoniae. n=4	4/100	1/25	3/75	3/75	3/75	2/50	0/0	4/100	1/25	3/75	2/50	0/0	4/100
P.aurogino sa. n=1	1/100	1/100	1/100	1/100	1/100	0/0	0/0	1/100	0/0	1/100	1/100	0/0	0/0
Citrobacter spp. n=2	2/100	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/50	0/0	0/0	2/100
Salmonella spp. n=1	1/100	1/100	1/100	1/100	1/100	0/0	0/0	1/100	0/0	1/100	0/0	0/0	0/0
Total. n=14	14/100	6/43	11/79	10/71	10/71	4/29	1/7	12/86	1/7	12/86	8/57	0/0	12/85

Key: CRO: Ceftriaxone, AMP: Ampicillin, E: Erythromycin, SXT: Trimethoprim sulphamethoxazole, CN: Gentamycin, CAF: Chloramphenicol, CIP: Ciprofloxacin, TE: Tetracycline, DO: Doxycycline, CTX: Cefotaxime, CFP: Cefepime, AMC: amoxillin Clavunate, CAZ: Ceftazidime, CXT Cefotaxime, M: Meropenem.

5.9. Multidrug resistance pattern of bacterial isolates

Multidrug resistant strains were common for both gram negative and gram-positive bacteria.. Out of 30 bacterial isolate 29 (96.67%) were resistant to at least one antibiotics used in the susceptibility tests. Twenty three (76.67%) isolates were resistant to at least one antibiotic from three different categories (MDR) with resistance pattern varied from 3-10 drugs. Hundred percent of *K. pneumoniae* and *E. coli* was multidrug resistant with resistance pattern varied from 4-9 drugs and 6-10 drugs respectively. About 63% of isolated *S. aureus* was multidrug resistant with resistance pattern varied from 5-9 drugs. All gram negative bacteria isolates (except single isolate of *Citrobacter Spp.*) were multidrug resistant bacteria. A single isolate of *E. coli, S.aureus* and *CoNS* were resistant to 8 drugs of different categories (table 7).

Table 7: Multidrug resistance pattern of bacterial isolated of adult patients of having suspected blood stream infection at Jimma University Medical Center from March15, 2019-September 30, 2019.

	Antibiogram	Frequency
S.aureus	AMP, SXT	1
	P, AMP, AMC	1
	P, AMP, SXT	1
	P, AMP, AMC, CLN, E	1
	P, AMP, AMC, CRO, SXT	1
	P, AMP, AMC, CLN, SXT, E	1
	P, AMP, AMC, CRO, FOX, CXT, C, E	1
	P, AMP, AMC, CRO, CN, FOX, C, SXT, DO	1
CoNS	P, AMP, AMC	1
	P, AMP, FOX	1
	P, AMP, AMC, CRO, FOX, E	1
	P, AMP, AMC, CRO, FOX, TE, E	1
	P, AMP, AMC, CLN, C, SXT, TE, E, DO	1
	P, AMP, CRO, CLN, FOX, SXT, TE, E, DO	1
S.pyogene	AMP, CLN, SXT, E	1
E. coli	AMP, CRO, CAZ, CXT, E, TE	1
	AMP, CRO, CAZ, CXT, E, TE, CFP, SXT	1
	AMP, AMC, CRO, CAZ, CXT, E, TE, CFP, SXT	1
	AMP, CRO, CXT, CIP, E, TE, CFP, SXT	1
	AMP, AMC, CRO, CAZ, CXT, CAF, E. TE, CFP, SXT	2
K.pneumoniae	AMP, E, CFP, SXT	1
	AMP, CRO, CAZ, CXT, CAF, E, TE, SXT	1
	AMP, AMC, CRO, CAZ, CXT, E, TE, CFP, SXT	1
	AMP, CRO, CAZ, CXT, C, E, CLN, TE, SXT	1
Citrobacter spp.	AMP, SXT	1
	AMP, SXT, TE	1
P.auroginosa	AMP, AMC, CRO, CAZ, CXT, E, TE, CFP	1
Salmonella spp.	AMP, AMC, CRO, CAZ, CXT, E, TE,	1

Key: CRO: Ceftriaxone, P: Penicillin G, AMP: Ampicillin, E: Erythromycin, SXT: Trimethoprim Sulphamethoxazole, CN: Gentamycin, CAF: Chloramphenicol, CIP: Ciprofloxacin, TE: Tetracycline, DO: Doxycycline, AMC: amoxillin Clavulanic acid, FOX: Cefoxitin, CLN; Clindamycin, CFP: Cefepime, CAZ: Ceftazidime, CXT: Cefotaxime.

5.10. Multidrug resistant bacteria isolated from inpatients verses outpatients

Out of 30 bacterial isolates 23 (76.67%) were multidrug resistant (MDR). Among isolates from inpatients 18/22 (81.81%) were MDR while isolates from respondent in outpatient department 5/8 (62.5%) were MDR (figure 3).

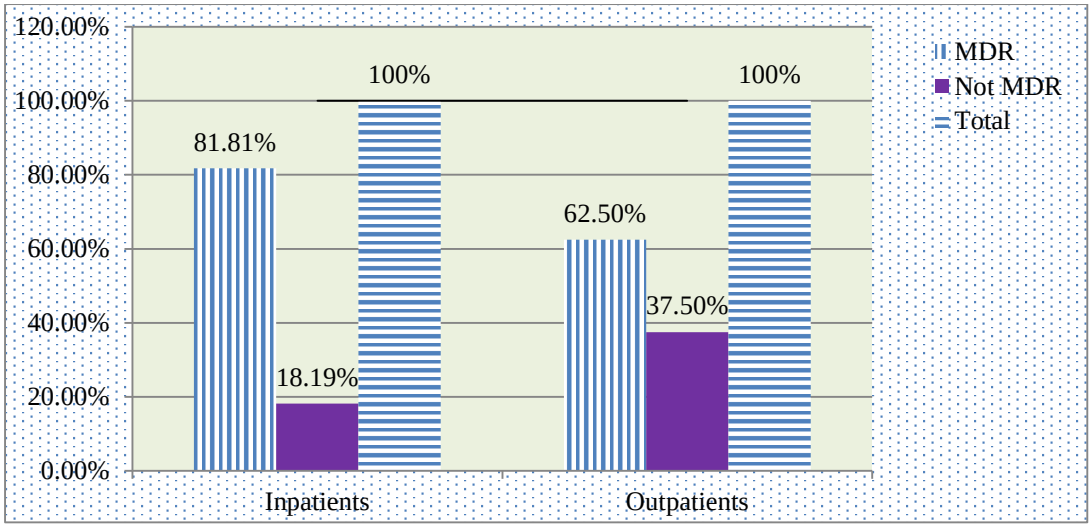


Figure 3: Multidrug resistant bacteria isolated from inpatients versus outpatients among adult patients of having suspected blood stream infection at Jimma University Medical Center from March15, 2019-September 30, 2019.

5.11. Prevalence of ESBL and/or AmpC beta-lactamase

Of the 14 isolated gram-negative rods prevalence of ESBL and/or AmpC beta-lactamase was 9 (64%). All are due to *E. coli* and *K. pneumoniae.* Among ESBL and/or AmpC producer: 4(44.4%) and 1(11.1%) were extended spectrum beta-lactamase (ESBL) and AmpC beta-lactamase positive respectively. The rest 4 (44.4%) were positive for both extended spectrum beta lactamase (ESBL) and AmpC beta-lactamase. All isolated *E. coli* 6 (100%) and 3 (75%) of isolated *K. pneumoniae* were ESBL and/ or AmpC (table 8).

Table 8: Table that show prevalence of ESBL and/or AmpC bacterial isolates of adult patients of suspected blood stream infection at Jimma University Medical Center from March15,2019-.September 30,2019.

Isolated bacteria	ESBL	AmpC	Both ESBL and AmpC	Total
E.coli (n=6)	3	1	2	6
K.pneumoniae (n=4)	1	0	2	3
Total	4	1	4	9

6. Discussion

Clinically, BSI is associated with high morbidity and mortality and considerably impacts on health care costs, especially when caused by antimicrobial-resistant bacteria. In our study the overall isolation rate of bacteria from blood culture of 171 adult patients was 30 (17.54%). This was relatively comparable with previous study conducted in Jimma 15.8% [32], Gondar 18.2% [38], two study in India 18.6% [43] and 18.62% [44]. It was higher than study reported from other study in Jimma 8.8% [40], Tanzania 13.4% [31], New York City (12.6%) [8] and Nepal 13.8% [45]. However, our finding is lower than finding of others research done in different part of Ethiopia like Addis Ababa 32.8% [22], Mekelle 28% [46] and Bahirdar 39.2% [20] and other country like Egypt 36.86% [47], two study in India 26 [48] and 31.2% [35]. The most possible explanation for variation in BSI rates among these studies could be due to the difference in study population, blood culture system, volume/number of blood culture, Content of used media [49], geographical location, the study design, and application of infection control policies within/between countries .

The result of this study showed that Gram-positive bacteria 16 (53.33%) were more frequently isolated from blood than Gram-negative bacteria 14 **(**46.67%). Although there was difference in terms of prevalence others study also report predominance of gram positive bacteria. Two studies in Jimma 60.9% and 39.1% [40], 53.3% and 46.7% [32], Gondar 64.34% and 35.66% [39] and 69% and 31% [38], Addis Ababa 74.2 % and 23.8% [22] and other countries like Tanzania 82.1% and 17.9 [31], India 57.8% and 42.2% [37] which represent gram positive bacteria and gram negative bacteria respectively . However, our finding was in contrast to other studies reported where gram negative bacteria were more frequently isolated than gram positive bacteria such as in Bahirdar (52.3% and 47.7%) [20], two studies in India (58.3% and 41.9%) [43] and (69.2% and 30.8%) [50]. This dissimilarity might be due to epidemiological variation of the bacteria responsible for bloodstream infection and the incidence and etiology of BSI have continuously changed over the period of time [51].

Among blood isolates of the present study *S. aureus* was the predominant isolate which accounts 8 (22.72%). Even though there was prevalence difference, others study also show *S. aureus* as a predominant blood isolates: like in, Jimma 40% [32], Addis Ababa 50% [22], Bahirdar 22.7% [20], Mekelle 37.5% [46]. But this was different from other studies in which *CoNS* was their predominant blood isolates such as in Jimma 26.1% [40], in Gondar 31.6%

[39] and 42.3% [38], in Brazil 40.7% [52] and in Tanzania 67.4% [31]. *CoNS* were second most common gram positive isolates in our finding. It has long been considered as blood contaminant however currently it become an important pathogen in hospital acquired bloodstream infections as results of the expanding use of invasive medical devices. The alternative reason for highest prevalence of these two bacteria could be: they commonly found in the hospital environment which might be contaminate among admitted patients and also found as the most common skin commensal that may get assess to blood during medical procedure and increase the infection rate since most of our respondents were from admitted patients.

E. coli is the most common blood isolate of gram negative bacteria in our study. Similar study in Tanzania [31] and India [50] [37, 53] showed that *E. coli* as the most common blood isolate of gram negative bacteria. In contrast to our finding other study in Australia [54] and Nepal [51] reported that *Salmonella Spp.* as the most common blood isolate of gram negative bacteria, which is the least prevalent in our study. Other study in India report *P.aeruginosa* as predominant blood isolate gram negative bacteria [55]. Reason for predominance of *E. coli* in our study may be due to most common isolate of hospital acquired infection in study area [34], and its relation with high-risk of surgical procedures, especially in the digestive or urinary tract that releases bacteria into the blood. Disparity in prevalence of these etiologic agents of bloodstream infection in different studies also might be due to epidemiological variation / difference in etiologic agents and seasonal variation.

Most of our blood isolates (80%) were from patient who had chronic illness. According to our study, blood of patients who had underlined comorbidity were 4.580 times more likely to be positive when compared with those who had no underlined Comorbid (AOR=4.580, 95% CI=1.701-12.332, P=0.003). In consistence to our study others studies also agree that patients who had underlined chronic illness were more risk to develop BSI [56, 57]. This may be related with immune compromised status of such patients, in frequently use of invasive procedure, frequently hospitalization status for management of their chronic illness [58]. The odds of being blood culture positive among respondent who had no formal education was 9.630 times more likely as compared to those who had secondary school and above (AOR= 9.630, 95% CI = 2.046-45.32, P =0.04). This may be due to gap of knowledge about prevention of infection.

In our study another important point was high antibiotics resistance rate that may be causes a serious therapeutic challenges to the management of bloodstream infections. Antibiotic susceptibility pattern of gram positive bacteria shows that they have a high level of resistance against Ampicillin 15 (94%) and Penicillin G 11 (81%), intermediate level of resistance to amoxicillin clavulanic acid 10 (63%) and, low level of resistance to gentamycin 1 (6%), doxycycline 2 (13%), chloramphenicol 3 (19%), clindamycin 4(25%). However, no resistant was observed to Ciprofloxacin which is in line with the previous studies conducted in Jimma [40]. Different study also report similar finding that low level of resistance to gentamycin (28.6%) [40], clindamycin (3.4%) [22] and High level of resistance to penicillin (83.5%) [22], (85.7%) [40] and ampicillin (90%) [39]. In contrast to our result others studies report low level of resistance to ampicillin (40.8%) and penicillin (51%) [39]. This variability may be related to frequency of use of these drugs, its cost and practice of self-medication and implementation of policies regarding to controlling emergency of drug resistant bacteria vary greatly across a country [59] .

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a worldwide issue associated with significant morbidity and mortality [60]. There were 8 and 6 isolate of *S. aureus* and *CoNS* in our study of which 2 (25%) and 4 (66.67%) were Methicillin resistant respectively (Cefoxitin disc was used). This is similar with study in Tanzania (23.3%) MRSA [31]. Lower when compared to other previous study in same area that reveal 100% and 33.33% isolate were MRSA and MRCoNS respectively [40]. Other study like study in New York City 6 (40%) and 21 (75%) [8] and Brazilian 38.5% and 100% [58] MRSA and MRCoNS respectively also report higher finding. But lower finding was reported from Eastern Nepal (40%) MRCoNS [45], 50.8% of Brazilian. This may be due to incidence of MRSA bloodstream infection (BSI) shows high geographical variability as well as temporal variation [61].

According to our study most of the GNB show a high resistance to ampicillin (100%), erythromycin (85.71%), tetracycline (85.71%) and trimethoprim sulphamethoxazole (85.71%). Similar result from other study that high resistance to ampicillin (100%), tetracycline (88.9%) and trimethoprim sulphamethoxazole (88.9%) [40], ampicillin (88.5%) and trimethoprim sulphamethoxazole (80%) [22]. However other study reveal that low level of resistance to erythromycin (35.0%) and trimethoprim sulphamethoxazole (25.0%) [31]. In our study low level of resistance rate to ciprofloxacin 1 (7.14%) and gentamycin 1 (7.14%) and no resistant gram negative bacteria to meropenem. Greater than two third of gram negative bacteria in our study were resistant to used cephalosporin drugs. It is obvious that

cephalosporin drugs are one of the most frequently used antibiotics for both inpatients as well as outpatients. This could be the reason for high level of resistance since positive linear relationship between frequency of antibiotic use and antibiotic resistance [62].

The overall multidrug resistance in present study was 23 (76.67%). It was consistent with the previous studies conducted in the same area (80%) [32]. This was higher when compared to finding of other study like study in Mekelle (59%) [46] but lower than finding of study in Jimma (86.96%) [40]. Among 23 (76.67%) of multidrug resistant bacteria 13 (56.5%) were due to gram negative bacteria and the rest 10 (43.5%) were by gram positive bacteria. This indicate rapid emerging of multidrug resistant gram negative bacteria than gram positive bacteria which was in agreement with similar study in the area [40].

In our finding all *K. pneumonia*, all *E. coli* and 62.5% of isolated *S. aureus* were multidrug resistant. This might be due to hospital environment favors the circulation of drug resistant bacteria since most of our isolates were from inpatients and most common cause of health care associated infection in study area were by this three bacteria [34]. The other possible factors that may determine high prevalence of multidrug resistance of gram negative bacteria in our study were: high prevalence of ESBL producer *E. coli* and *K. pneumoniae*. Plasmid coding for ESBL enzyme may also harbors additional beta lactamase and furthermore gene conferring resistance to other antimicrobial classes results limit response of bacteria to different antibiotics [63].

BSIs caused by ESBL-producing *E. coli* and ESBL producing *K. pneumoniae* are usually severe and have been associated with increased rates of treatment failure, high mortality and high hospitalization costs [64]. Five (83.3%) and 3 (75%) of our isolated *E. coli* and *K. pneumoniae* were ESBL producer. Higher when compared with study in China that 355 (55.5%) and 46 (16.5%) of isolated *E. coli* and *K. pneumoniae* were ESBL producers respectively [65] and Mexico City 22 (39.3%) and 3 (23.1%) of isolated *E. coli* and *K. pneumoniae* were ESBL producers respectively. According to our study all ESBL producer among gram negative were *E. coli* and *K. pneumoniae* which is similar with study in India [44]

7. Strength and Limitation of the study

7.1. Strength

- ✓ Aseptic technique in every procedure was performed
- ✓ Two blood sample for every respondents were taken

7.2. Limitation of the study

- ✓ Unable to isolate anaerobic bacteria pathogens because of lack of facilities needed.
- ✓ Lack of MIC for Methicillin
- ✓ Minimum requirement of blood volume was used.
- ✓ Since the study was cross sectional study, study population was not systematically selected and relatively low number of blood cultures the result may not be truly representative.
- ✓ Use manual blood culture system

8. Conclusion and Recommendation

8.1. Conclusion: Based on our result the following conclusion have been drawn

The overall culture confirmed prevalence of blood isolates in adult patients suspected of having bloodstream infection was high. *S. aureus* and *E. coli* were the most common Gram-positive and Gram-negative bacteria causing adult bloodstream infection, respectively. Ciprofloxacin, Gentamycin and Meropenem were the most effective drugs for the treatment of bacterial bloodstream infection of adult patients. Having comorbidity and educational level of respondents were independent associated factors for blood culture positivity. More than $3/4^{th}$ of the blood isolated bacteria among adult patients of bloodstream infection were multidrug resistant.

8.2. Recommendation

Most of blood isolated bacteria show resistance to the commonly used drugs so that hospitals and treating physicians should have based on result of blood culture and sensitivity to treat patient with bloodstream infection. Our results suggest to hospital, antimicrobial stewardship, antimicrobial surveillance, control of antibiotic use and antibiotic quality control to intervene local management of blood stream infection especially a frequent empirical use of 3^{rd} generation cephalosporin. Findings of our present study may not represent profile and antimicrobial resistance pattern of bacteria causing bloodstream infection in the future due to

its dynamic characteristics. Therefore further/routine study with large sample size should be essential to come up with real updated information about profile along with their antimicrobial resistance patterns of bacteria causing bloodstream infection. Finally high multidrug resistant bacteria in our result that may further limit therapeutic option call all others stakeholder to take a role in antimicrobial resistance stewardship to control the spread of such drug resistant strains of bacteria.

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ANNEX

JIMMA UNIVERSITY

INISTITUTE OF HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY

Annex I- Information sheet

1. Information sheet in English version

Title: bacterial profile and antimicrobial resistance pattern among adult patients of suspected blood stream infection at JUMC, south Ethiopia.

Principal investigator:-Dassalegn Muleta

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Introduction

The main aim of this research is to assess the bacterial profile and antimicrobial resistance pattern among adult patients of suspected blood stream infection at JUMC. The study will be undertaken by Dassalegn Muleta candidate master of medical microbiology school of medical laboratory institute of health science at Jimma University. This consent form contains all the information you will need to know about the study to be undertaken before you decide to consent to take part in the above-mentioned study.

Participation

We are asking you and others to voluntarily participate in this study. What is expected from you is to respond some question which take about five minutes and give 10 ml of venous blood. The blood samples are collected using sterile and disposable equipment.

Risks

While you are participating, you are likely to have some risks. The risks associated with this study could be some discomforts and in a rare occasion a hematoma may be developed when we collect 10ml of venous blood from you. However, these things are not produce serious pain and if in case any problems arises during and following sample collection, we shall offer you necessary medical interventions until you fully recover.

Benefits

If you are positive for bacterial infection during investigation, opportunities for treatment will be arranged and you will be lucky.

Confidentiality

Any information that we will collect about you during this study will be kept confidential information about your identity will be put away after re-coding your file and kept in a secured place. Only the principal investigators will be able to link your identity with the code number, if this becomes necessary to assist you in any way.

Right to refuse

Since participation in this study is entirely voluntarily, you can refuse to participate in this study at any time. Your refusal will not affect your taking health care.

2. Information sheet in afaan oromoo version

Mata duree: profayila fi damdamachu qorichaa bakteeriya kan ga'eyyii hospitaala Unibarsiiti Jimmaatti bakteriyaan dhiiga isaani keesssa seenun dhukuba(blood stream infection) jedhamu fiduu, Kibba Itoophiyaa

Qorannoo kan gaggeessu: Dassalaanyi Mul'ata

Tessoo: Jimma Unibarsittiiti inistitiwuti fayyaa muummee medical laboratorii

Lakk. Bilbilaa;-+251921423109

E-mail: -dmuleta4all@gmail.com

Seensa: Kayyoo qorannoo kana profaayila bakteriyaati fi damdamachu qorichaa inni dhukkubsatoota ga'eyyi hospitaala Jimma unibarsititti godhu ibsuuf. Qorannichi kan gageeffamu barata Dassalanyi mul'ata kan jedhamun Jimma unibarsitiitti inistitiwuti fayyaatti muummee medical laboratory keessaa digrii lammaffaa medikal mayikrobiyologidhan kan ta'eni. Guchni waligaltee kun oddeeffannoo wa'ee qorannichaaf si barbachisuu hundaa waan qabuuf osoo waligaltee kana hin xumurin

Hirmaannaa: Isinis warren kan biras qoraannoo kanarraati hirmaachun feedhi keessaan ta'uu kabajan isin gafanna.Isin irraaa kan eegamu daqiiqa 5nif gaafilee gafatamtanif deebi lachuu fi dhiiga 10ml ta'e qofa. Dhiigichi kan warabamu haala qulqullina qabun fi meesha al fayyaadamun gatamuun waan ta'ef balaa tokkoo illee isin irra hin qaqabsiisu.

Miidhaa Yeroo qorannoo kanarratti hirmaatu midhaa muraasa wayi sirraa ga'u danda'a. miidhan kanan wal qabatus yeroof sitti tolu dhiisu fi iddoon dhiigni irraaa fudhame tiqqoo eshee si dhitahu. Hha ta'u malee wantootni kun kan baay'ee sin miine fi yaalin barbachisuu waan siif latamuf balaa cimaarra siin qaqabsiisu.

dura sirriitti dubbisi.

Fayiidaa: Yoo bakteriyaan dhiiga kee keessatti argame qorichi barbachiisu siif ni laatama.

Iccitii : Odeeffannoon wa'ee kee walitti qabame hundi bifa dhokatan koodin ittii waan gadhamuf kan qorannicha gaggeessuu qofatu wa'ee odeeffannoo sirra gurame beeka malee namni bira tokko iyyuu hin beeku. Firiin dhiigaa keerraa argamee doktoraa kan kee qofatti kan kennamudha.

Mirga diduu: Hirmannan kee guutuma guututti fedhiidhan waan ta'ef yeroo barbadetti dhiisu ni dandeessa. Qorannicha adda kuutun kee yaali argachu keerratti dhiibba tokkoolee hin qabu.

3. Information sheet in Amharic version

ርዕስ: - በደቡብ ምዕራብ ኢትዮጵያ በሚገኘው JUMC ብለድስትሪም ኢንፌክሽን በተጠረጠሩ የአዋቂ ህመምተኞች መካከል የባክቴሪያ መገለጫ እና የፀረ-ተሕዋሳት መቋቋም ሂደት።

ዋና መርማሪ -Dassalegn Muleta

አድራሻ-የጃማ ዩኒቨርሲቲ የጤና ሳይንስ ተቋም የሕክምና ላብራቶሪ ክፍል

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ኢ-ሜል: -dmuleta4all@gmail.com

መግቢያ

የዚህ ምርምር ዋና ዓላማ በ JUMC ብለድስትሪም ኢንፌክሽን በተጠረጠሩ የአዋቂ ህመምተኞች መካከል የባክቴሪያ መገለጫ እና የፀረ-ተህዋሲያን የመቋቋም መገምገም ነው ። ጥናቱ የሚካሄደው በጅማ ዩኒቨርስቲ የጤና ሳይንስ ተቋም የህክምና ላብራቶሪ ኢንስቲትዩት ሜዲካል ማይክሮባዮሎጂ እጩ የሆነ ዳሰለኝ ሙለታ ነው ። ይህ የስምምነት ቅጽ ከዚህ በላይ በተጠቀሰው ጥናት ለመሳተፍ ለመስማማት ከመወሰንዎ በፊት ስለሚደረገው ጥናት ማወቅ ያለብዎትን ሁሉንም መረጃዎች ይይዛል ።

ተሳትፎ

እርስዎ እና ሌሎች በፈቃደኝነት በዚህ ጥናት ውስጥ እንዲሳተፉ እንጠይቃለን። ከአንተ የሚጠበቀው ነገር ለአምስት ደቂቃ ያህል የሚወስደውን ጥያቄ መመለስ እና 10ml ደም ነው ። የደም ናሙናውዎች በቀላሉ የማይበከሉ እና ሊጣሉ የሚችሉ መሳሪያዎችን በመጠቀም ይሰበሰባሉ ።

ጉዳት

እርስዎ በሚሳተፉበት ጊዜ ምናልባት አንዳንድ ጉዳት ሊኖሩ ይችላሉ ። ከዚህ ጥናት ጋር ተያይዘው የሚመጡት ጉዳት አንዳንድ ችግሮች ሊሆኑ ይችላሉ እና አልፎ አልፎ ደግሞ 10ml በምንሰበስብበት ጊዜ ሄማቶማ ሊፈጠር ይችላል ። ሆኖም እነዚህ ነገሮች ከባድ ህመም አይደሉም ፣ እናም የናሙና ክምችት በሚሰበሰብበት እና በሚከተለው ጊዜ ውስጥ ቢነሱ ቢከሰቱ ሙሉ በሙሉ እስኪያገግሙ ድረስ አስፈላጊ የሕክምና ዕርዳታዎችን እንሰጥዎታለን ።

ጥቅሞች

በምርመራው ጊዜ በባክቴሪያ ኢንፌክሽኑ አዎንታዊ ከሆኑ ለህክምና እድሎች ይዘጋጃሉ እና ዕድለኛም ይሆናሉ ።

ምስጢራዊነት

በዚህ ጥናት ወቅት ስለ እርስዎ የምንሰበስበው ማንኛውም መረጃ ስለ ማንነትዎ ሚስጥራዊ መረጃ የሚቀመጥ ሲሆን ፋይልዎን በተጠበቀ ቦታ ይቀመጣል ። በማንኛውም ሁኔታ እርስዎን ለማገዝ አስፈላጊ ከሆነ ዋና ማንነትዎን ከኮዱ ቁጥሩ ጋር ማገናኘት የሚችሉት ዋና መርማሪዎቹ ብቻ ናቸው ።

የለማሰታፍ መብት

በዚህ ጥናት ውስጥ ተሳትፎ ሙሉ በሙሉ በፍላጎት ስለሆነ በዚህ ጥናት ውስጥ በማንኛውም ጊዜ የለማሰታፍ ሙሉ መብት አሉ ። እምቢታዎ የጤና እንክብካቤዎን አይጎዳውም ።

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Annex II. Consent/Assent Form

1. Consent/Assent Form in English version

Participant consent

I have read/hear the forgoing information and the purpose of the study explained to me. I had the chance to ask questions about the study and all questions have been answered to my understanding. I have been informed and have understood that participation is entirely voluntary and withdrawing this consent at any time is interest based. I consent so that voluntarily to participate in this study as a respondent.

Participant code: _____ Participant signature----------

Date: _____ Care giver signature----------

2. Consent/Assent Form in afaan oromoo version

Kayyoo qorannoon kun gaggeefamuf fi odeeffannoo barbaachisan dubbiseen jira. Carraa gaffii wa'ee qorannichaa gaafatan gafatamun hanga hubannoo kooti deebi debiseen jira. Odeffannoo fi hubannaa wa'ee qoranichan walqabatan feedhi koo guutudhaan yeroon barbadu hirmachudhan yeroon hin barbanne dhiisudhan fedhi horadhen jira. Kanaaf waligaltee kana fedhi koo gutuudhan akka hirmata qarannichati waligalerra.

Lakk. Dhokataa kan hirmaata---------- Mallattoo hirmaata----------

Guyyaa---------- Mallattoo dhukubsachiisa----------

3. Consent/Assent Form in Amharic version

የተላለፈውን መረጃ አንብቤያለሁ / ሰማሁ እንዲሁም የጥናቱ ዓላማ አብራራልቶኛል ። ስለ ጥናቱ ጥያቄዎች የመጠየቅ እድል ነበረኝ እናም ሁሉም ጥያቄዎች ለገባኝ መልስ ተሰጥተዋል። መረጃው ሙሉ በሙሉ በፍቃደኝነት ላይ የተመሠረተ መሆኑንና ይህን ከፈለግኩ በማንኛውም ጊዜ ፈቃዴን መሰረዝ እንደምችል ተረድቻለሁ ። እንደ አመላካች በዚህ በፈቃደኝነት ለመሳተፍ እስማማለሁ።

ቀን: - _____ የተሳታፊ ፊርማ ----------

የተሳታፊ ኮድ: - _____ የእንክብካቤ ሰጪው ፊርማ ----------

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Annex III-Questionnaire

1. Questionnaire in English version

Thesis title: bacterial profile and antimicrobial resistance pattern among adult patients of suspected blood stream infection at JUMC, South west Ethiopia. Please give your responses by accordingly. Your concise and clear responses would facilitate smooth data analysis. All information provided will be treated as confidential.

Date ----------unique ID number----------patients location----------		
Socio demographic information	**Sex**	1. Male 2. Female
	Age	---------- in years
	Marital status	1. Married 2. Single 3. Divorced 4. Widowed
	Educational status	1. No formal education 2. 1-4 grade 3. 5-8 grade 4. 9-12 grade 5. 12+
	Residence area	1. Rural 2. Urban
	Patients location	1. Medical ward 2. Surgical ward 3. Outpatient department 4. Intensive care unit
	Had you start antibiotics?	1. Yes (for how long----------) 2. No

Clinical data	Suspected origin of infection	1. Gastro intestinal tract 2. Urinary tract 3. Respiratory tract 4. Skin/elsewhere Wound 5. Cardiovascular system 6. Nervous system 7. Unknown origin
	Comorbidity	1. NO 2. Yes 3. if yes identify----------

YUNIBARSIITI JIMMA

DHAABBATA SAAYINSI FAYYAA

KUTAA BARUMSA MEDICAL LABORATORII

2. Questionnaire in afaan Oromoo version

Mata duree: profayila fi damdamachu qorichaa bakteeriya kan ga'eyyii hospitaala Unibarsiiti Jimmaatti bakteriyaan dhiiga isaani keesssa seenun dhukuba(blood stream infection) jedhamu, Kibba Itoophiyaa. Deebin isiin nuuf kennatun gaaffii gaafatamtanif gabaaba fi ifa yoo ta'e qorranno keenyaa dhugaan bu'ureessa waan ta'ef maloo hangaa dandeessanitti akka nuu gargartan irraa debinee isiin yaadachifna.

Guyyaa----------ID----------hospitaala keessaa iddoo itti argamu----------		
Odeeffannoo waa'ee Sociodemographic	**Saala**	1. Dhiira 2. Dhalaa
	Umrii	---------- Waggaadhan
	waa'ee ga'ilaan wal qabate:	1. hin fuunne/herumne 2. fuudhe/herumte 3. wal hikaan 4. abban manaa/haati manaa kan irraa du'e /duute
	Sadarkaa barnoota	1. hin barannee 2. Kutaa 1-4 3. Kutaa 5-8 4. Kutaa 9-12 5. Kutaa 12 ol
	Eessaa dhuftee	1. Baaddiyyaa 2. Magaala
	Hospitaala keessatti eessati argamaa?	1. Kutaa wal'ansa waliigalaa 2. Kutaa baqaqsanii hodhuu 3. Kutaa deddebii 4. kutaa yaalin addaa kennamuf
	Qoricha(antibiotics) eegaltee?	1. Eeyyee. Hagamif addaa baasi---------- 2. Lakki

Daata biraa	Madda dhukkubichaaf shakkame.	1. Mar,imaan garaa 2. Ujummoo fincaani 3. Ujummoo hargansuu 4. Gogaa/ madaa iddoo biraa jiru 5. Sirna dhiiga 6. Sirna Narvii 7. Maddii isaa hin beekamu
	Dhukkuba dabalataa qabaa/qabdii?	1. Lakkii 2. Eeyyee 3. Yoo eeyyee ta’e adda baasi----------

ጂማ ዩኒቨርስቲ

የጤና ሳይንስ ተቋም

ሜዲካል ላብራቶሪ ትምህርት ክፍል

3. Questionnaire in Amharic Version

ርዕስ: - በደቡብ ምዕራብ ኢትዮጵያ በሚገኘው JUMC ብለድስትሪም ኢንፌክሽን በተጠረጠሩ የአዋቂ ህመምተኞች መካከል የባክቴሪያ መገለጫ እና የፀረ-ተሕዋሳት መቋቋም ሂደት። እባክዎ ምላሾችዎን በዚሁ መሠረት ይስ ጡት። የእርስዎ አጭር እና ግልጽ ምላሾች ጠቀሚ የመረጃ ትንተና ያመቻቻል። የተሰጠው መረጃ ሁሉ በሚስጥር ይያዛል ።

ቀን ---------- ልዩ መታወቂያ ቁጥር ---------- ታማሚ የሚገኝ ቦታ ----------		
የሶሺዮድሞግራፊክ መረጃ	ፆተ	1.ወንድ 2. ሴት
	ዕድሜ	----------
	የጋብቻ ሁኔታ	1. ያገባ 2. ያላገባ 3. ያፍታ/ች 4. ባሏ የሞተ/ሚስት ያሞተች
	የትምህርት ደረጃ	1. መደበኛ ትምህርት የለም 2. 1-4 ደረጃ 3. 5-8ክፍል 4. 9-12 ክፍል 5. 12+
	የመኖሪያ ቦታ	1. ገጠር 2. ከተማ
ክሊኒካል መረጃ	የታካሚዎች ቦታ	1. የሕክምና ክፍል 2. የቀዶ ጥገና ክፍል 3. የተመላላሽ መምሪያ 4. የከባድ እንክብካቤ ክፍል
	ፀረ ተሕዋስያንን ወስደዋል?	1. አዎ, ከሆነ ግላፅ ---------- 2. የለም

	የተጠረጠረ የኢንፌክሽን ምንጭ	1. የጨጓራ አንጀት 2. የሽንት ቧንቧ 3. የመተንፈሻ አካላት 4. ቆዳ / ሌላ ቦታ ቁስለት 5. የልብና የደም ቧንቧ 6. የነርቭ ስርዓት 7. ያልታወቀ መነሻ
	ሌለ በሽታ	1. አይ 2. አዎ 3. አዎ ከሆነ ግላፅ ----------

Annex IV: Laboratory Procedures

General principle of blood culture and susceptibility test:

Bacteria enter the blood from extra-vascular sites via different mechanism, when the bacteria multiply at a rate exceeding the capacity of the immune system to remove them they cause bloodstream infection. The purpose of a blood culture is isolation and identification of bacteria circulating in the vascular system. Once bacterial pathogen isolated from blood disk diffusion method on Muller Hinton Agar without/with blood is used to perform antimicrobial sensitivity test. Antibiotic-impregnated disk, placed on agar previously inoculated with the test bacterium, pick-up moisture and the antibiotic diffuse radially outward through the agar medium producing an antibiotic concentration gradient. The concentration of the antibiotic at the edge of the disk is high and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory for the organism, which then grows freely. A clear zone or ring is formed around an antibiotic disk after incubation if the agent inhibits bacterial growth and that clear zone is measured by dial caliper/rural and interpreted according to predetermined standard.

1. General protocol of culture media Preparation

1. Weighing and dissolving of culture media
2. Sterilization and sterility testing
3. Addition of heat sensitive ingredients
4. pH testing of culture media
5. Dispensing of the culture media
6. Quality assurance of culture media
7. Storage of culture media

2. Blood collection, transportation and culturing

1. Identify the subject and prepare
2. Tie tourniquet. Select vein puncture site, then release tourniquet.
3. Cleanse the selected venipuncture site with alcohol and 2% of tincture of Iodine
4. Clean rubber caps of blood culture containers with alcohol. Let caps dry.
5. Retie tourniquet without touching the prepped area and withdraw blood.
6. After bleeding stops, if any iodine remains on the skin, clean venipuncture site with alcohol to remove it.

7. Repeat the procedure for each blood culture set ordered, selecting a different site for each venipuncture, if possible
8. Inoculate blood into desired broth
9. All blood culture bottles must be labeled in front of the patient.
10. Well labeled Blood cultures are transported at room temperature.
11. Incubate at 37^0c for 7 days

3. Gram staining principle and procedure

Principle: Following staining with a crystal violet and treatment with iodine, the crystal violet-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 by acetone alcohol. So that gram positive bacteria retain color of crystal violet while gram negative bacteria become stained with counter stain (safranin dye).

Procedure

1. Make smear on dry clean slide.
2. Wait till air dry
3. Fix with flaming
4. Flood slide with crystal violet and then wait for a minute
5. Rinse slide by clean tap water
6. Flood slide with Gram's iodine and then wait for a minute
7. Rinse slide by clean tap water
8. Apply acetone alcohol and then wait for 30 second
9. Rinse slide by clean tap water
10. Flood slide with safranin and then wait for a minute
11. Rinse slide by clean tap water
12. Allow air dry
13. Place a drop of immersion oil on the slide and view with 100x oil-immersion objective.

4. Biochemical tests

4.1. Catalase test

Principle: Staphylococci bacteria produce enzyme called catalase while comman pathogen streptococci do not. This enzyme used as a catalyst in the breakdown of hydrogen peroxide to water and Oxygen. Produced Oxygen form bubble the mixture. So that it differentiate those

bacteria that produce the catalase staphylococcus from non-catalase producing bacteria such as streptococci.

Procedure:

1. Add 2ml of 3% hydrogen peroxide solution into sterile test tube
2. Using a sterile wooden stick pick colonies of the test organism
3. Mix with hydrogen peroxide solution then look for bubbling.
4. Bubble indicate positive test

4.2. Coagulase tests

The coagulase test differentiates Staphylococcus aureus which produces the enzyme coagulase (convert fibrinogen to fibrin), from other strains of Staphylococcus which do not produce coagulase. S.aureus strains are capable of coagulating plasma while other not.

Procedure

Slide test method

1. Place a drop of distilled water on slide
2. Emulsify a colony of the test organism to make suspension.
3. Add a drop of EDTA ant coagulated plasma to the suspensions and mix gently.
4. Look for clumping of the organisms within 10 seconds.

Test tube method: performed for those slides test negative.

1. Take sterile test tubes and add undiluted plasma into tube.
2. Add the test broth culture to tube.
3. Mix gently and incubate the tubes at 37^{o}C.
4. Examine for clotting after 24 hour.

Results

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

No clotting …… . . . . . Negative

4.3. Optochin Test

For identification of alpha-hemolytic streptococci as S. pneumoniae

Procedure:

1. Using an inoculating loop, streak two or three pure colonies on 5% sheep blood agar plate.
2. Place a P disk (5 μg) and Incubate at 37°C with 5% - 10% CO_2 for 18 to 24 hours.
3. Measure zone of inhibition

Result: sensitivity indicates S. pneumoniae

4.4. Indole test

The test organism is cultured in a medium which contains tryptophan. Bacteria that produce an enzyme called tryptophanase degrade the amino acid tryptophan and produce Indole. Added Kovac's reacts with the Indole produce a red colored compound.

Procedure:

1. Inoculate few colonies of the culture into peptone water.
2. Incubate at 37^0c for 24 hours.
3. Add a few drops of Kovac's reagent and then look a color change.

Result: If the layer of indicator reagent turns to red within 1 minute, it is Indole positive and if it remains yellow it is Indole negative

4.5. Urease test

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease-producing, the enzyme will break down the urea to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline a change of indicator into pink-red was produced.

Procedure

1. Make suspension with saline
2. Inoculate heavily over the entire surfaces of urea agar in test tube
3. Incubate at 37^0c for 12-16 hours.
4. Observe for color change

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

4.6. Kligler Iron Agar

Procedure:

1. Make a saline suspension from pure colonies
2. Using a sterile inoculating wire, stab the butt of the KIA and then slant surface of the agar with the organism.
3. Incubate at 37^0c for 18 to 24 h.
4. Look for the color change

Result:

- ✓ If acid slant–acid butt (yellow–yellow): glucose and lactose fermented.
- ✓ Alkaline slant–acid butt (red–yellow): glucose fermented only.
- ✓ If alkaline slant–alkaline butt (red–red): glucose not fermented.
- ✓ The presence of black precipitate (butt) indicates hydrogen sulfide production, and presence of splits or cracks with air bubbles indicates gas production.

4.7. Citrate utilization test

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator.

Procedure

1. Make saline suspension of pure colonies
2. Streak the surface of the slant
3. Incubate at 37oc aerobically for 18 to 48 hours.

Result: Blue color indicates a positive reaction and green color indicate negative reaction.

4.8. Motility Test

This medium is used for checking the motility of organisms.

Procedure:

1. Motility agar will be prepared and inoculated with a straight inoculating needle making a single stab about 2cm down into the medium.
2. The motility will be examined after incubated at 37^0c for 24 hour.

Result: Motility will be indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation.

4.9. Lysine decarboxylase (LDC)

The acids produced by the bacteria from the fermentation of glucose will initially lower the pH of the medium and cause the pH indicator to change from purple to yellow. The acid pH activates the enzyme that causes decarboxylation of lysine to amines and the subsequent neutralization of the medium. This results in another color change from yellow back to purple. Bacteria that decarboxylate lysine turn the medium purple. In addition bacteria that produce H_2S appear as black colonies.

4.10. Oxidase test

The oxidase test is used to assist in the identification of bacteria which produce the enzyme cytochrome oxidase.

Procedure:

1. Piece of filter paper impregnated with oxidase reagent.
2. A colony of the test organism is then smeared on the filter paper.
3. Look for a color change
4. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple color.

4. AST procedure:

1. Select a pure colony of the organisms to be tested.
2. Aseptically emulsify a pure colony from the plate in the sterile saline solution and mix
3. Repeat until its' turbidity match that of the standard turbidity 0.5Mcfarland.
4. Take a sterile swab and dip it into the saline suspension of organism.
5. Gently squeeze the swab against wall of the tube to remove excess suspension in the swab.
6. Streak the test organism onto a sterile Mueller-Hinton agar (MHA) plate.

7. After the streaking is complete, allow the plate to dry for 5 minutes.
8. Place Antibiotic discs on the surface of the agar using sterile forceps.
9. Gently press the discs onto the surface of the agar by flame sterilized forceps
10. Carefully invert the inoculated plates and incubate for 16-18 hours at 37° C.
11. After incubation, use a metric ruler/dial caliper to measure diameter of the zone of inhibition for each antibiotic used.
12. Compare the measurement obtained from the each antibiotic with the standard table to determine the sensitivity zone.
13. Interpret the measured inhibition zone as sensitive or resistant according to predetermined standard table of CLSI.

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DECLARATION

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

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