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Bacterial etiology, antimicrobial susceptibility patterns and the prevalence of nosocomial infection in different clinical sample from patients attending intensive care unit in Tikur Anbessa Hospital,Addis Ababa,Ethiopia

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This is to certify that the thesis prepared by Jemal Mohammed, entitled: **Bacterial etiology, antimicrobial susceptibility patterns and the prevalence of nosocomial infection in different clinical sample from patients attending intensive care unit in Tikur Anbessa Hospital,Addis Ababa,Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public health microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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V.Acronyms and Abbreviations

ATCC: American Type Culture Collection

BSI: Blood stream infection

CCU: Critical Care Unit

CDC: Center for disease control and prevention

CLSI: Clinical Laboratory Standard Institution

CoNS: Coagulase-negative Staphylococcus

DAI: Device Associated Infection

DDST: Double disk synergy test

DRERC: Department Research and Ethical Review Committee

EPIC: European prevalence of infection in intensive care

ESBL: Extended Spectrum Beta-Lactamase

GNB: Gram-Negative Bacilli

HAI: Hospital acquired infection

HAP: Hospital acquired pneumonia

HCA: Health care associated

HCW: Health care wards

ICU: Intensive care unit

INICC: International Nosocomial Infection control consortium

MDR: Multi drug resistance

MRSA: Methicillin resistant Staphylococcus aureus

NHSN: National Healthcare Safety Network

NICU: Neonatal intensive care unit

NIs: Nosocomial infections

NNIS: National Nosocomial infection surveillance system

RTI: Respiratory tract infection

SOPs: Standard operating procedures

SPSS: Statistical Package for Social Sciences

SSI: Surgical site infection

TASH: Tikur Anbessa Specialized Hospital

UTI: Urinary tract infection

VAP: Ventilator associated pneumonia

VRE: Vancomycin- resistant Enterococci

WHO: World Health organization

VI. Abstract

Background: Nosocomial infections are widespread health problems in the world including in developed and developing countries which are the most important aggravating agents of mortality, morbidity, length of hospital stay and cost in the world. Nosocomial infections are becoming difficult to treat due to the increasing trend of antibiotics resistance, especially the critically ill patients in the intensive care unit (ICU). Relatively, few data are available from Ethiopia to indicate present health care associated infections (HAIs) status of situation.

Objective: To determine bacterial etiology, antimicrobial susceptibility patterns and the prevalence of nosocomial infection in different clinical sample from intensive care unit patients in Tikur anbessa hospital

Methods: A cross-sectional study was conducted from January to December 2018 in intensive care unit of Tikur Anbessa Specialized Hospital. Among 612 patients admitted to intensive care unit, 192 study participants (patients) who were suspected of bacterial nosocomial infections were included based on WHO guidelines for nosocomial infections using convenient sampling technique. Bacteria were isolated using culture and biochemical tests. Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion method as per CLSI. ESBLs confirmation was done by double disk synergy diffusion method. Data was analyzed with SPSS version 23.0.

Results: During a 12 months period from January to December 2018, 612 patients admitted to intensive care unit to Tikur Anbessa Hospital were studied for prevalence of nosocomial infection. A total of 192 patients were selected based on their clinical ground as per WHO 2002 guidelines. Total 14 % (87/612) patients were confirmed by culture to have nosocomial infection. Of the 77 patients were 70.1 %(61) male and 29.9% (26) females. The distribution of nosocomial infection among positive cases was blood stream infection (38.5%). Respiratory tract infection(20.2%),urinary tract infection(17.4%) and surgical site infection(11.0%).A total of 109 bacterial strains were isolated ,*K.pneumoniae* accounted for (26.6%) of total isolates, followed by *Acinitobacter spps (17.4%), E.coli* (16.5%) and *CoNS* (9.2%). A large majority of bacteria isolates (82.6%) were gram negative. High multi drug resistances (MDRs) bacteria were observed in ICU and Overall, multi drug resistance was observed in 71.6 %(78/109) of isolates. Gram positive and Gram negative isolates showed 52.6% and 75.6% MDR respectively. Both

Gram-positive and-negative isolates expressed resistance to most of the penicillin and cephalosporins tested. Amikacin and Meropenem were the most effective antibiotics against gram negative bacteria isolates (except *Acinitobacter spps*). *Acinitobacter Spps* showed highly resistance for most drugs including amikacin and meropenem.High rates of the extended spectrum beta lactamases (ESBLs) of *K. pneumonia* and *E. coli* were observed (31.9% and 21.3% respectively). Low rates (1.8%) of methicillin resistant *staphyloccus aures* (MRSA) was observed, whereas no vancomycin resistance *Enterococcus spps* were detected.

Key terms: nosocomial infection, bacterial isolates, Antimicrobial susceptibility patterns, Intensive care unit, *K.pneumoniae, E.coli, Acinitobacter spps*

AddisAbaba, Ethiopia

1 .Introduction

1.1 Back ground

Nosocomial infections are widespread health problems in the world including in developed and developing countries. Nosocomial infections also called “hospital-acquired infections” is variously described as an infection acquired in hospital by a patient who was admitted for a reason other than that infection or as-an infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at admission. It refers to an infection which comes from hospital environment after 48 hrs of admission [1]. This includes infection acquired in the hospital but appearing after discharge. Its source could be from other patients, health care workers, and/or hospital equipment devices [2].

Nosocomial infection (NIs) are frequent problem particularly in intensive care units (ICU) because of various invasive therapeutic or diagnostic interventions are frequently and for extended period used such as the use of wide spectrum antibiotics , mechanical ventilation, central venous catheterization, invasive pressure monitoring and urinary catheterization .[3] .

The U.S. Centers for Disease Control and prevention, estimates that HAIs in American hospital account for approximately 1.7 million infections and 99,000 associated death each year.Based on a study of a large sample of the U.S. acute care hospitals, on any given day, approximately 1 in 25 patients has at least one health care associated infection.Thus these infections are contribute to serious adverse event, including increased length of hospital stay, additional treatment with antibiotics, susceptibility to further infections, advanced medical intervention, high mortality, morbidity and cost in the world [4].

The center for disease control and prevention (CDC) has a National Nosocomial infection surveillance system (NNIS). This system receives monthly reports from hospital in USA (more than 270 institutes). From their new report it was estimated that health care associated infections accounts for 1.7 million infections and 99,000 associated death each year [5] .In addition, the WHO states that noscomial infections are one of the leading causes of death . But in other previous studies conducted in United States showed that the incidence of device associative hospital acquired infection can be reduced by as much as 30% which would result in correlative

reduced health care costs. This implies appropriate use of medical devices prevent incidence of hospital acquired infection [6] of inappropriate empiric antimicrobial treatment associated with infection [7].

Nosocomial infection rates are higher among critically in patients than in the general patient population [3] Evidence suggested that epidemiology of nosocomial infection varies from one country to another, even from one hospital to another. Previous study conducted in Pakistan Rawalpindi ICU (intensive care unit) of Tertiary care Hospital shown RTIs (respiratory tract infections) (47.95%) and UTIs (urinary tract infection) (25.3%). In study conducted at Karachi, the frequency of RTIs (respiratory tract infection) and UTI (urinary tract infection) was 21% and 44.6% respectively [16]. In another study carried out at Hyderabad, the frequency of RTIs and UTIs was 30.1% and 39.1% respectively [8]. The occurrence of ICU related infections does not appear to have changed substantially over the years despite improved knowledge about their etiology and the implementation of targeted preventive strategies [9, 10]

The burden of health care associated infection in developing countries is high prevalence of Health care associated (HCA) infection is much higher than proportions reported from Europe and the USA. The overall health care associated infection density in adult intensive care units was 47.9 per 1000 patient days, being at least three times as high as densities reported from USA [11].

In developing countries, the risk is two to twenty times higher and the proportion of infected patients frequently exceeds 25%.[12] In low and middle income countries, the burden of hospital acquired infection is unknown due to lack of reliable data [13]

In Africa, literature review shows that hospital wide prevalence of HAI varies between 2.5% and 14.8% in surgical wards, the cumulative incidence ranging from 5.7 to 45.8 % [14].Infrastructure of hospitals, low compliance of hand hygiene, understaffing, overcrowding heavy work load, misuse of personal protective equipment, late establishment of infection control programmes are major problems in resources limited countries these problems cause high infection rates and spread of multidrug resistant pathogens. [15]

Infection that can affect any system of the human body and the location of these infections usually depends on the nature of a patient's hospital procedure. The common include urinary

tract infection (UTI). Surgical site infection (SSI), respiratory tract infection (RTI) and blood stream infections (BSI) [2].

Study conducted in Pakistan 2010 in medical intensive care unit showed that the most frequent HAIs in ICU patients are respiratory infection followed by urinary tract infection and blood stream infection [16].

It is well recognized that most hospital in developing countries, especially Africa including Ethiopia, have little or no effective infection control programme due to several factors such as lack of awareness of the problems, lack of trained personnel, poor water supply, erratic electricity supply, ineffective antibiotic policies leading to emergence of multiple antibiotic resistance microbes to poor Laboratory back up, poor funding and non adherences to safe practices by health workers [17]. Though bacterial Nosocomial infection expected high, less attention has been given in Ethiopia. This problem is one of the health's related problems in the regional state. It might be one of the reasons for mortality and morbidity in the study hospital. The study was having importance in terms of addressing the bacterial etiology, antimicrobial susceptibility patterns and the prevalence of nosocomial infection in the study area.

1.2 Problem of Statements

Health care associated (or nososcomial) infection is a major problem in hospital worldwide particularly the incidence rate is high in ICUs compared to non ICU wards in hospital and the prevalence is higher in developing countries compared to Europe or USA [11].

Overall mortality is very high among ICU patients with ICUs associated nosocomial infection and it causes complication which result in death due to loss of immunity to prevent infection and excessive use of invasive procedures such urinary catheterization, central venous catheterization, mechanical ventilation and surgical procedures in developed and developing countries. It increases risks to patients. These infections have serious health and financial cost in an estimated incidence of 2 million infections and 20,000 deaths per year and added cost of $ 2 billion per year in the American health care system. [16]

Following effective standard infection prevention precautions in hospital plays a key role in improving patient health, in controlling and in preventing the spread of health care-associated infections. Bacterial contamination and cross transmission of microorganisms from inanimate surfaces, health care worker's hands, environmental contamination and have a significant role for ICU acquired colorization and infections. Health workers not only contaminate their hands after direct patient contact but also after touching inanimate surfaces and equipments in the patient zone. Inadequate hand hygiene results in cross transmission of pathogens. Ineffective infection regulation program is one of the most important contributing factors to HA infections. [18]

There is increasing drug resistance against the commonly used antimicrobial agents globally especially among the patients in critical units. The ongoing emergency of resistance in the community and hospital is consider a major threat to public health, study conducted in Belgium, Ghent hospital from 2009-2010 addressed that intensive care unit patients are the "epicenter" of nosocomial infection and it is also described as a factory for creating, disseminating, amplifying and developing antimicrobial resistance. The additional cost of multidrug resistance in hospitalized patients with infections has been estimated at $ 6,000 to $ 30,000 per patient. [19]

Moreover, several bacterial nosocomial infections carry a substantial economic burden due to high antimicrobial use and increased length of hospitalization. This burden of resistance, however, is probably more due to the high rate Antimicrobial drug resistance is a major global

problem affecting both developed and underdeveloped countries as well as problem of both in the community and health institutions. Along with the problem of nosocomial infection comes with the burden of "multidrug" antimicrobial resistance. The ongoing emergency of resistance in the community and hospital is considerable a major threat for public health. Due to the specific risk profile of its residents, the ICU also is deemed the epicenter of resistance development. Both infection and MDR result in a considerable clinical and economic burden. This increase cost of health care, morbidity and mortality. As such, the presence of MDR boosts the deleterious impact of nosocomial infection. [20] The rate of resistance varies across different studies due to organism isolated from different specimens. [21*] Pseudomonas aeruginosa* is the most resistant in the hospital environment such as intensive care units. It is frequently resistance including multiple classes of antimicrobial agents so the best control measure is early prevention of the infection. [22]

More over the distribution of the bacteria is different in different part of the world and studying the microbial factors that cause these infection in-all geographical regions, shows its dispersion. In study conducted from intrusive care unit patient samples in Iran noted that both gram negative and positive bacteria are responsible for Nosocomial infection. The most frequent gram negative microgranisms are *P. aeruginosa* and *Klebsiella Spp* as well as *S.aureus* among gram positive organism.[23] In another study in India showed that E.coli is found to be the predominant isolates followed by *K.pneumonia, Proteus mirabilis, Citrobacter, Fruendi, P aeruginosa, P, vulgaris, Acinetobacter species and S. typhi .[24]*

Nosocomial infection can affect any system of the human body; predisposition is dependent on the frequency of exposure of any system to microbial contamination via invasive medical procedure, presence of foreign body and susceptibility of the host. There are different types of nosocomial patient is treated with broad spectrum antibiotics in the study hospital. This might result in an increases in resistance among both gram positive and gram negative bacteria to common used to tract bacterial nosocomial infection. Apart from clinical presentations microbiological analysis with cultures and antimicrobial sensitivities is important for selection of a specific antimicrobial therapy. In the study hospital culture and drug susceptibility test is commonly practiced but the common problems are clinicians start patient treatment with broad spectrum drugs before susceptibility test delivered and the other problem is service interruption

due to budget constraint. It demands attention from health practitioners as it results in mortality, morbidity and affect the well being to patients.

Most published studies of HAI originate from hospitals in developed nations. Relatively, few data are available from Ethiopia to indicate present HAI status of situation. [25, 26] In Ethiopia Health care associated infections are estimated to be 40%. [27]

In the study setting there is no such as comprehensive research that highlight bacterial etiology, antimicrobial susceptibility patterns and the prevalence bacterial nosocomial infection in ICUs in Tikur Anbessa hospital.

1.3. Significance of the study

As clearly indicated in the statement of the problem, ICU patients are more vulnerable to nosocomial infection causing pathogens. Therefore the expected multiple benefits of this study was

Firstly, it was important in identifying patients with nosocomial bacterial infection and taking appropriate treatment after testing drug susceptibility test. Secondly, it was provide up-to-date information on frequently isolated bacteria from intensive care unit patients in the study hospitals. Thirdly, it increases the awareness of health professionals and patients and enhances the prevention of the disease in the hospital. Fourthly, it was assist hospitals in planning hospital antibiotic policy and physicians was also give due attention to diagnostic and microbiological analysis with cultures and antimicrobial sensitivities for the selection of a specific antimicrobial therapy. Finally, the study was used as a base line data for clinicians, health sector administrators and researchers from local to the federal level.

2. Literature Review

2.1. Epidemiology

Hospital-acquired infection has been estimated to be 5-10% in developed countries, and 10-30% in developing countries. The rates usually vary between countries, within the country, within the districts and sometimes even within the hospital itself, due to complex mix of the patients, aggressive treatment and local practices. [1]

In the United States, it is estimated that about 10% of hospital patients or more than 2 million hospitalized patients annually suffer from hospital infection with estimated annual death rate of 20,000, which may reach up to 88,000 deaths per year. The hospital-acquired infection follows basic epidemiologic patterns that can help to direct prevention and control measures. [28]The pathogens that cause hospital infection have reservoirs, transmitted by predictable routes and require a susceptible host. The reservoirs and sources of the infection could be the inanimate environment such as surgical instrument and the operative theatre and the animate environment such as infected or colonized health care workers, patients and hospital visitors.

The possible mode of transmission for hospital-acquired infection are either cross-infection due to indirect spread of the pathogens via patients contact stay or autoinfection from an endogenous flora found in the patient. [18]

In Morocco, the prevalence of HAI was reported as 17.8%. The prevalence was higher in intensive care units (50%) and the most frequently infected sites were urinary tract (35%) and surgical wounds (32.5%).[29] In India, reported a higher incidence rate of HAI (26. 1%).The incidence of NI was higher among the patients of medical (28%) ward than surgical (24.5%) ward, with the isolation rates of *Acinetobacter baumanii, Escherichia coli, Klebsiella pneumoniae. Pseudomonos aeruginosa, Enterococcus faecalis, Staphylococcus aureus* and Coagulase negative *staphylococci were 89.1%,89.1%,50%,*43.5%, 73.9%, 34.8% and 17.4% respectively.[30] *I*n Ethiopia, an overall incidence of hospital acquired infections of 17.8% was reported with 18.1% episodes of bacterial infections. Urinary tract and surgical site infections were detected in 48% and 45.6% of the cases, respectively. [31]

In Nigeria reported a prevalence of nosocomial infection of 2.6% over a 5 year period in Ibadan (South Western, Nigeria) with Surgical and Medical wards having the highest figures of 48.3%and20.5% respectively. UTI and SSI were the most prevalent (43.9% and 30.7% respectively) with UTIs significantly higher in surgical and medical wards, surgical site infections in obstetrics and gynaecology wards. [32]

In another similar study covering the period of 2000-2009 in Ile-Ife, (South Western, Nigeria), a prevalence 3.0% of HAI was reported. The highest prevalence of 9.0% was reported in the year 2006.The Intensive Care Unit (ICU) had the highest period prevalence of 14.7% followed by Orthopaedics ward (7.7%).Surgical ward contributed the highest number of cases with 38.3% followed by medicine (18.4%), Orthopaedics (13.9%), Obstetrics and Gynaecology (13.6%), ICU (6.4%), paediatrics (5.0%), and neonatal contributed the least (4.3%).[33]

In Nepal reported a Prevalence of bacteria causing nosocomial infection was 34.4%. In this study, the most common nosocomial infection was found to be UTI (39.30%) followed by LRTI (25.20%), SSI (25.20%), VAP (7.7%) and BSI (2. 6%).The most common isolates were *Escherichia coli* followed by *Acinetobacter species*, *Klebsiella pneumonia* and *Staphylococcus aureus.[34]*

In Iran at *Tehran University of Medical Sciences reported that higher rates of nosocomial infection was observed in ICU* The majority of reported isolates were Gram negative bacilli with a rates 20.8%. Other organisms causing nosocomial infections in these hospitals were *Candida* spp., and gram positive bacteria.The most frequent pathogens were *E.coli (32.8%),followed by K.pneumoniae(31%),P.aeruginos(12.8%) and Acinetobacter spp(9.1%).[35]*

Another study conducted in university of Sao Paulo Brazil that compares the rates nosocomial infection between ICU and non ICUs admitted patients showed that the rates of nosocomial infection is slightly higher in ICU admitted patients(30.6%), especially for respiratory infections. Among the commonly isolated pathogens, *Enterobacteriaceae* (33.8%),was the most prevalent followed by *Pseudomonas aeruginosa* (26.4%), *Staphylococcus aureus*16.9%; Streptococci (7.5%), coagulase-negative staphylococci (5.6%), and *Candida* species (7.5%).100% resistant to methicillin *Staphylococcus aureus* was reported but all were susceptible to vancomycine.Among 14 *Pseudomonas aeruginosa isolates were* for gentamicin, with 50% resistance; for imipenem,

with 30% resistance; for ceftazidime, with 50% resistance; for ciprofloxacin, with 41.6% resistance; and for amikacin, with 41.6% resistance was reported.[36]

2.2. Causative etiology

Most hospital-acquired infections are caused by pathogens common in the general population, in whom they cause little disease compared to hospitalized patients. The patients in ICU are especially at risk to develop infections.

There are many potentially organisms which can cause nosocomial infections, including bacteria, viruses, fungi and parasites.

Bacteria:

They are by far the most common nosocomial pathogens. Their reservoir can be endogenous or exogenous. Endogenous bacteria are from patient's own flora and exogenous are often from another patient (cross infection), from hospital staff (infected or colonized) or from the environment such as air-conditioning and vaporizers.

A further distinction can be made between commensal bacteria and pathogenic bacteria. *Commensal* bacteria, also called opportunistic, are part of normal flora and have a protective role by preventing colonization by pathogenic organisms. They do not cause disease in normal or healthy settings. Only when introduced into unprotected tissue or if the patient is immunocompromised, they may cause infection. *Pathogenic* bacteria are organisms with higher virulence, which cause infections regardless of host health status.

The bacteria that commonly cause nosocomial infections include *Staphylococcus (S.) aureus*, *Streptococcus* spp., *Bacillus cereus*, *Acinetobacter* spp., *coagulase negative staphylococci*, enterococci, *Pseudomonas (P.) aeruginosa*, *Legionella* and members of the Enterobacteriaceae familysuch as *Escherichia (E.) coli*, *Proteus mirabilis*, *Salmonella* spp., *Serratia marcescens* and *Klebsiella pneumoniae*. But the most frequently reported nosocomial pathogens have been *E. coli*, *S. aureus*, enterococci and *P. aeruginosa* .[37]

Paraful SP et al during 2016 in their study reported isolation of bacteria in 35.34%. Among them 127 isolates were gram negative bacilli and 69 isolates were gram positive cocci. *S. aureus (29.1%) and Klebsiella spp (26. %)* were the most common isolates followed by *E.coli(17.3%)., Pseudomonas spp(13.8%) streptococcus spp(5.1%),Acinetobacter(4.1%),Citrobacter(2.6%), Proteus spp (1%) and Coagulase negative staphylococcus(1%).[38]*

Mahin J et al (2009) reported the majority of nosocomial infection cases in ICUs were caused by gram negative organism Pseudomonas aeruginosa was the most common (43.2%). Other gram negative organisms were *Klebsiella spp (33.7%), Acinetobacter spp (8.8%) and E. coli* (8. 2%).Among gram positive pathogens *S. aureus* (39.2) was the common isolates followed by coagulase negative *staphylococci*. [23]

2.3. Nosocomial Infections

Based on the body systems involved there are different sites of nosocomial infection; the most common ones are Urinary tract infections (UTIs), Bloodstream infections (BSIs), surgical site infection and respiratory site infection which together account for more than 80% of all hospital-acquired infections [1].

2.3.1 Nosocomial Urinary Tract Infections

. Urinary Tract Infection (UTI) remains the most common bacterial infection in human population and is also one of the most frequently occurring nosocomial infections which accounting of 32 to 40% of all the HAIs occurring in approximately 1.7 million patients annually worldwide [CDC 2015]

Urinary tract infection may be defined as a condition in which bacteria are established and multiplying within the urinary tract. [39]

Infection classified by sites of infection as the kidneys (pyelonephritis), bladder (cystitis), prostate (prostatitis), and urethra (urethritis) or may affect the blood stream (bacteremia, septicemia).and can be a source of 10% to 15% of nosocomial bloodstream infections in medical or surgical intensive care units (ICUs).[40]

The National Healthcare Safety Network (NHSN) reports that nearly 75% of UTIs acquired in hospitals are associated with urinary catheters. Indwelling urinary catheters are often essential in critically ill patients and are used in 65% of patients in Critical Care Units (CCU) and in approximately 20% patients on the general wards. The use of urinary catheters is warranted in many cases, but many catheters are used unnecessarily and for prolonged periods of time. Inappropriate use of urinary catheters puts patients at an increased risk for both infectious and noninfectious complications, leads to overuse of antimicrobials and contributes to the rise of antimicrobial resistance, and increases healthcare costs. [41] A study conducted from April 2005-June 2010 in Spain, Barcelona intensive care unit among 2329 patients indicated that gram

negative bacilli were the predominant etiology of all the catheter related Urinary tract infection (*UTI*) and with a particularly important presence of *E. coli*. Among the gram positive cocci, particularly *E. Faecalis from enterococci* were seen during the study period .[42] In long-term catheterization, bacteriurias is inevitable and are the most common source of Gram-negative bacteraemia in hospitalized patients.[1] The most common pathogens causing Urinary Tract Infection (UTI) were *Escherichia coli* and other *Enterobacteriacae*, which accounts for approximately 75% of the isolates.*[43]* Moreover, a cross sectional study conducted from September 2003-June 2008 in Ethiopia, Felege Hiwot Referal Hospital, Bahirdar, across 529 patients urine on resistance of bacterial isolates from urinary tract infection shown that urinary tract infection is highly associated with antimicrobial resistance species and this depends up on age, sex, catheterization, hospitalization and previous exposure of antimicrobials. [43]
The infection may be symptomatic UTI or asymptomatic bacteriuria. Symptomatic UTI has signs and symptoms of infection such as fever, urgency, frequency and dysuria. Infection in children less than one year may show additional symptoms like hypothermia, apnea, bradycardia and lethargy. In asymptomatic bacteriuria, urine culture is positive with 10^5cfu per ml of urine with no more than two types of microorganisms. [44]

Bacterial contamination of the hands of personnel, hand towels, fluids for bladder irrigation, bottles used for urine collection, bedpans, rectal thermometers, lubricants for catheter insertion, and also dust and air from the ward atmosphere have been implicated in hospital cross infections.[28]

Ordinarily, the healthy urinary bladder is sterile, which means it does not have bacteria or other microorganisms in it. There may be bacteria in or around the urethra possibly because the urinary tract is in direct contact with the exterior, but normally these bacteria do not enter the bladder. During insertion, catheter can pick up bacteria from the urethra and allow them into the bladder, causing an infection to start. The most common sites of UTI are the urethra and the urinary bladder. From these sites, the infection may ascend into the ureters and subsequently involve the kidney. [2]

UTIs are associated with less morbidity than other types of nosocomial infections, but can occasionally lead to bacteraemia and even death. It has been reported to be the second most common cause of bacteraemia in hospitalized patients [1]. Infections are usually defined by

microbiological criteria such as: a positive quantitative urine culture of $\geq 10^5$ cfu per ml and not more than two species of microorganisms. [5]

2.3.2 Nosocomial Surgical Site Infections

Surgical site infections (SSIs) which account17% of all health care-associated infections are the second most common HAIs next to urinary tract infections [50]. Surgical site infection (SSI) is an infection that occurs at the incised site after operation procedure According to the Center for Disease Control and prevention (CDC) SSIs are classified into the following categories upon assessment at 30 days post operative surgery.

A.incisional surgical wound Infection; this is superficial and involve only the skin and sub cutaneous tissue of the incision, with visible signs of inflammation.

B. Deep Incisional Infections; These are also defined at 30 days post operative surgery or at one year, if organ implant is involved. It involves infection present in the deep soft tissues of the incision.

C. Organ space Infections; these involve any part of the anatomy other than the incision itself.

In general, a wound can be considered infected if purulent materials drain from it, even without confirmation of positive cultures

Surgical procedures increase a patient's risk of acquiring an infection in the hospital. Microorganisms can gain access into a wound either by direct contact air borne dispersal or by self contamination.

Surgery directly invades the patient's body, giving bacteria a way into normally sterile parts of the body. An infection can be acquired from contaminated surgical equipment or from healthcare workers. Following surgery, the surgical wound can become infected. [1] The risk of developing a surgical wound infection is largely determined by three factors: the amount, type of microbial contamination of the wound and host susceptibility. Almost all surgical sites are contaminated with bacteria, based on the degree of contamination, wounds are classified as clean, clean-contaminated, contaminated, dirty or infected and many studies have revealed that the risk of infection increase with degree of contamination . Surgical site infection rate per 100 operations was reported to be 2.1% for clean, 3.3% for clean contaminated, 6.4% for contaminated and 7.1% for dirty or infected wounds .[46]

Study conducted in Nigeria from January –March 2006 ,out of a total of 45 surgical wound specimens the prevalence of , *Staphylococcus aureus* , *Pseudomonas aeruginosa*, *Proteus*

mirabilis and *Escherichia coli* was 42.30%, 32.90%, 12.80%, and 12.80%, respectively.[47] According Nigatu Endalafre *et al.* in 2011, patients developed different forms of nosocomial infections during study period (June2007-April 2008) in Addis Ababa, Tikur Anbessa Hospital among 215 patients of which Surgical site infection comprised of 29.8%. Bacterial pathogens identified as cause for SSI were *Escherichia coli* , P. *aeruginosa* , *K. pneumoniae* , *P. vulgaris*, *E.cloacae* , *K. oxytoca* , C. *braakii* , S. *aureus* and *Coaglase negative saphlococcus aureus* .[48] Study conducted in University of Gondar teaching hospital from November 2010- February 2011 among 220 post operative wound specimens**.** The organisms isolated were S. *aureus, Klebsiella* species, *Proteus* species, *Escherichia coli*, *Enterobacter* species, coagulase negative staphylococci, (CoNS) accounting for 45 (25.4%), 32 (18.1%), 30 (16.9%) and 26 (14.7%) of the isolates *Pseudomonas aeruginosa* and *Citrobacter* species. [49]

In another study carried out in Hawassa referral hospital southern Ethiopia on 100 surgical patients with post surgical wound revealed that the most frequent isolates causing post surgical site infections were Staphylococcus *aureus, Klebsiella* spp., *Escherichia coli* and coagulase negative staphylococci respectively. Other bacteria isolated include *Pseudomonas aeruginosa* (9.0%), *Proteus* spp. (6.8%), Streptococci (5.1%), *Citrobacter* spp. (2.3%) and *Enterobacter* spp. (1.7%). In this study the rate of resistance reported that 173 (97.7%) were resistant to at least 1 antimicrobial, while 164 (92.7%) were resistant to ≥2 antimicrobials. [50] Antimicrobial resistance has been a problem in the field of surgery, as advances in control of infections have not completely eradicated the problem of drug resistance. [47]

The diagnosis of SSI is mainly clinical: purulent discharge around the wound or the insertion site of the drain, or spreading cellulitis from the wound. The infection is usually acquired during the operation itself; either exogenously (e.g. from the air, medical equipment, surgeons and other staff), endogenously from the flora on the skin or in the operative site or, rarely, from blood transfused at surgery. The main risk factor is the extent of contamination during the procedure, which is to a large part dependent on the length of the operation, and the patient‘s general condition. [5]

For most SSI, the sources of pathogens are endogenous flora of the patient’s skin, mucous membrane or hollow viscus. When mucous membrane is incised, the exposed tissues are at risk for contamination with endogenous flora. These organisms are usually aerobic gram-positive

cocci (e.g. *staphylococci*), but may include faecal flora (e.g. anaerobic bacteria and gram-negative aerobes) when incisions are made near the perineum or groin. [51]

When a gastro-intestinal tract (GIT) organ is opened during an operation and becomes the source of pathogens, gram-negative bacilli (e.g. *E. coli*), gram-positive organisms and sometimes anaerobes (e.g. *Bacillus fragillis*) are the typical SSI isolates. Seeding of the operative site from a distant focus of infection can be another source of SSI pathogen, particularly in patients who have prostheses or implants placed during the operation. Such devices provide a nidus for attachment of the organism. [51]

Exogenous sources of SSI pathogens include surgical personnel (especially members of the surgical team), operating room environment (including air), and tools, instruments, and materials brought to the sterile field during an operation. Exogenous floras are primarily aerobes, especially gram-positive organisms (e.g. *staphylococcus* and *streptococci*). Fungi from endogenous and exogenous sources rarely cause SSI and their pathogenesis is not well-understood. [51]

2.3.3. Nosocomial pneumonia

Hospital-acquired pneumonia (HAP) is the second most common nosocomial infection after urinary tract infections. The incidence of HAP ranges from 5 to 15 cases per 1000 hospital admissions, and is a frequent problem in general wards (incidence ranging from 1.6 to 3.67 cases per 1000 admissions). In the case of patients admitted to an intensive care unit (ICU), HAP occurs in up to 25% of patients with approximately 70 to 80% of episodes occurring during mechanical ventilation. Invasive mechanical ventilation means that an endotracheal tube or cannula is inserted in the natural airway to ensure air way patency and to enable positive pressure ventilation. This maneuver increases the risk of microorganisms entering the lower respiratory tract resulting in ventilator associated pneumonia (VAP). [52]

The microorganisms come from contaminated equipment or the hands of health care workers. In addition respiratory intubation and suctioning of material from the throat and mouth were also increase the risk of acquiring nosocomial pnemoniae. The introduced microorganisms quickly colonize the throat area and form colony, but do not yet cause an infection. It is easy for a patient to inhale the microorganisms into the lungs where it causes infection. The incidence of HAP in the ICU varies geographicaly. In the United States, the National Nosocomial Infection

Surveillance data found that 31% of all nosocomial infections in combined medical–surgical ICUs were due to pneumonia, with 83% of cases being ventilator-associated pneumonia (VAP) (53). In Europe, a large Italian study in 125 ICUs (which included 34,472 patients) reported that 9.1% of all admitted patients developed nosocomial infections, and pneumonia (specifically VAP) was the most prevalent (48.7%) ICU-acquired infection. [54] In a prevalence study of 254 ICUs in Mexico, 23.2% of patients had an ICU acquired infection, and VAP was the most prevalent infection (41.2%) [55]

According to a study in Asian hospitals, the proportion of ICU-acquired respiratory infections ranges from 9 to 23%. The principal sources of pathogens in HAP cases are the health care environment and the patient's own microbial flora. The microbial etiology of HAP in the ICU varies according to patient population, hospital ICU settings, the country, and the type of presentation [56]

A study conducted in Assiut University Hospitals, Egypt from January to December 2010 on Clinical and Microbiological Profile of Nosocomial Infections in Adult Intensive Care Units demonstrated that among admitted, 5979 patients 900 patients developed nosocomial infections. The overall rate of NIs among adult ICU patients was 15%. The commonest type of NI was lower respiratory tract infection (59.9%). The most frequently isolated microorganisms were gram negative bacteria (54.2%) amongst which, *Klebsiella* spp. was the most common. Gram positive bacteria accounted for 45.8% with methicillin resistant *Staphylococcus aureus* (MRSA) being the predominant (23.6%). Several risk factors including burns, endotracheal tubes, mechanical ventilation urinary catheters, intravenous catheters and hospital stay for more than 2 weeks were highly significant for acquiring nosocomial infections [57]. Similarly, in the study carried out in Imam Reza teaching Hospital at the northwest of Iran from March 2010 to January, 2012 on antimicrobial susceptibility patterns among bacteria isolated from intensive care units reported that respiratory tract (51.7%) was the common sites of infection followed by urinary (24.8%), and blood (10.4%). *Enterobacter aerogenes* (50.6%), *Escherichia coli* (16.7%) *Pseudomonas aeruginosa* (7.5%) and *Staphylococcus aureus (*39.7%). were the most frequent isolated pathogen [58]

The diagnosis of pneumonia may be based on clinical and radiological criteria which are readily available but non-specific: recent and progressive radiological opacities of the pulmonary

parenchyma, purulent sputum, and recent onset of fever. Risk factors for infection include the type and duration of ventilation, the quality of respiratory care, severity of the patient‘s condition (organ failure), and previous use of antibiotics [1].

2.3.4 Nosocomial Bacteraemia

These infections represent a small proportion of nosocomial infections (approximately 5%) but case fatality rates are high — more than 50% for some microorganisms [1]. Infection may occur at the skin entry site of the intravascular device, or in the subcutaneous path of an intravenous catheter. Bacteria transmitted from the surroundings, contaminated equipment, or healthcare workers' hands can invade the site where the catheter is inserted. A local infection may develop in the skin around the catheter. The bacteria also can enter the blood through the vein and cause a generalized infection. The main risk factors are the length of catheterization, level of asepsis at insertion, and continuing catheter care [5]

Gram positive bacteria are more predominant causative agents of blood stream infection than Gram negative bacteria in Brazil Teaching Hospital study from 2005-2006 among 243 patients. This might be due to the greater use of intravascular device and antibiotics in critical patients. [59] Moreover, Acinetobacter baumannii is most important cause of nosocomial blood stream infection in intensive care unit ward in China, sun Yat-sen University study conducted from 2006-2012 among 258 patients. Frequently use of invasive procedures increase the occurrence of *Acineto bacter baumanni* bacteremia [60].

According to a study conducted in Upper Egypt from 2006-2007 pointed out that the etiologic agents of blood stream infection from blood culture among 295 patients were coagulase negative *staphylococci,* S. *aureus* and *Klebsiella pneumoniae* . Blood stream infection (BSI) is among serious nosocomial infection that demands continuous follow up and it results in prolonged hospital stays, high health care costs, and significant mortality. Due to repeatedly occurring pathogenic organisms, leads to resistance to many antibiotics, particularly antimicrobial resistance caused by Gram positive bacteria. It is causing high mortality in Affiliated Urban University Hospitals [61*]*

Blood stream infection is among the major life-threatening infections in hospitals. Many cases of hospitalization are reported in blood stream infection in each year in hospitals by P. *aeruginosa*

and S. *ureus*. These results in prolonged hospital stay, increased mortality and cost in hospitals [62].

2.4. Factors influencing the development of nosocomial infections

2.4.1 The microbial agent

The patient is exposed to a variety of microorganisms during hospitalization. Contact between the patient and a microorganism does not necessarily result in the development of clinical disease. Other factors also influence the nature and frequency of nosocomial infections. The likelihood of exposure leading to infection depends partly on the characteristics of the microorganisms, including resistance to antimicrobial agents, intrinsic virulence, and amount (inoculum) of infective material. Many different bacteria, viruses, fungi and parasites may cause nosocomial infections. Infections may be caused by a microorganism acquired from another person in the hospital (cross-infection) or may be caused by the patient‘s own flora (endogenous infection). Some organisms may be acquired from an inanimate object or substances recently contaminated from another human source (environmental infection). [1]

Before the introduction of basic hygienic practices and antibiotics into medical practice, most hospital infections were due to pathogens of external origin (foodborne and airborne diseases, gas gangrene, tetanus, etc.) or were caused by microorganisms not present in the normal flora of the patients (e.g. diphtheria, tuberculosis). Progress in the antibiotic treatment of bacterial infections has considerably reduced mortality from many infectious diseases. Most infections acquired in hospital today are caused by microorganisms which are common in the general population, in whom they cause no or milder disease than among hospital patients (*Staphylococcusaureus*, coagulase-negative staphylococci, enterococci, Enterobacteriaceae) [1].

2.4.2. Patient susceptibility

Important patient factors influencing acquisition of infection include age, immune status, underlying disease, and diagnostic and therapeutic interventions. The extremes of life (infancy and old age) are associated with an inadequate/decreased resistance to infection. Patients with chronic disease such as malignant tumours, leukaemia, diabetes mellitus, renal failure, or the acquired immunodeficiency syndrome (AIDS) also have an increased susceptibility to infections

with opportunistic pathogens. The latter are infections with organism(s) that are normally innocuous, e.g. part of the normal bacterial flora in the human, but may become pathogenic when the body‘s immunological defenses are compromised. Immunosuppressive drugs or irradiation may also lower resistance to infection. Injuries to skin or mucous membranes bypasses natural defense mechanisms poses risk of infection [1].

Malnutrition is also a risk factor. Many modern diagnostic and therapeutic procedures, such as biopsies, endoscopic examinations, catheterization, intubation/ventilation and suction and surgical procedures increase the risk of infection. Contaminated objects or substances may be introduced directly into tissues or normally sterile sites such as the urinary tract and the lower respiratory tract. [1]

2.4.3 Environmental factors

Health care settings are an environment where both infected persons and persons at increased risk of infection congregate. Patients with infections or carriers of pathogenic microorganisms admitted to hospital are potential sources of infection for other patients and staff. Patients who become infected in the hospital are a further source of infection. Crowded conditions within the hospital, frequent transfers of patients from one unit to another, and concentration of patients 32 highly susceptible to infection in one area (e.g. newborn infants, burn patients, and intensive care) all contribute to the development of nosocomial infections. Microbial flora may contaminate objects, devices, and materials used in one procedure which subsequently contact susceptible body sites of other patients. In addition, new infections associated with bacteria such as waterborne bacteria (atypical mycobacteria) and/or viruses and parasites continue to be identified [1].

2.4.4 Bacterial resistance

Many patients receive antimicrobial drugs. Through selection and exchange of genetic resisntance elements, antibiotics promote the emergence of multiple antibiotic resistant strains of microbes; microorganisms in the normal human flora sensitive to the given drug are suppressed, while resistant strains persist and may become endemic in the hospital. The widespread use of antimicrobials for therapy or prophylaxis is the major stimulant of resistance.[1]

Antimicrobial agents are, in some cases, becoming less effective because of resistance. As an antimicrobial agent becomes widely used, bacteria resistant to this drug eventually emerge and may spread in the health care setting. Many strains of pneumococci, staphylococci, enterococci, and tuberculosis are currently resistant to most or all antimicrobials which were once effective. Multiple resistant *Klebsiella* and *Pseudomonasaeruginosa* are prevalent in many hospitals [63].

This problem is particularly critical in developing countries where more expensive second-line antibiotics may not be available or affordable [1]

2.5. Nosocomial infection and the magnitude of Drug resistance

According to World Health Organization, antimicrobial resistance is existent in all parts of the world and new resistant bacteria develop and spread globally .The prevalence of antibiotics resistance have showed a wide variation in MDR with in different countries. But MDR in developing countries are complex and higher than developed countries. This is due to many developed nations have implemented surveillance networks and monitoring systems to track the spread and increase of antimicrobial resistance. But there are few numbers from developing countries because monitoring resistance often requires laboratory equipment, trained personnel and financial resources, which lower income countries lack [WHO 2008].

Multi drug resistance is defined as the resistance to at least three classes of first line agents including Ampicillin, Chloramphenicol, Trimethoprim-Sulfamethoxazole, Fluroquinolones (Ciprofloxacin and Ofloxacin), and Cephalosporins (Cefotaxime, Ceftriaxone and Ceftazidime) [65]

Different study conducted in different countries that showed various results on MDR .in Iran Hadi et al 2016 reported that multi drug resistance (MDR) gram negative bacteria were documented in 25.8% Acinetobacter, 20% of klebsiella and 16.6% of pseudomonas. More over the rate of methicillin resistant staphylococcous aureus (MRSA) was 87.5%.[58] In Dhaka at tertiary care hospital showed that 50% of third generation cephalosporin resistant E.coli, klebsiella and 50% of impenem resistant pseudomonas and Acinetobacter were observed. Among them Acinetobacter was remarkably resistant to most antibiotics[66].Another study in Rawalpindi, revealed that the majority (> 50%) of the gram negative isolates were resistance to

many of antibiotics tested such as ofloxacin, ciprofloxacin ,cefotaxime ,ceftriaxone and ceftazidime[16]

A prospective multicentre observational study was conducted in Ghent University Hospital Belgium from February 2006-june 2007 among 198 intensive care unit patients. The result confirmed that Multi drug resistance (MDR) highly occurs among the patients with excessive exposure use of drugs. Hospitalization in another ward prior to intensive care unit (ICU) admission was also recognized as significantly associated with multi drug resistance (MDR) involvement [67]

Moreover, emergence of multi drug resistance is high in intensive care unit (ICU) patients of Belgium, Ghent University Hospital study from 2009 - 2010 among 260 patients due to excessive use of broad spectrum antibiotics. More than 60% of all Intensive care unit patients take antibiotic during hospital stay but the epidemiology of Multi drug resistance (MDR) is complex in nature. During the past few decades, it appeared that some antibiotics have a higher risk of causing antimicrobial resistance, example, third-generation cephalosporins, vancomycin, imipenem and intravenous fluoroquinolones [19].

A study conducted in Al Ansar hospital Saudi Arabia in the ICUs patients revealed that multi drug resistant (MDR) bacteria are becoming increasingly prevalent in ICU environments. Several factors are reported which contributing to the spread of antimicrobial resistance, mainly extensive use of antibiotics, new mutation, selection of resistant strains, suboptimal infection control and treat patients with out susceptibility pattern of agents. In this study, pseudomonas aeruginosa (16.3%) E.coli (13.6%) Acinetobacter baumanni (10.4%) klebsiella pneumonia (8.5%) and S, aureus (6.3%) were isolated in decreasing order. A.baumannii was highly resistance to third generation of cephalosporin. In addition P.aeruginosa and E.coli showed resistance to cefotaxime, cefotriaxone, tetracycline, piperacillin and co-trimoxazole. High frequencies of multi drug resistance bacteria occur in ICU.In gram negative bacteria, the resistance is mainly due to rapid increase of ESBLs in K.pneumonia, E.coli and proteus spps and high rates of ESBLs producer K .pneumonia and E.coli were observed (49%and 40%). Among gram positive organisms, higher resistace MRSA and VRE isolates also observed (43% and 14% respectively). [68]

Antimicrobial Resistance Surveillance study among Intensive Care Units of a Tertiary Care Hospital in Southern India indicated that there is the increasing trend AMR among the hospital acquired pathogens such as MDR-GNBs, MRSA and VRE which pose a great threat to HCWs as well as to the other critically ill patients of the ICUs.The result of this study showed that about 22.2% infections were HAIs, out of which pneumonia (6.24%) was the most common. Analysis of antimicrobial susceptibility pattern revealed that most of Gram-Negative Bacilli (GNB) was Multi Drug Resistant (MDR) i.e., resistant to three or more class of antibiotics such as cephalosporins, carbapenems, aminoglycosides, tetracyclines and fluoroquinolones. The prevalence of Methicillin- resistant *Staphylococcus aureus* (MRSA) and Vancomycin- resistant *Enterococci* (VRE) were found to be 40.6% and 11.9% respectively. *Acinetobacter* spp was the most resistant organism [69]

"The prokaryotic cell is versatile and capable of adapting to the introduction of antibiotics into the environment. The inherent genetic variation ensures a fair amount of heterogeneity that ensures survivors in antibiotic charged environments. Thus, survey of bacterial isolates from the pre-antibiotic days in India showed the presence of resistant organisms, even though in small numbers" [70].

A cross sectional study carried out in Mulago National referral hospital and International Hospital Kampala.Uganda two intensive care units a 58% of multi drug resistance prevalence was reported. In this study 50% *Escherichia coli* and 33.3% *Klebsiella pneumoniae* were extended spectrum beta lactamase or AmpC beta lactamase producers and 9.1% *Acinetobacter species* were extensive drug resistant [71].

More importantly, study carried out in Ethiopia, Jimma University Hospital from June – December 2011 among 322 patients confirmed that there is Multi drug resistant (MDR) bacterial infection which are resistant to a number of antimicrobial drugs belonging to different chemical classes. Antimicrobial drug resistance can be acquired as a result of mutation or of resistance genes via horizontal gene transfer, or can be an innate feature of an organism that is encoded [62]. Similarly study in Jimma University, conducted from May- September 2013 among 150 specimens to see drug susceptibility pattern indicated that the isolated bacteria were found to have high frequency of resistance to ampicillin, penicillin, cephalothin and tetracycline. 71% of Gram positive and 97.4% Gram negative isolates showed Multi drug resistant (MDR) [72].

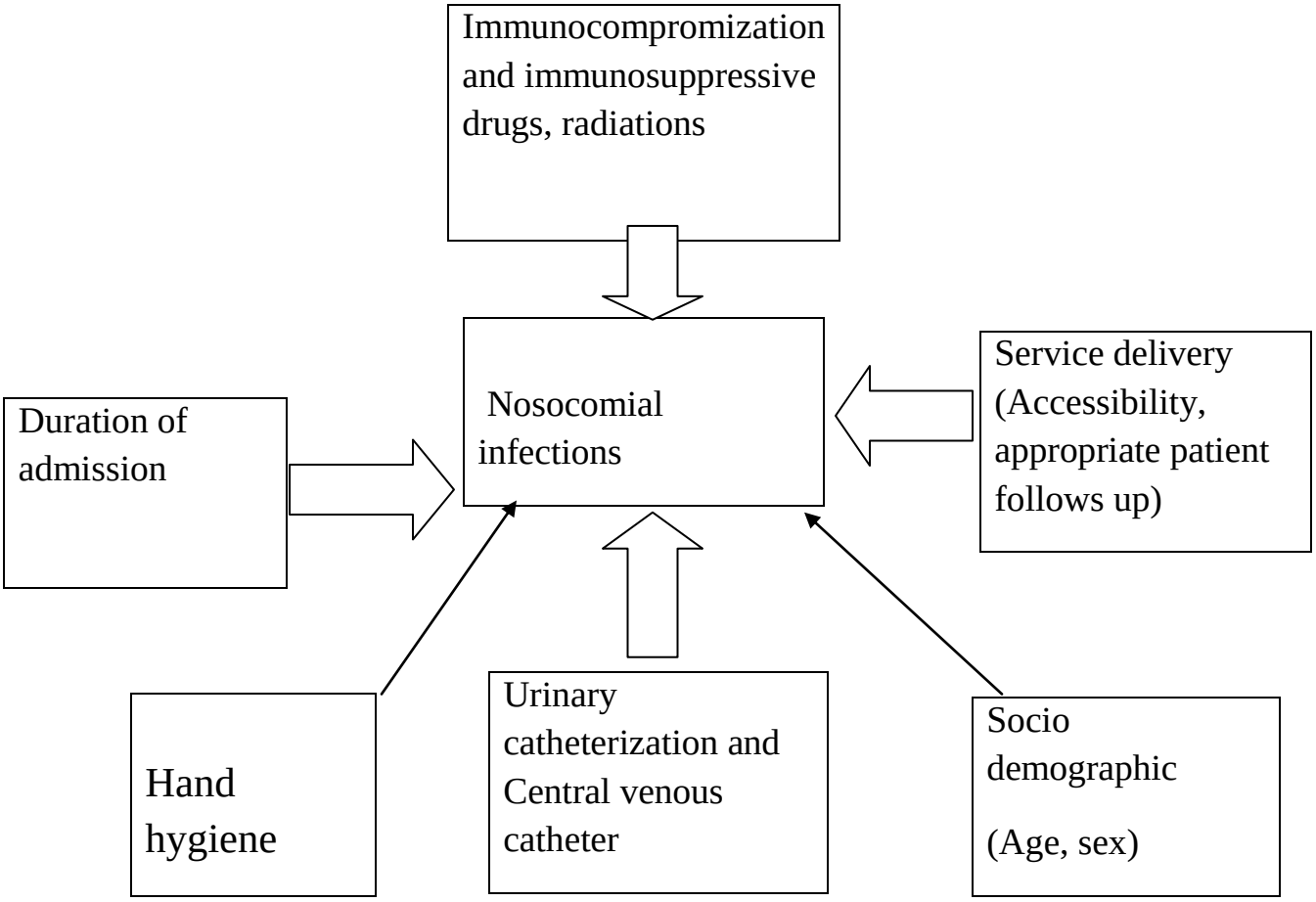
Study conducted in Addis Ababa Tikur Anbessa Specialized Hospital from March to August 2015 among 192 patients result indicated that 75.6%(149/197) were culture positive and the predominant bacterial isolates were S .aureus (33.3%) and E.coli (14.3%).Of all bacterial isolates, the multidrug resistance level was 65.5% .Gram positive and Gram negative bacteria showed MDR level of 55.3% and 73.9% respectively. Amoxacillin(93.5%),Ceftriaxone(85.3%) and Penicillin(84.5%) were least effective.[99]Another cross sectional study conducted in Addis Ababa Tikur Anbessa Hospital from September 1/2016 to October 30/2017 among 422 specimens reveled that there is an increase of resistance to common drugs in blood stream infections.Penicillin(86.7 %) was the least effective antibiotics against Gram positive bacteriawhile ampicillin(85.7%) and amoxicillin clavulinic acid(77.14%) were the least effective antibiotics against Gram negative bacteria. Result from this study indicated that 55.2% and 74.3% Gram positive and Gram negative bacterial isolates were multidrug resistant respectively. [100] Similarly study conducted in University of Gonder hospital from February 1 to May 31 2016 among the 260 patients to determined multidrug resistant bacterial isolates of nosocomial infection indicted high prevalence of hospital acquired infections. A rate of 66.5% was reported from Gonder. Results from this study showed that S.aures (35.6%), E.coli and Klebsiella spp were the commen isolated pathogen. More over the leading multidrug resistant Gram negative isolates were observed in Citrobacter spp (100%), Klebsiella spp (79.3%) and E. coli (75.3%) where as 67.5% was methicillin resistant S. aures and the overall MDR rate was 70.4%.[97]

Study conducted in University of Gondar Hospital from November 2010- February 2011 among 220 specimen results revealed that among isolated Gram-negative rods were highly resistant to Ampicillin, cotrimoxazole, doxycycline, tetracycline, chloramphenicol, Nalidixic acid and gentamicin. S. aureus confirmed high level of resistance to tetracycline while, ceftriaxone and ciprofloxacin were found to be relatively sensitive to all the isolates [49].

Another cross-sectional study in Harar hospital eastern Ethiopia from February to March, 2015 showed that out of 162 total patients, 54 were confirmed by culture to have nosocomial infection; the prevalence of bacterial nosocomial infection in the study hospitals was 28%. Klebseiella species were the predominant bacterial isolates accounted 20.7%. A large majority of bacteria isolates (69%) were gram negative and isolates were found to be resistant to Amoxicillin-Clavulanic acid (90%), Tetracyclin (90%), Amoxicillin (100%), Trimethoprim-

sulphamethoxazole (80%) and Chloramphenicol (72.5%). Gram positive isolates were found to be resistant to Methicillin (94.4%), Penicillin (88.6%), Amoxicillin (94.4%), Doxycyclin (83%), Oxacillin (94), Tetracyclin (83%) and Chloramphenicol (83.4%). On the other hand, both gram positive and gram-negative isolates were sensitive to Ceftriaxone (72.2%) and Ciprofloxacin (66.4%). [73]

Figure 1: Conceptual Frame Work.



3. Objectives of the study

3.1 General objective

To determine bacterial etiology, antimicrobial susceptibility patterns and the prevalence of nosocomial infection in different clinical sample from patients attending in intensive care unit in Tikur anbessa hospital

3.2 Specific objectives

1. To determine the bacterial etiologic agent causing nosocomial infection in CCU patients in Tikur Anbessa Specialized Hospital

2. To determine the prevalence of nosocomial infections among CCU patients in Tikur Anbessa Specialized Hospital

.3. To determine the antimicrobial susceptibility patterns among CCU patients in Tikur Anbessa Specialized Hospital.

4. Research questions

1. What are the bacterial etiology and the prevalence of nosocomial bacterial infection among the CCU patients at Tikur Anbessa Specialized Hospital?

2. What are the antimicrobial susceptibility patterns of specific organisms causing nosocomial infections among CCU patients at Tikur Anbessa Specialized Hospital?

5. Materials and methods

5.1 Study area

The study was conducted at Tikur Anbessa Specialized Hospital adult and pediatric ICU in Addis Ababa Ethiopia. Tikur Anbessa Specialized Hospital is one of the largest tertiary referral teaching hospital located in Lideta Kefle Ketma in Addis Ababa capital city of Ethiopia, which has 800 beds and has its own adult and pediatric ICU. The hospital has 6 surgical intensive care unit beds, 6 pediatrics intensive care unit beds and 6 medical intensive care unit beds and the average patient flow per day is 1200 and per year is 370,000-400,000. It has a total of 1644 staffs of which the TASH has 200 doctors, 379 nurses and 115 other professionals and also 950 administrative staffs. This hospitals accommodiates different departments includes OPD, surgery, familyplanning,ART,laboratory,pharmacy,ANC,oncology, anesthesiology,ENT,delivery and neonatal care and it provides services for the community in and out side the capital as referral services

5.2 Study design and period

- A cross-sectional study was conducted from January2018 to December 2018.

5.3. Population

5.3.1 Source Population

- All patients who were admitted in critical care unit at Tikur Anbessa Specialized Hospital with in the study period.

5.3.2. Study Population

- patients in critical care unit who were suspected bacterial nosocomial infection after 48 hours of admission or patients with sign and symptom of bacterial nosocomial infection in the Tikur Anbessa Hospital during the study period.

5.4. Inclusion and Exclusion criteria

5.4.1. Inclusion Criteria

- Patients who developed bacterial nosocomial infection (Urinary tract infection (UTI), surgical site infection (SSI), Blood stream infection (BSI), Respirtory tract infection (RTI) after 48 hours admission were eligible and included in the study.

5.4.2. Exclusion criteria

- Patient's acquired infection before admission to the hospital or the first case of admission (Community acquired).
- The repeat specimens and stool samples were not included in the study

5.5. Study variables

5.5.1. Dependent variables

- Bacterial nosocomial infection
- Antimicrobial susceptibility patterns

5.5.2. Independent variables

- Age, sex,

5.6. Sample size calculation and Sampling method

5.6.1. Sample size calculation

The sample size was determined using single population proportion formula considering the 95% confidence level taking the prevalence as 13% from previous study conducted at Addis Ababa Gedebou *et al*, 2009 and Degree of allowable error 0.05. The sample size was therefore calculated as follows:

Total study subjects**:** $n = z^2 p (1-p)$

$$\frac{}{d2}$$

Where P = prevalence of nosocomial bacterial infection (13%) [74] In Addis Ababa Tikur Anbessa Teaching Hospital.

D = degree of accuracy desired (0.05)

$\mathbf{z}^2$ 1-α/2 = the standard normal deviation (1.96)

$n = (1.96)^2 * 0.13(1-0.13)$

$$\frac{}{(0.05)^2} = 174.$$ The 10 % non-response rate is 17.4

Therefore, the sample size will be: 192

5.6.2. Sampling Method

The sampling technique was convenient. Of a total of 612 patients admitted in the ICU, 192 patients who suspected of nosocomial infection were included in this study. To select target population, careful clinical examination was conducted by physician, intern students and senior nurses to all ICU patients who admitted in pediatric intensive care unit, surgical intensive care unit and medical intensive care unit. A guideline from WHO was adapted and it was provided to the physician inorder to evaluate those patients and a guideline which serve as to assess those patients for the development of nosocomial infections during the study period.

Between January 2018 to December 2018, a total of 109 isolates were recovered from various clinical specimens obtained from patient who were hospitalized in the ICU of Tikur Anbessa hospital addis ababa Microbiological cultures for clinical samples comprised blood, urine, tracheal aspirates, csf, sawb, aspirates and discharge of pus and wound and others were done. Cultures were processed using standard microbiological methods. For the blood cultures, the Bactec 9120 blood culture instrument (Becton Dickinson, Baltmore, Md., USA) was used. The blood culture bottles were incubated in the Bactec system as recommended by the manufacturer for seven days. When the positive blood bottle, three drops of blood culture samples taken up with sterile syringe and were inoculated onto blood agar, chocolate agar and Macconkey agar. All the plates were incubated for 24h at 35ºC aerobically overnight. Urine samples were cultured on blood agar, and MaConkey agar, respiratory samples were cultured on blood agar, chocolate agar, and Macconkey agar and also others samples. All the cultures were incubated aerobically. Identification of the bacteria was carried out based on Gram staining, and standard biochemical tests. After identification, all isolates were subcultured on Muller Hinton agar (MHA). The

organisms were suspended in saline to turbidity 0.5 McFarland standards. A swab of the cell suspension was then spread in three directions on entire surface of MHA plate, and left for 15 min to allow moisture absorption at room temperature before applying the multi-disk on the agar. The agar plates were then incubated at 35°C for 18-24 h. E. coli (ATCC 25922) and S. aureus (ATCC 25923) were used as controls and P.aeruginosa (ATCC 27853). The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute.

Antibiotic susceptibility testing for Gram-positive bacteria included ampicillin, clindamycin, erythromycin,, penicillin, ,cefoxitin, cotrimoxazole, ciprofloxacin, and vancomycin. For the Gram-negative bacteria, the antibiotics tested were ampicillin,, amikacin, augmentin, cefotriaxone, cefotaxime,ceftazidime, ciprofloxacin, cefepime, tobramycin, gentamicin, meropenem, piperacillin/tazobactam, chloramphenicole and co-trimoxazole for in vitro susceptibility of bacterial isolates to these antibiotics.

Test for ESBL production all the strains showing diameter of less than 27mm for cefotaxime, less than 22 mm for Ceftazidime and less than 25mm for ceftriaxone were selected for checking ESBL production.

Detection of Extended Spectrum Beta Lactamases (ESBL) was done by the double disc synergy test (DDST) method according to CLSI guidelines as follow: Standardized inoculums (0.5 McFarland) of the test organisms were inoculated on Muellar Hinton Agar (MHA) using sterile swab stick. Amoxicillin/clavulanic acid disc (30ug,) was placed at the center of the inoculated MHA.Aztreonam(30ug), Ceftrixone(30ug),Ceftazidime (30ug,) Cefepime(30ug),Cefotaxime (30ug) and Cefpodoxime (10ug) were placed 15mm center to center from the Amoxycillin/clavulanic acid discs. The plates were incubated at 37^{o}C for 24 hours. After incubation, enhancement of zone of inhibition of any one of the antibiotics the Aztreonam, Ceftriaxone, Ceftazidime ,Cefepime,Cefotaxime and Cefpodoxime discs towards the Amoxycillin/Clavulanic acid discs is indicative of ESBL production, often showing shape zone referred to as keyhole.The enhancement is due to inhibition of ESBL by clavulanic acid and subsequent action of the extended spectrum cephalosporins.

Definition of resistance we defined MDR strains for this study as those that exhibit resistance to 3 or more classes of antibiotics: antipseudomonal, cephalosporins/penicillins, macrolides, carbapenems, fluoroquinolones, and aminoglycosides. Clinically Important Resistance (CIR)

were recognized when such strains showed resistance to extended spectrum cephalosporins, meticillin and vancomycin.

Definition of nosocomial infection is shown in Table 1.

Table 1: Simplified criteria for surveillance of nosocomial infections

Type of nosocomial infections	Simplified criteria
Surgical site infection	Any purulent discharge,abscess,or spreading Cellulitis at the surgical site during the month After the operation
Respiratory infection	Respiratory symptoms with at least two of the following signs appearing during hospitalization -Cough -Purulent sputum -New infiltrate on chest radiograph consistent with infection
Vascular catheter infection	Inflammation, lymphangitis or purulent discharge at the insertion site of the catheter
Urinary infection	Positive urine culture(1 or 2 species) with at least 10^5 bacteria/ml,with or with out clinical symptoms
Septicaemia	Fever or rigours and at least one positive blood culture

SOURCE -WHO, 2002

5.7. Operational definition

Nosocomial infection refers to an infection developed from hospital environment after 48 hours admission or infections acquired during hospital stay which are not present during admission.

Antimicrobial sensitivity pattern carried out to determine which antibiotic will be successful in treating a bacterial infection and measured as sensitive, intermediate and resistant based on criteria set by Clinical and Laboratory Standards Institute (CLSI).

Invasive procedure refers to a diagnostic or therapeutic technique that requires skin penetration or entry of a body cavity.

Intensive care unit also known as an intensive treatment unit refers to a special department of a hospital or health care facility that provides intensive care medicine**.**

Multi drug resistance: Definition of resistance. According to the CDC system of MDR classification, MDR strains are those which showed resistance to 3 or more classes of antimicrobial agents.Extensive drug resistant strains are those which showed resistance to all, but 1 or 2 classes of antimicrobial agents, while pan-drug resistant strains are those which showed resistance to all classes.

ESBLs are an enzymes hydrolyzing most pencillins, cephalosporins and monobactum (Aztreonam) but not cephamycins and carbapenems and susceptible to beta-lactamase inhibitors (clavulanate, sulbactum and tazobactum)

MRSA is resistance to the antistaphylococcal penicillinase-stable penicillins (eg methicillin, nafcillin and oxacillin) has been referred to as methicillin resistance and the acronyms MRSA (for methicillin-resistant S.aureus) are still commonly used, even though methicillin is no longer the agent of choice for testing

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5.8. Measurement and Data collection

5.8.1. Data collection procedure

Socio-demographic data was collected from study participants request forms

5.8.2 Laboratory Analysis

5.8.2 .1.Sample collection and Examination

Specimen was collected from intensive care unit (ICU) admitted patients that are suspected by physician developing nosocomial infection based on standard procedures and guidelines through quality assurance program. The sample was collected by trained laboratory personnel and nurses from the appropriate site of infection and appropriate time of collection. Time of collection depending on the types of specimen was collected. Blood was collected during patient temperature rises. The first urine passed by patient at the beginning of the day mid stream urine was collected for examination. Pus from abscess was collected using sterile cotton when abscess is incised and drained, or after it has ruptured naturally. All specimens were collected using appropriate container and labeled with patient name, date and time of collection. The container was tightly close so that its contents do not leak during transportation. Arrangements were made for immediate transportation of the specimen to the laboratory (44).

5.8.2.2 .Blood sampling and processing

In this study 10ml of venous blood was collected of which 5ml was inoculated immediately in Tryptone soya broth and incubated aerobically at 37 ^{0}C for 10 days. The inoculated culture was being followed daily for visible growth (Annex III).

5.8.2.3. Urine specimens

Mid stream urine for bacteriological examination was collected from the patient directly or from catheter into sterilized container. The bladder and urinary tract are normally sterile, however the urethra may contain a few commensals and also with female patients, the urine is contaminated with organisms from the vagina. Colony count $\geq 10^5$ CFU/ml of urine was considered indicative of significant infection (Annex III).

5.8.2. 4.Swab from wound infection

Wound specimen was collected from appropriate site of infection aseptically using sterile swab. It was cultured on Manitol salt agar, blood agar and MacConkey agar, first gram stain followed by biochemical test was conducted in order to isolate the causative agents. Detail procedure is attached in the annex (Annex I).

5.8.2.5 Culture and Gram staining

Blood specimens were inoculated in Trypto soya broth where as urine and swab specimens were inoculated on blood agar and MacConkey agar. Both culture media incubate aerobically at 37°C for 24-48 hrs but Trypto soya broth was incubated for 2 weeks, examine daily up to 14 days for visible growth (show turbidity, hemolysis, gas production and clots) and sub cultured on Blood agar, Chocolate agar and MacConkey agar. A positive culture was identified by their colony characteristics, Gram-staining reaction and finally was confirmed by biochemical test (44).

5.8.2.6. Biochemical tests

Biochemical tests such as coagulase, catalase, bactericin and optochin susceptibility tests were used to identifay gram positive bacteria. Family gram negative bacteria were identified by indole production, H_2S production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization. (Annex I)

5.8.2.10. Drug susceptibility testing of bacterial isolates

Antimicrobial susceptibility testing was conducted for bacterial isolates using agar disk diffusion method on Mueller-Hinton agar. This test was performed by taking 3-5 colonies of bacteria from a pure culture, and transferred to tubes containing 5ml of nutrient broth and mixed gently. It was incubated at 37 ^{0}C until the turbidity of the suspension became adjusted to 0.5 McFarland standards. Then using a sterile cotton swab the standardized suspension was swabbed on to the Mueller-Hinton agar for non-fastidious organisms and Mueller-Hinton agar with 5% sheep blood will be used for fastidious organisms. The antimicrobial impregnated disks were placed with sterile forceps on the agar surface in such a way that each disk was placed at least 24 mm away from each other to avoid the overlapping zone of inhibition. After the disk is placed, the plate was allowed to stand for 30 minutes for the antibiotic to dissolve in the media. Then, the plates were inverted and incubated at 37for 24 hours and read for the diameter of zone of inhibition.

Grades of susceptibility pattern were recorded as sensitive, intermediate and resistant by comparison of zone of inhibition as indicated in the manufacturer's guide (44).

The following commonly prescribed antibiotics were used for drug susceptibility test. Antibiotics forgrampositivebacteriaincludes;
Ampicillin(AM)(10ug),Clindamycin(CD)(2ug),Erythromycin(EM)(15ug) Ciprofloxacin (CIP) (5 µg) , Trimethoprim-sulphamethoxazole (SXT) (1.25/23.75µg), Cefoxitin(FOX)(30ug), Penicillin (P)(10 units) and Vancomycin(VA)(30ug) were be used.

Antibiotics were used for gram negative bacteria consists of Ampicillin(AM)(10ug), AmoxicillinClavulanate(AUG)(20/10µg),Gentamicin(GN)(10µg)Torbamycin(TN)(10ug),Amikacin(AMK)(30ug),Trimethoprimsulphamethoxazole(SXT)(1.25/23.75µg),Ceftriaxone(CRO)(30µg),Cefotaxime(CTX)(30ug),Ceftazidime(CAZ)(30ug),Cefepeme(CPM)(30ug)Chloramphenicol(C)(30µg),Meropenema(MRP)(10ug),Piperacillin./Tazobactuma(P/T)(100/10ug)andCiprofloxacin (CIP) (5µg).

5.9. Data Quality Assurance

Appropriate specimen was collected from the appropriate sites with correct volume. All specimens were collected, handled, transported and stored according to the standard operating procedure to ensure the accuracy of data then verification of all specimen were done regarding with the information on the requisition form and container label and was processed immediately. All new batch stains (gram stain) was tested with appropriate organisms to ensure correct staining reactions and was used before expiry dated

The most frequently encountered equipments in microbiology laboratory include microscope, centrifuge, refrigerator, incubator, autoclave, water bath and heat block. The operating temperature refrigerators and incubators were monitored and charted daily. Equipment used was clean and calibrated correctly. Performance of the equipment was checked by pilot test, using standards and daily quality control. The manufacturer`s instruction strictly followed during preparation of culture media .the PH ,sterility and performance testing on prepared media was performed. Standard Operating Procedures (SOP) were strictly followed verifying that media meet expiration date and quality control parameters per CLSI. Visual inspections of cracks in media or plastic petridishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination was

performed. QC was performed to check the quality of medium. .Performance of all prepared media was also checked by inoculating international standard-strains such as E. coli (ATCC 25922), S. aureus (ATCC 25923) and P. aeruginosa (ATCC 27853). To standardize the inoculums density of bacterial suspension for the susceptibility test, 0.5 MacFarland standard was used. After collection, all data including laboratory results were checked for completeness by principal investigator each day.

Standardized report formats used in reporting gram stain, culture and antimicrobial susceptibility tests. Any preliminary report of gram stain results or isolation of a pathogen from primary culture was delivered to concerned body soon. Final reports were checked for correctness and clarity and signed by a person in charge before leaving the laboratory.

Socio-demographic characteristics of the patient were collected appropriately from request paper of patient. Samples were collected in accordance with SOPs and were going soon for analysis using transport media. Culture results were recorded carefully before entry to statistical tool. Before analysis the data was double checked.

5.10. Data analysis and interpretation*:*

The data was entered and double checked before analysis. Then the data was exported to SPSS version 23 for analysis. The descriptive statistics (mean, percentages, frequency or cross-tabulation) was calculated and were used to summarize the collected data. Comparisions were made using Chi-square test.

The prevalence of hospital acquired infection was determined by using descriptive statistics. The prevalence of HAI was calculated number of patients with HAI divided by the total number of study population with 95% CI and drug susceptibility test was measured as sensitive, intermediate and resistant based on criteria set by Clinical and Laboratory Standards Institute (CLSI, 2018)

5.11. Ethical considerations

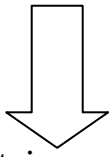
The study was approved by "Department Research and Ethical Review Committee(DRERC)" of the Department of Medical Laboratory Science, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University.. The purpose and procedures of the study was explained to the study participants within the study period. Those patients who were give informed consent was selected and enrolled as the participants of the study. All the information obtained from study participants were kept confidential and the specimens collected from the patients were used only for this study purpose. There was not any direct payment for participating in the study. But any positive finding in laboratory examination result was reported to their physician for appropriate treatment and management. All cases of nosocomial infections were treated based on antibiotic sensitivity results.A letter informing the medical director of the hospital about the objective of the study was written from the university prior to actual data collection period. Permission letter was also obtained from the study site.

5.12. Dissemination of the result

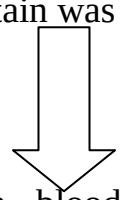
The results of the study were submitted to the Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, and Addis Ababa University. In addition, the results were submitted to the study site. Abstract was submitted (like EMA, EPHA and EMLA) and other international associations to present the results during continuous medical education events or conference organized by these associations.

6. Work flow

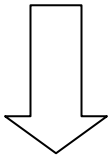
Clinical samples were collected and brought to the laboratory under aseptic precautions with clinical data



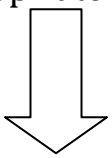
Gram stain was done



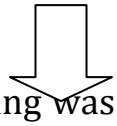
Culture on primary plate media, blood agar, Mac Conkey, Nutrient agar by standard procedures



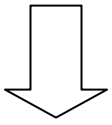
Incubate the plate for the appropriate time in the correct temperature and atmosphere



Identification of bacterial was done on the basis of standard recommended procedures (biochemical test)



Antimicrobial susceptibility testing was done on Muller Hinton agar plates as per Clinical and Laboratory Standard Institutes (CLSI)



Report results

Figure 2- Work flow

7. Result

7.1. Characteristics of Study Participants

In this study among 612 patients admitted to intensive care unit, 192 patients were selected based on their clinical ground, after a careful clinical examination to assess the bacterial etiology, antimicrobial susceptibility patterns and prevalence of Bacterial nosocomial infection of in different clinical sample from patients attending intensive care unit. On admission all patients were carefully examined clinically to exclude community-aquired infections as per WHO guidelines 2002. A total of 192 patients (88 from medical intensive care unit, 63 from surgical intensive care unit and 41 from pediatrics intensive care unit) were selected and included in this study as per WHO guidelines 2002. A total of 332 samples were collected from 192 suspected patients in ICU. Of 192 patients, 61.5 %(118) were male and 38.5 %(74) were female. Sex and Age distribution of patients admitted to the ICU is shown in Table 2. Majority, 29.2 %(56) of the patients were found in age group of 30-49 years. (Table 2).

Table 2. Age and sex distribution of study participants per medical units (n=192)

	SEX		
AGE GROUP	**MALE**	**FEMALE**	**TOTAL**
<5	**12(6.3%)**	**11(5.7%)**	**23(12.0%)**
5-14	**14(7.3%)**	**7(3.6%)**	**21(10.9%)**
15-29	**16(8.3%)**	**18(19.4%)**	**34(17.7%)**
30-49	**31(16.1%)**	**25(13.0%)**	**56(29.2%)**
50-59	**21(10.9%)**	**5(2.6%)**	**26(13.5%)**
60-69	**14(7.3%)**	**7(3.6%)**	**21(10.9%)**
>70	**10(5.2%)**	**1(0.5%)**	**11(5.7%)**
TOTAL	**118(61.5%)**	**74(38.5%)**	**192(100%)**

SOURCE OF AGE GROUP CLASSIFICATION: WHO 2018

7.2 Patterns of nosocomial infection

Eighty seven patients were confirmed by the laboratory to have nosocomial infection which is 14.2 %(87/612) the prevalence bacteria cause nosocomial infection. Of the 87 Patients, 70.1% (61) were males and 29.9% (26) were females. (Figure 2)

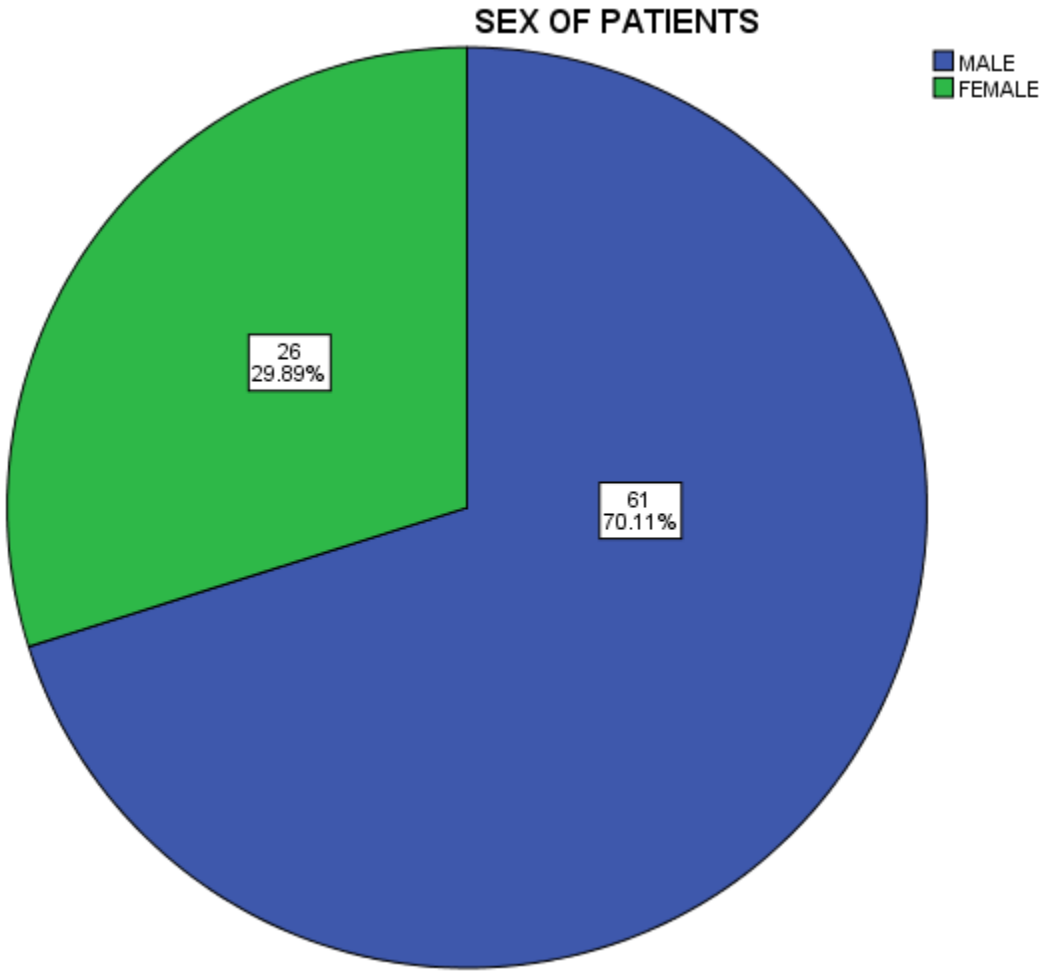


Figure 3: Distribution of nosocomial infections by sex.

7.3 Etiologic agents

A total of 109 bacteria were isolated from nosocomial infection after exclusion of repeated samples from the same patients collected from, 135 blood specimens, 82 urinary specimens, 38 respiratory specimens, 26 wound/pus, 26 cerebrospinal fluid (CSF) specimens and 25 other body fluid. Out of 332 samples, 32.8% (109) were culture positive and 67.2% (223) were culture negative. The majority of culture positive patients had one organism (34.9%) and less than one third of culture positive patients had multiple organisms (10.4%). Of the 109 isolates, 82.6% (90/109) were Gram-negative bacilli and 17.4% (19/109) were Gram-positive cocci.

The most common organisms isolated are shown in Table 3. The most frequent isolated gram negative bacteria were: *K. pneumonia 26.6%(29/109), Acinitobacter spps17.4%(19/109), E. coli 16.5%(18/109), Psedomonas Spps 8.3%(9/109),and K.Oxytoca 4.6%(5/109)* . Other less frequent gram-negative bacteria included *Serratia spps 2.8%(3/109), P.aeroginosa 1.8% (2/109),Enterobacter cloacae1.8%(2/109) , K.oxanae 0.9%(1/109),A. baumannii 0.9%(1/109*) , and *P.miribalis 0.9%(1*/109).

Gram-positive isolates were less frequent as compared to gram-negative isolates and included *coagulase negative Staphylococcus (CoNs) 9.2% (10/109) methicillin-sensitive Staphylococcus aureus (MSSA) 2.8%(3/109), methicillin-resistant Staphylococcus aureus (MRSA) 1.8%(2/109), Enterococcus 1.8%(2/109), and Streptococcus spp 1.8%(2/109). Vancomycin-resistant Enterococcus spps* were not detected during the study period.

Table 3:.**Distribution of number of organisms per patients, frequent isolates and number of specimens per patients** (n=192)

	Microorganisms	Number	Proportion (%)
Number of organisms per patient(N=192)	Patients with one organism	67	34.9%
	Patients with two organisms	18	9.4%
	Patients with three organisms	1	0 .52%
	Patients with four organisms	1	0.52%
	Patients with no organisms	105	54.7%
Isolates(N=109)	*K.Pneumoniae*	29	26.6%
	Acinitobacter spps	19	17.4%
	E.Coli	18	16.5%
	CONS	10	9.2%
	Psedomonas spps	9	8.3%
	K.Oxytoca	5	4.6%
	Serratia Spps	3	2.8
	MSSA	3	2.8
	MRSA	2	1.8
	E.clocae	2	1.8
	Viridians streptococcus	2	1.8
	Enterococcus Spps	2	1.8
	P .aeroginsa	2	1.8
	A.baumannii	1	0.9
	P.mirbalis	1	0.9

	K.Oxaneae	1	0.9
Number of specimens per patient	Patients with one specimen	99	51.6%
	Patients with two specimens	53	27.6%
	Patients with three or more specimens	40	20.8%

Percentage of positive specimens are shown in Figure 3 .The rates of positivity in specimens were blood (38.5%), respiratory (20.2 %), urinary tract (17.4 %), Wound/pus (11.0 %), CSF (6.4%) and others (6.4%). The most common isolate from blood culture specimens were *K. Pneumoniae, Acinitobacter spps and CoNS* form 22.9% of the blood isolates, while the most common isolates from respiratory specimens *K.Pneumoniae (9.2%) The Acinitobacter spps, MSSA and K.pneumoniae* were the most common isolate from wound and pus specimens (6.4%), while *E.coli* was the most commonly isolated organisms from the urinary tract specimens (7.3 %). The *Klebissela spps (K .Pneumoniae,K.oxytoca,and K.ozanae*) were the most common isolates of CSF specimens(3.6%) while *E.coli and K.pneumoniae* were form 3.6 % of the other body fluid specimen. The majority of patients (51.6%) had one anatomical infection site. 27.6% and 20.8% of patients had two site and three infection sites respectively (see Table 3).

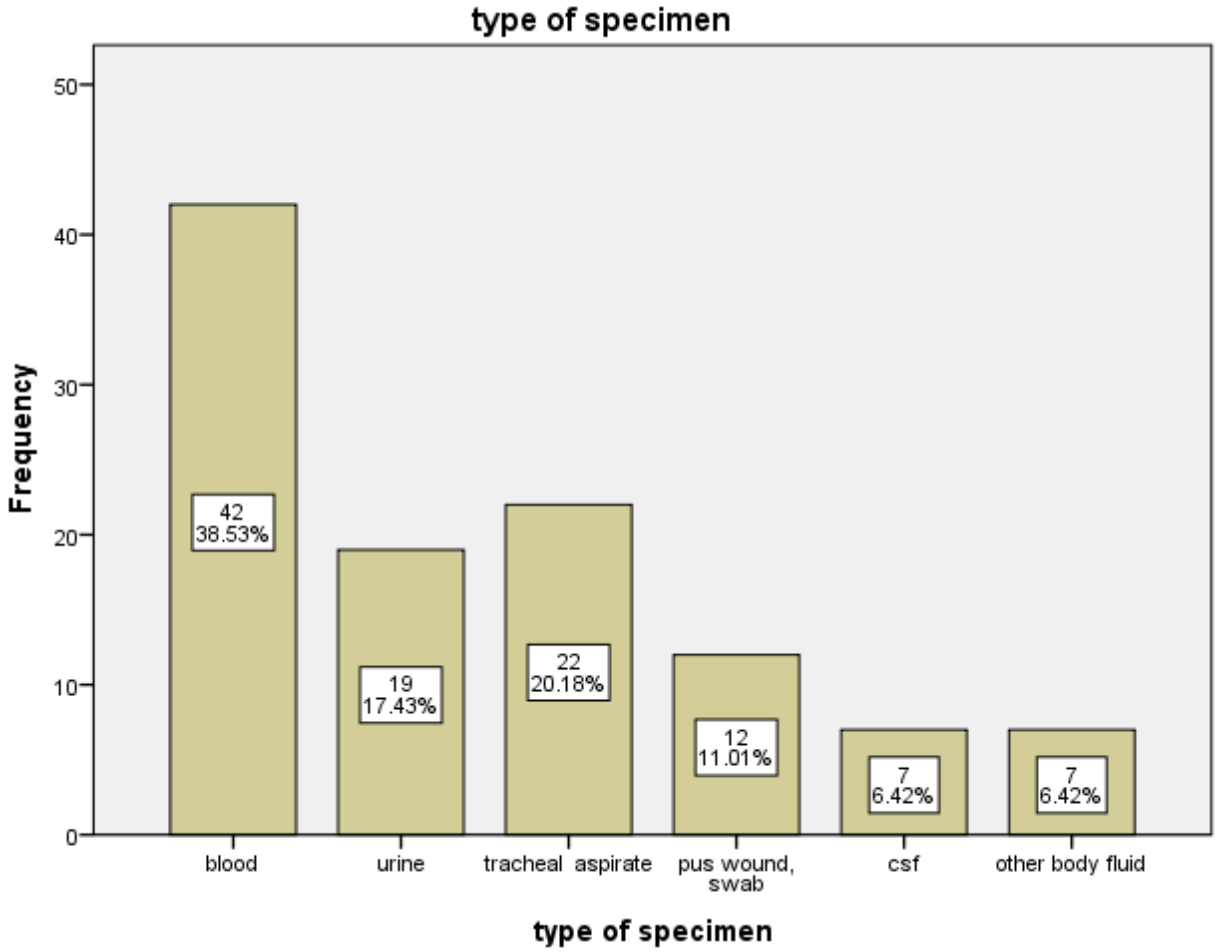


Figure 4: percentage of positive specimen

The distribution of nosocomial infection among sites of infection showed that 38.5% (41) were blood stream infections, 20.2%(22) respiratory infections ,17.4%(19) urinary tract infections, 11% (12) surgical site infection and 12.8%(14) other sites.(see Table 4).

Table 4:Sites of nosocomial infection and bacterial isolates from patients, who admitted in intensive care unit in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia from January 2018 to December 2018, (n=192)

Site of nosocomial infections	Organisms	Frequency	Percent
BSI(41)	*K.pneumoniae*	*9*	*22.0%*
	Acinitobacter spps	*8*	*19.0%*
	CoNS	*8*	*19.0%*
	E.coli	*5*	*12.2%*
	K.oxytoca	*2*	*4.9%*
	Serratia spps	*2*	*4.9%*
	MRSA	*2*	*4.9%*
	Viridians streptococcu	*2*	*4.9%*
	Enterococcus spps	*2*	*4.9%*
	Psedomonas spps	*1*	*2.4%*
	MSSA	*1*	*2.4%*
RTI(22)	*K.pneumoniae*	*9*	*40.9%*
	Psedomonas spps	*6*	*27.3%*
	Acinitobacter spps	*4*	*18.1%*
	E.coli	*1*	*4.5%*
	K.oxytoca	*1*	*4.5%*
	P.aeroginosa	*1*	*4.5%*
UTI(19)	*E.coli*	*9*	*47.4%*
	K.pneumoniae	*3*	*15.5%*
	Acinitobacter spps	*2*	*10.8%*
	A.baumannii	*1*	*5.3%*
	P.miribalis	*1*	*5.3%*
	E.clocae	*1*	*5.3%*
	K.oxytoca	*1*	*5.3%*
	Psedomonas spps	*1*	*5.3%*
SSI(12)	*K.pneumoniae*	*3*	*25.0%*
	Acinitobacter spps	*3*	*25.0%*
	MSSA	*2*	*16.7%*
	Serratia spps	*1*	*8.3%*
	CoNS	*1*	*8.3%*

	E.coli *P.aeroginosa*	*1* *1*	*8.3%* *8.3%*	
OTHERS(14)	*K.pneumoniae* *E.coli* *Acinitobacter spps* *Psedomonas spps* *E.clocae* *K.oxytoca* *K.oxanae* *CoNS*	*5* *2* *2* *1* *1* *1* *1* *1*	*35.7%* *14.3%* *14.3%* *7.1%* *7.1%* *7.1%* *7.1%* *7.1%*	

BSI-Bloodstream infection,UTI-Urinary tract infection ,RTI-Respiratory tract infection,SSI-Surgical site infection,MSSA-Methicillin sensitive staphylococcus aureus,MRSA-Methicillin resistant staphylococcus aureus,CoNS-Coagulase negative staphylococcus

7.4. Antimicrobial Susceptibility pattern of bacterial isolates

7.4.1. Gram positive

Most of the isolates were found to be resistant to Trimethoprim-sulphamethoxazole (64.7%), Ampicillin (63.2.0%), Ciprofloxacin (63.2.0%), Penicillin (53.3%), and Oxacillin (46.7%). On the other hand, most of the gram positive isolates were sensitive to Clindamycin (66.7%) and Erythromycin (66.7%). (See Table5). In gram-positive isolates, for MSSA Clindamycin (0% resistance), Erythromycin (0% resistance), Penicillin (0% resistance) and Trimethoprim-sulphamethoxazole (33.3% resistance) were the most effective antibiotics, whereas, for MRSA, Cefoxitin, Trimethoprim-sulphamethoxazole Erythromycin, Penicillin showed 100 % resistance and Clindamycin showed 50% resistance. For *Enterococcus Spps* Ampicillin(50% resistance) Trimethoprim-sulphamethoxazole(50% resistance) and Ciprofloxacin(50.0%) showed 50% resistance whereas no vancomycin resistance Enterococcus spps were detected.(see Table 5)

Table -5 Resistance patterns of gram positive bacteria (n=192)

DRUGS	AM	TS	CIP	CD	E	FOX	P	VA
ORGANISMS	R(%)	R(%)	R(%)	R(%)	R(%)	R(%)	R(%)	R(%)
MSSA	1(33.3)	1(33.3)	1(33.3)	0(0)	0(0)	0(0)	0(0)	-----
MRSA	2(100)	2(100)	2(100)	1(50)	2(100)	2(100)	2(100)	-----
CoNs	7(70)	7(70)	7(70)	4(40)	3(30)	5(50)	6(60)	-----
Streptoccous spps	1(50)	------	1(50)	------	-----	-----	-----	-----
Enteroccous spps	1(50)	1(50)	1(50)	------	-----	-----	-----	0(0)
TOTAL	12(63.2)	11(64.7)	12(63.2)	5(33.3)	5(33.3)	7(46.7)	8(53.3)	0(0)

R-Resistant,AM-Ampicillin,TS-Trimetoprime-Sulfamethoxazole,CIP-Ciprofloxacin,CD-Clindamycin, E-Erythromycin,FOX-Cefoxitin,P-Pencillin,VA-Vancomycin

7.4.2. Gram negative

In this study Gram negative isolates, 14 commonly prescribed drugs were used to test antimicrobial susceptibility pattern of 90 Gram negative bacteria isolatets from nosocomial infection. These isolates were highly resistant to Ampicillin (96.6%), Trimethoprim-sulphamethoxazole (88.3%), Augumentin (79.7%), Cefotaxime (78%), Ceftazidime (75.6%) and Ceftraxone (76.3%) These bacterial isolates were resistant to Ciprofloxacin (68.9%), Cefepime (67.8%), Torbamycin (63.3%), Chloramphenicol (61%) and Gentamicin (60%). On the other hand, the isolates were sensitive to Amikacin (62.2%) and Meropenem (61.1%) (See Table 6). Among gram-negative isolates, K.pneumoniae was resistant to Trimethoprim-sulphamethoxazole 86.4%(25/29),cefotaxime82.8%(24/29),ceftraxone79.3%(23/29),Augumentine79.3%(23/29),Ciprofloxacin75.9%(22/29),Ceftazidime 73.1%(19/29) and for *K.pneumoniae,* Amikacin was the most effective antibiotic (31% resistance), followed by Meropenem (37.1% resistance) and Piperacillin-tazobactum (51.7% resistance).E.coli was resistant to Ampicillin 94.4%(17/18) Trimethoprim-sulphamethoxazole 94.4%(17/18),Cefotaxime 88.9%(16/18),Ceftraxone 88.9%(16/18),Ceftazidime88.2%(16/17),Augumentine83.5%(15/18),Cefepime77.8%(14/18),Ciprofloxacin77.8%(14/18) and for *E.coli*, Meropenem (11.1% resistance), amikacin (27.8%resistance), and Gentamicin (33.3% resistance) showed low resisistance. *Acinitobacter Spps* showed highly resistance for most drugs include Ceftazidime(84.2%),Gentamycine(84.2%resistance),Ciprofloxacin(78.9%resistance),Torbamycin(78.9% resistance),Cefepeme(73.7% resistance) and Meropenem(68.4% resistance) and Amikacin(57.9%) where as *Acinitobacter spps* were showed relatively low reistance to Piperacillin- Tazobactum(47.4 %resistance).on the other hand acinitobacter spps were one of MDRos in study period. For *Pseudomonas Spps* , low resistance was seen for Amikacin(33.3% resistance), Meropenem(33.3% resistance) and Piperacillin –tazobactum(33.3% resistance) and Ciprofloxacin(33.3% resistance),where as *Psedomonas Spps* showed resistance ceftazidime (66.7% resistance),Gentamicin(66.7%), and Cefepem(44.4%).

Table-6 Resistance patterns of gram negative bacteria (n= 192)

DRUGS	AM	AMK	AUG	CRO	CXT	CAZ	CPE	PTZ	TS
ORGANIS	R(%)	R(%)	R(%)	R(%)	R(%)T	R(%)	R(%)	R(%)	R(%)
E.coli	17(94)	5(27.8)	15(83.3	16(88.9	16(88.9	15(88.2)	14(77.8)	9(50)	17(94.4)
K.pneumoniae	29(100	9(31)	23(79.3	23(79.3	24(82.8	19(73.1)	21(72.4)	15(51.7)	25(86.2)
K.oxytoca	5(100)	1(20)	3(60)	2(40)	2(40)	2(40)	2(40)	2(40)	5(100)
K.oxane	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
E.clocae	2(100)	0(0)	2(100)	1(50)	1(50)	1(50)	1(50)	1(50)	2(100)
P.miribalis	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)
Serratia spps	2(66,7	1(33.3)	2(66.7)	2(66.7)	2(66.7)	2(66.7)	1(33.3)	2(66.7)	2(66.7)
Psedomonas spps	------	3(33,3)	-------	------	-------	6(66.7)	4(44.4)	3(33.3)	-------
Acinitobacter spps	------	11(57.9	-------	------	-------	16(84.2)	14(73.7)	9(47.4)	1(100)
A.baumannii	------	1(100)	------	------	-------	1(100)	1(100)	1(100)	------
P.aeroginosa	------	0(0)	------	------	------	2(100)	2(100)	------	-------
TOTAL	57(96.6	32(35.6	47(79.7	45(76.3	46(78)	65(75.6)	61(67.8)	42(47.7)	53(88.3)

DRUGS	GM	TN	MEP	CHL	CIP
ORGANISIMS	R(%)M	R(%)	R(%)	R(%)	R(%)
E.coli	6(33.3)	11(61.1)	2(11.1)	10(55.6)	14(77.8)
K.pneumoniae	18(62.1)	19(5.5)	11(37.9)	20(69)	22(75.9)
K.oxytoca	1(20)	2(40)	0(0)	2(40)	2(40)
K.oxane	0(0)	0(0)	0(0)	0(0)	0(0)
E.clocae	2(100)	1(50)	0(0)	1(50)	2(100)
P.miribalis	1(100)	1(100)	0(0)	1(100)	1(100)
Serratia spps	3(100)	3(100)	1(33.3)	2(66.7)	2(66.7)
Psedomonas spps	6(66.7)	4(44.4)	3(33.3)	------	3(33.3)
Acinitobacter spps	16(84.2)	15(78)	13(68.4)	------	15(78.9)
A.baumannii	1(100)	1(100)	1(100)	------	1(100)
P.aeroginosa	0(0)	0(0)	2(100)	------	0(0)
TOTAL	54(60)	57(63.3)	33(36.7)	36(61)	62(68.9)

R-Resistant, AM-Ampicillin,AMK-Amikacin,AUG-Augumentine,CRO-Ceftraxone,CXT-Cefeotaxime,CAZ-Ceftazidime,CPE-Cefepime,PTZ-Piperacillin-Tazobactum,TS-Trimethoprim-Sulfamethoxazole,GM-Gentamycin,TN-Torbamycin,MEP-Meropenem,CHL-Chloramphenicol,CIP-Ciprofloxacin

Finally, both gram positive and gram negative isolates were found to be multi drug resistant. The overall multidrug resistance level (MDR ≥3 different classes of antibiotics) of all bacterial isolates was 78/109(71.6%). But gram negative isolates were found to be more resistant compared to gram positive isolates 68/90(75.6%) gram negative bacteria isolates and10/19(52.6%) gram positive bacteria isolates were MDRos and out of 30 ESBLs producer enterobacteroceaes 29 were MDRs. The most common MDRs gram negative bacterial isolates were *K. pneumoniae (36.8%), E.coli (22.1%), and Acinitobacter Spps* (19.1%).

Table-7 **Distributions of MDRos among gram negative and gram positive bacteria isolates (n=192)**

	Microorganisms	*Number*	*Percents*
GN MDRos(N=68)	*K.pneumoniae*	*25*	*36.8%*
	E.coli	*15*	*22.1%*
	Acinitobacter spps	*13*	*19.1%*
	Psedomonas spps	*4*	*5.9%*
	Serratia spps	*3*	*4.4%*
	K.oxytoca	*2*	*2.9%*
	E.clocae	*2*	*2.9%*
	P.aeroginosa	*2*	*2.9%*
	P.miribalis	*1*	*1.5%*
	A.baumannii	*1*	*1.5%*
GP MDRos(N=10)	*CoNS*	*7*	*70.0%*
	MRSA	*2*	*20.0%*
	Enterococcus spps	*1*	*10.0%*

GN MDRos –Gram negative multi-drug resistant organisms, GP –MDRos- Gram positive multi-drug resistant organisms, CoNS-Coagulase negative staphylococcus, MRSA-Methicillin resistant staphylococcus aureus

Frequency of ESBL producer isolates is shown in Table 8.In this study, ESBLs positivity was found to be 33.3 %(30/90). The rates ESBL producer isolates were high in both blood and urine specimens each accounted for 33.3% while both respiratory and wound site specimens were 10%.Others specimens and CSF accounted for least each was 6.7%.The rates of the ESBLs of *K. pneumonia*, *E. coli* and other enterobacteraceae were high observed (31.9%, 21.3% and 10.6%,

respectively). The isolated bacteria showed a very high rate of resistance to the third generation cephalosporins namely ceftriaxone, cefotaxime and ceftazidime.

Table 8: **Frequency of ESBLs and non ESBLs producer gram negative bacteria**

Organisms	Frequency	Percents
ESBL *K.pneumoniea*	15	31.9%
ESBL *E.Coli*	10	21.3%
ESBL Others Enterobacterocea	5	10.6%
Negative ESBLs	17	36.2%
Total	47	100%

ESBL =Extended spectrum beta lactamase

8. DISCUSSION

Nosocomial infection is one of the major health problems in developing and developed countries that cause death, disability and economic impact by prolonged hospital stay and also leads to drug resistance by frequent drug administration during hospitalization [90]. Despite the scarcity of data, Health care associated infection (HCAI) in sub Saharan Africa appears high and less attention was given by Sub Saharan Africa countries. Studies on these public health problems are scanty. [91]

In this study overall prevalence of bacterial nosocomial infection was 14%. This is similar with another study conducted in Addis Ababa (13%) [74], studies in Egypt (15%) [57] and in India (12%). [89] In contrary, this prevalence finding is lower than a research conducted in Nepal 34.4 %. [34] and India (29.3%) [8]. However, Higher than another study in Bahirdar city of Ethiopia, (10.9%) [45] and higher than the previous studies in the same hospital (9%) by Negatu E .et.al [48]. The high prevalence of Nosocomial infections in our study areas might be due to poor infection prevention and inadequate service delivery in ICU of the hospital.On the other hand the present study focused on ICU ward unlike other previous studies in the same hospital which focused only surgical ward.

In our study we have compared the positivity of nosocomial infection in different sites. Among of nosocomial infection, a large number 38.5% (42) of positive patients for bacterial nosocomial infection were found in blood stream infections (BSIs). This is more than respiratory infections (RTI) 20.2% (22), urinary tract infection (UTI) 17.4% (19) and surgical site infections (SSI) 11% (12). This result is similar to a study in Fiji [79], in Uganda [80], in Iran [81] and in Yauonda; Camerron [82].This result is different to a study in Ethiopia Bahrdare [45] and Adiss Ababa [74]. In the above mentioned two studies were the surgical site infection was found to be the main site of bacterial nosocomial infection. In Ethiopia, studies showed that the BSI prevalence was 2.1% in Surgery patients, the lower prevalence in may be due to the focus only in specific patients [45] and previous studies were focused on only surgical site infections on surgery and obstetrics wards. The reasons for high number of positive patients in blood stream infection this might be critical patients use intra vascular catheter in order to obtain fluids, nutrients and blood transfusion and easily acquired during the medication due to immunity loss. Because of the presence of an intravenous catheter is an important risk for developing nosocomial BSI. The

percent of nosocomial BSI among all nosocomial infections has increased and there have been changes in antimicrobial resistance [84-86]. In this BSIs (n=41), the majority of the isolates were *K.pnumoniae 22.0% (9), Acinitobacter Spps 19.0% (8), CONS 19.0% (8), E.coli 12.2% (5) and K. oxytoca, Serratia spps, MRSA, viridians, streptococcus, Enterococcus Spps* each accounted for 4.9% (2) and *Pseudomonas Spps and MSSA* each 2.4% (1). This finding was similar with another study in Fiji[79], in India([87], in Italy[88] and Yauonda,Camerron [82].In this study, gram negative bacteria were most involved in nosocomial BSI than gram positive bacteria, but in several studies, CoNS followed by *S.aures* comprised the most prevalent bacteria isolated from BSIs[59.61].

In this study the second site of nosocomial infection is respiratory tract infections (RTI) 20.2% (22). The most commonly observed HAI was respiratory infections in Mexico (41.2%) [55], Egypt (59.9%), [57] and Iran (51.7 %%) [58] Whereas in this study, among all the HAIs, respiratory infections occupied the second position (20.2%). The highest proportion of this discrepancy was observed in the above studies of respiratory infections in ICU may be due to high complex mixed and crowded patient population, hospital ICU type and settings. Among respiratory tract infections (RTI) (n=22) isolates, *K.pnumoniae 40.9% (9), Pseudomonas Spps 27.3% (6), Acinitobacter spps 18.1% (4) and Escherichia coli, K.oxytoca, P.aeroginosa* each 4.5% (1).In this study gram negative isolates comprised the predominant of nosocomial respiratory infections causing bacteria. This finding similar to other studies conducted in Pakistan [16], in Brazil [36], in Egypt [57] and in Saudi Arabia [68]

The third site of infection is Urinary tract infections (UTI) 17.4% (19). Based on this study, UTI was more common in females at 54.5 percent than in males at 31.8 percent. In urinary tract infection females are more infected than males due to the shorter and wider urethera in females as confirmed in other studies by Fantahun Biadglegne & Bayeh Abera et al (2009) [43] in Bhirdar,Ethiopia. Among urinary tract infections (UTI) (n=19) isolates, *E.coli 47.4% (9), Klebsiella pneumonia 15.8% (3), Acinitobacter Spps 10.5% (2) and A.baumannii, P.Mirabils, E.clocae, K .oxytoca, P.aeroginosa* each 5.3% (1). UTIs are the most frequent infections worldwide among hospitalized patients, and Enterobacteriaceae (mainly *E.coli*) are generally the causal agents. In the current study, the main pathogens involved in UTI were *E. coli* (47.4%). Many researchers had declared that E.coli was the most commonly encountered isolate from urine samples.[40,42] It is to be expected that *E. coli* is the common colonizing or infecting agent of the UT. This agrees with research conducted by Mulu Wondimagegn et al. (2012) [45] in Ethiopia, who reported that E.coli was the most common isolated organism from UTI.

Surgical site infections (SSI) was the least frequent occurs in ICU of this study11% (12). In contrast to this study high proportion of surgical site infections was observed studies conducted in Bahirdar [45] and Adiss Ababa [74]. Ethiopia (49.4%). This may be due to the reason that these studies were conducted on ward-specific infections particularly the surgical procedure underwent in most of the patient in surgical ward. Early transfer of patients from surgical ICU to other wards and a better modern wound care management after surgery made much lower

proportion of SSI in the study subjects in this study. In this SSI (n=12), the majority of the isolates were *K.pnumoniae 25% (3), Acinitobacter Spps 25% (3), MSSA 16.7% (2) and Serratia spps, CONS, E.coli, P.aeroginosa 8.3%* (1).

In terms of the overall number of bacterial isolates, *Klebsiella pneumonia* 26.6%(29/109) were the predominant bacteria followed by *Acinitobacter spps*17.4%(19/109*), E. coli* 16.5%(18/109) and *coagulase negative Staphylococcus* (CoNs) 9.2% (10/109) .This finding was consistent with studies result in Egypt [57] ,Eastern Ethiopia[73]and India[75] . However it showed disagreement with a studies done in India[24] and Iran[35] where the most common isolated bacteria were *E. coli* followed by *K.pneumonia*.Moreover, Gram negative bacteria were the dominant isolates accounting for 82.6%(90) isolates than Gram positive 17.4%(19). This is also supported by other studies in Addis Ababa, Ethiopia [48, 74], in Egypt [57] and in Tanzania [3], in Rawanpindi, Pakistan [16]. This is might be gram negative bacteria including entrobacteriaceae and non fermentive gram negatve bacilli are significant pathogenic for critical patients and might be due to gram negative bacteria resistance to third generation of cephalosporin and carbapenems has increased while methicillin resistance in S .aureus has decreased. The research conducted in Hungary reported that MRSA was the most frequently reported pathogens but while its incidence seemed stabilize after 2007, notifications of MDR gram negative organisms have significantly increased from 2005-2010 and MDR *K.pneumoniae* and MDR *E.coli* accounted for approximately 70% reports [83].This is most likely due to the increased occurrence, spread and microbiological diagnosis of gram negative organisms

The most common Gram-positive bacteria in this study were *Cogaulase negative Staphylococcus* (9.2%) and followed by *S.aures* (4.6%) together both formed approximately 12.8 % of the total isolates. Similar studies, *coagulase negative sthaphylococcus* followed by *S. aureus* comprised the most prevalent bacteria isolated from blood [92, 93]. This results different with other studies conducted in Iran[23] in Moroco [29], in Nepal[34] ,in Brazil[36]and in India[38] that *S.aures* followed by *coagulase negative staphylococcus* comprised the most prevalent bacteria among gram positive total isolates from ICU. In this study among the 5 *S. aureus* isolates, two isolates (1.8%) were methicillin-resistant strains (MRSAs). This low rate of MRSA is unusual and is probably due to low proportion of samples from wounds and surgical sites as these are the sights commonly infected by MRSA [94]. In this study, resistance rate for MRSA isolates were high of

Cefoxitin, Trimethoprim-sulphamethoxazole, Erythromycin, Penicillin showed 100 % resistance and showed moderate Clindamycin resistance (50%) This is in agreement with other studies conducted in India [38], Rawalpindi Pakistan [16] and Saudi [76] intensive care units. No VRE detected in our study of all *enterococci.*

This study results revealed that gram negative isolates showed high level resistant to Ampicillin (96.6%), Trimethoprim-sulphamethoxazole (88.3%),Augumentin (79.7%), Cefotaxime (78%), Ceftraxone(76.3%). and Ceftazidime(75.6%), Similarity was found with other studies previously done in Pakistan[16],Dhaka[66] and Saudi[68]. These bacteria showed better susceptibility for Amikacin (62.2%) and Meropenem (61.1%). In gram-negative isolates, for *K.pneumoniae*, Amikacin was the most effective antibiotic (31% resistance), followed by Meropenem (37.1% resistance) and Piperacillin-tazobactum (51.7% resistance). For *E.coli*, Meropenem (11.1% resistance), amikacin (27.8%resistance), and Gentamicin (33.3% resistance) showed low resistance. These finding are consistent with those previously reported by other researchers [23, 24, 35, and 58]. *Acinitobacter Spps* showed highly resistance for most drugs include Ceftazidime(84.2%),Gentamycine(84.2%resistance),Ciprofloxacin(78.9%resistance),Torbamycin(78.9% resistance),Cefepeme(73.7% resistance) and Meropenem(68.4% resistance) and Amikacin(57.9%). Similar study conducted in Dhaka reported that *Acinetobacter* was remarkably resistant to most antibiotics including imipenem(66) .For *Pseudomonas Spps* , low resistance was seen for Amikacin(33.3% resistance), Meropenem(33.3% resistance) and Piperacillin –tazobactum(33.3% resistance) where as *Psedomonas Spps* showed resistance ceftazidime (66.7% resistance),Gentamicin(66.7%), and Cefepem(44.4%).This finding similar to study conducted from Iran[68]

It is well known that multidrug resistant (MDR) bacteria are becoming increasingly prevalent in ICU environment as a result of extensive use of antibiotics. The overall 71.6% (n=78/109) multidrug resistance level of our finding was similar from other studies done in Ethiopia which showed MDR level 70.4% [97] However our study result was higher than a study done in Uganda and study done previously at the same hospital, Tikur Anbessa Specialized Hospital, which showed 58.0% and 65.5% the over all MDR level of bacteria respectively [71, 99]. This MDR increment showed that antimicrobial resistance is changing over time which might suggest a very high resistance gene pool due to gross misuse and inappropriate usage of antibacterial

agents [6] together with incorrect administration of antimicrobial agents in empirical therapies and lack of appropriate infection control strategies [19]In Gram positive and Gram negative isolates showed 52.6% (n=10/19) and 75.6% (n=68/90) MDR level respectively which was similar with studies done in the same Hospital Addis Ababa, Ethiopia [99,100] and Gram negative bacteria were most resistant than gram positive. The most common MDR GN isolates were *K. pneumoniae (36.8%) ,E.coli(22.1%), and Acinitobacter Spps* (19.1%) which are resistance to at least three first line drugs Similar result was obtained in a studies in Rawanpindi, Pakistan [16], in Iran[58],in India[69],in East and west Ethiopia[72,73]. But it contradicts with another study in Addis Ababa Teaching Hospital [62] which states that gram positive bacteria were most resistant than gram negative. This is might be due to unique outer membrane of gram negative bacteria exclude the action of drugs and it is rich in molecule lipopolysacride often refers to endotoxin. All Gram-negative bacteria isolates showed high frequency of resistance to multiple antibiotics but maximum resistance was observed in *K. pneumonia* followed by *E.coli* and Acinitobacter spps. The present study indicates that E. coli is still the most common cause of urinary tract infection. This finding is consistent with the other studies from our country. [45, 48].

ESBL-producing microorganisms are an increasing problem in ICU worldwide. In this study ESBLs prevalence was found to be 33.3 %(30/90) and high rates of ESBL in *K. pneumonia* (31.9%) and *E. coli* (21.3%) were detected by double disk synergy diffusion test. This was in agreement with the results obtained in Egypt [77], in a study also in Saudi Arabia [68]. On other hand, other study in Nigeria [78] reported that *E. coli* are becoming more common than ESBL-producing *Klebsiella spp*. In European countries and in the USA, the rates of ESBL positivity in *E. coli and K. pneumonia* isolates were lower than this study [95, 96].

The increased incidence of multidrug resistance among ICU patients may be due to reasons, such as prior antibiotic use, long antibiotics exposure, and inadequate antibiotic therapy. Resistance to antibiotics poses a serious and growing problem, because such resistant bacteria are becoming more difficult to treat.

9. Conclusion and Recommendation

9.1 Conclusion

The overall prevalence of bacterial nosocomial infection was high. High proportions of infected patients were reported in blood stream infection. High percentages of gram negative isolates were identified. The most common bacterial isolates were *Kellbesella pneumonia, Acinitobacter spps and E. coli. E.coli* was the most frequently encountered isolate in urine .Multi drug resistance is relatively high. High rates of multidrug resistant *K. pneumonia* were prominent in addition to *E.coli and Acinitobacter Spps*. Meropenem and Amikacin were highly effective compared to the other drugs tested in this study. *Klebsiella pneumoniae* was the most prevalent ESBL producer and all ESBL producer were resistant to third generations cephalosporin.This study therefore, found out that high proportion of patients developed nosocomial infection and high MDR organisms particularly GN ESBL producer in the study hospitals. This might relate to hospitals' overall sanitation, cleanliness, and poor infection prevention control, inappropriate usage of antibiotics and empirical treatement and inappropriate use of medical equipments.

9.2. Recommendations

From this study the following recommendations are forwarded:

1. Due to the increasing antimicrobial resistance rate in hospitals, antimicrobial susceptibility testing should be routinely employed to ensure appropriate antibiotic prescription, in an attempt to decrease antimicrobial resistance among critically ill patients and Physicians should decrease empirical therapy.
2. Laboratory methods for detection of ESBL producing pathogens should be done routinely for early diagnosis of these organisms especially among critically ill patients
3. Based on this study possible treatment of bacterial nosocomial infections are Carbapeneme (meropenem), Amikacin and Piperacillin-Tazobactum

10. Limitation of the Study

- Due to lack of budget, time and laboratory facility, this study did not include other important pathogens responsible for nosocomial infections such as anaerobes, fungi and viruses.
- The final confirmation of ESBLs isolates by genotypic methods could not be carried out due to limited resources.
- Patients discharge before 48 hours and low patient flow were the challenges faced during data collection

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12. Annexes

Annex I- English version of participant information sheet, consent form and incent form

1.1. Participant information sheet:

Name of the organization Department of Medical Laboratory Science, School of Allied Health Sciences, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

My name is----------. I am working as data collector for the study being conducted in this Hospital by JEMAL MOHAMMED who is studying for his Masters Degree in Public health and Diagnostic Micro Biology at Addis Ababa University Department of Medical Laboratory Science, School of Allied Health Sciences, Collage of Health Sciences. I kindly request you to lend me your attention to explain you about the study and being selected as the study participant.

Title of the Research Project

Bacterial etiology, antimicrobial susceptibility patterns and the prevalence of nosocomial infection in different clinical sample from patients attending intensive care unit in Tikur Anbessa Hospital,Addis Ababa,Ethiopia

Name of Investigator: Jemal Mohammed First of all we would like to thank you in advance for your cooperation and consent in participation in this study. Please take as much time as you need to read or listen the information sheet. If you have any question regarding the study please ask freely.

Background information

Nosocomial infections are widespread health problems in the world including in developed and developing countries which are the most important aggravating agents of mortality, morbidity, length of hospital stay and cost in the world. Nosocomial infection (NIs) are frequent problem particularly in intensive care frequent problem particularly in intensive care units (ICU) because of various invasive therapeutic or diagnostic interventions those are frequently and for extended period used such as the use of wide spectrum antibiotics , mechanical ventilation, central venous catheterization, invasive pressure monitoring and urinary catheterization The burden of health care associated infection in developing countries is high prevalence of HCA infection is much higher than proportions reported from developed countries. In developing countries, the risk is two to twenty times higher and the proportion of infected patients frequently exceeds 25%. In

low and middle income countries, the burden of hospital acquired infection is unknown due to lack of reliable data. Though bacterial Nosocomial infection expected high, less attention has been given in Ethiopia. This problem is one of the health's rated problems in Ethiopia. Relatively, few data are available from Ethiopia to indicate present HAI status of situation Moreover; several bacterial nosocomial infections carry a substantial economic burden due to high antimicrobial use and increased length of hospitalization. This burden of resistance, however, is probably more due to the high rate Antimicrobial drug resistance is a major global problem affecting both developed and underdeveloped countries as well as problem of both in the community and health institutions. Along with the problem of nosocomial infection comes with the burden of "multidrug" antimicrobial resistance. The ongoing emergency of resistance in the community and hospital is considerable a major threat for public health,

Purpose of the Research Project

We are asking you to take part in this study because we are trying to learn more about **bacterial profiles of nosocomial infection** and the drug susceptible profile in children and adults suffering from nosocomial infections .More over The finding of this study study will have para amount importance in prevention and control of bacterial nosocomial infection and in planning hospital antibiotic policy at Tikur Anbesa Specialized Hospital Medical science College, Addis Ababa University, Addis Ababa, Ethiopia

Potential benefits to subjects and/or to the society

You will not have any financial incentives or other inducements as the compensation from participating in the study. However, results will be given to their physician for treatment and management or to get counseling. Most importantly, the result of the study will be beneficial to provide information or data for future and further nationwide study and to develop health programs for health policy makers. Hence, you are indirectly benefiting other patients and the society in this respect.

Risks and complications

You will not be at any physical or psychological risk but during collection of the sample you may feel some discomfort, this does not produce serious pain

Confidentiality

In order to keep the confidentiality of the participants the sample will be labeled with code instead of giving name. No personal information will be disclosed to third party or will not appear in any report from this study. You can choose whether to be a part of this study or not and you have a right to get a laboratory diagnosis result for free

Rights:

Participation for this study is fully voluntary .You have full right to either participate or not in this study and it will never affect your right of getting appropriate treatment. If you feel uncomfortable with data and sample collection process you have full right to withdraw the data and sample collection process at any time.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project. If you have any question you can contact and ask at any time you want. You always well come at the following address given bellow.

Principal investigator

NAME JEMAL MOHAMMED

Address Department of Medical Laboratory Sciences, Collage of health Sciences, Addis Ababa University, and Addis Ababa, Ethiopia

Mobile +251921498597

E-mail: jemoha165@yahoo.com

1.2 Informed consent

I have read /was read tome that the participant information sheet. I have clearly under stood the purpose of the research, the procedure, the risks and benefits, issues of confidentiality, the right of participating and the contact address for any queries. I have been given the opportunity to ask questions for things that may have been unclear. I was informed that I have the right to with

draw from the study at any time or not answer any question that I do not want. Therefore, I declare my voluntary consent to participate in this study with my signature as indicated below.

Name of participant ----------

Signature of participant: ----------Date-------/-------/----------

Signature of data collector: ----------Date------/-------/---------

1.4. Incent form

This page contains an agreement signature to participate in the study entitle**d "Bacterial etiology, antimicrobial susceptibility patterns and the prevalence of nosocomial infection in different clinical sample from patients attending intensive care unit in Tikur Anbessa Hospital,Addis Ababa,Ethiopia"**

."So please read the following points and sign your signature at the end in the space provided.

1I understand the objective of the study in **Bacterial etiology, antimicrobial susceptibility patterns and the prevalence of nosocomial infection in different clinical sample from patients attending intensive care unit in Tikur Anbessa Hospital,Addis Ababa,Ethiopia**

1 I know that the left over sample (blood, urine, swabs ,sputum etc) that my child gave is going to be used for this study only.

2. I understand that, all the information and the results are confidential.

3. I understand that my child will not get any money for my participation.

4. All the information is explained by reception phlebotomist and Principal investigator.

Therefore, with full understanding of the situations I agree for my child to give blood for laboratory analysis.

Signature of the participant's parent: _____

Address of the participant: _____

Date: _____

Annex II.የጥናቱ ተሳታፊዎች የመረጃ ቅፅ እና የፈቃደኝነት መጠየቂያ ቅፅ (ወደ ተሳታፊዎች ቋንቋ የሚተረጎም)

<u>የጥናቱ ርእስ፡</u> የዚህ ጥናት ዋነኛ አላማ ሕመማ ን ሆስፒታል ውስጥ ተኝተው በሚ ታከሙበት ወቅት ተኝተዉ ከሚታከሙበት በሽታ ሌላ ሌሎች ህመም ሊያሰከትሉ የሚ ችሉ ተህዋሲያንን ለይቶ ማውጣትና እንዲሁም መድሀኒት የመቋቋም ባህሪያቸዉን ለማ ወቅ ነዉ፡፡፡

የጥናቱ ዓላማ : የዚህ ጥናት የመጨረሻ ዉጤት የተለያዩ ጥቅሞች ሲኖሩት አንደኛው በተህዋሲያን የሚመጡ ህመሞች ለመከላከል; ለመቆጣጠር እና ለሆስፒታል መድሀኒት እቅድ አፈጻጸም የሚጠቅም ሲሆን ሌላው ደግሞ ለሁለተኛ ድግሪ መመረቅያ ጽሁፍ የሚ ዉል ይሆናል፡፡

ለጥናቱ የሚያስፈልግ ናሙና አወሳሰድ እና የሚወስደው ጊዜ፡- ናሙና የሚሰበሰበው በሀኪሙ ህመሙ አላቸው ተብለው ከታሰቡት ህሙማን ሲሆን በሰለጠኑ ነርሶች እና ላቦራቶሪ ባለሞያ ከደም ; ከሽንት ከቁስል እና አክታ ናሙና በሚገባ ስአት እና ከሚገባ ቦታ ይወሰዳል፡፡ ናሙናው ከተሰበሰበ በኋላ ለምርምር ወደ ጥቁር አንበሳ ሆሰፒታል ላቦራቶሪ ይላካል፡፡ ገለጻ ካደረኩልዎት በኋላ ለትንሽ ጥያቄ መልስ እየሰጡኝ መጠይቁ እሞላለሁ ከመጠይቁ ብዛላ ናሙና ይሰጣሉ፡፡ መጠይቅ እና ናሙና መሰብሰቡ የሚወስደው ጊዜ 25 ደቂቃ ሲሆን ስለዚ ይህንን 25ደቂቃ ለመጠይቁ እና ናሙና ለመሰብሰብ ከኔ ጋር እንዲያሳልፉ በትህትና እጠይቃለሁ፡፡

ከጥናቱ ጋር ተያይዞ ሊይደርስ የሚችለው አደጋ እና የሚያገኙት ጥቅም ፡- ይህንን ናሙና በመስጠትዎ የሚደርስ አካላዊ ሆነ አእምሮአዊ ጉዳት አያስከትልም፡፡ ነገር ግን ናሙና ሲሰጡ ትንሽ ህመም ሊሰማ ዎት ይችላል፡፡ይህ የህመ ም ስሜ ት ምንም አይነት የከፋ ችግር አያመጣብዎትም፡፡ በዚህ ጥናት በመሳተፍዎ የሚያገኙት ክፍያ የለም ነገርግን በሽታ አምጪ ህዋሳት በሊቦራቶሪ መኖራቸው ከተረጋገጠ በኋሊ ተገቢውን መዴሃኒት እንዱወስደ ውጤቱ ለሀኪምዎ ተልኮ መዴኒኑቱን በሀኪምዎ ትዕዛዝ ይሰጥዎታል፡፡

<u>የመረጃ ሚስጢራዊነት-</u> ሁሉም ከተሳታፊዎች የሚሰበሰቡ መረጃዎች በሚስጢር የሚያዙ እና የሚጠበቁ ይሆናሉ፡፡የሚሰበሰበው ናሙና ለዚህ ጥናት አላማ ብቻ የሚዉል ሲሆን ለናሙናው ልዩ መለያ ምልክት ይሰጠዋል እንጂ የእርስዎ ስም አይጻፍም ፡፡

የተሳታፊዉ መብት፡ በጥናቱ ለመሳተፍ ሙሉ በሙሉ በተሳታፊዎች ፍቃደኝነት የተመ ሰረተ ነው፡፡ በዚህ ጥናት ባለመሳተፍዎ አግባብነት ያለው ህክምና የማግኘት መብትዎ አይከለክልዎትም፡፡ እንደዚሁም ደግሞ ተሳታፊዎች በማንኛውም ግዜ እና ስአት ካልተመ ቸዎት ጥናቱን ማቋረጥ ይችላሉ፡

አድራሻ፡

በማንኛዉም ጊዜ መጠየቅ የሚፈልጉት ጥያቄ ካለ ቀጥሎ ባለው አድራሻ መጠየቅ ይችላሉ፡፡

የተመራማሪው አድራሻ :

ስም፡ ጀማል መሀመድ

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አድራሻ፡ የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል፤ የጤና ሳይንስ ኮሌጅ ፤ አዲስ አበባ ዩኒቨርሲቲ

ለጥናቱ ተሳታፊዎች የፈቃደኝነት መጠየቂያ ቅፅ

የጥናቱ ተሳታፊዎች የመረጃ ቅጽ ካነበብኩት በኋላ (ካነበቡልኝ በኋላ) የሚደረገዉን ጥናት አላማ; ጥቅም እና ጉዳት; ለጥናቱ የሚያስፈልግ ናሙና አወሳሰድ እና የሚወስደው ጊዜ; የመረጃ ሚስጢራዊነት; የተሳታፊዉ መብት በሚገባ ተረድቻለሁ፡፡ ጥናቱ ላይ መሳተፍም ሆነ አለመሳተፍ በራሴ ፍቃድ የሚወሰን መሆኑም ተገልጾልኛል፡፡ በጥናቱ ስሳተፍ ግልጽ ያልሆነልኝ ነገር ካለ ባድራሻው መሰረት መጠየቅ እንደምችል እድሉ ተሰጠኛል፡፡ በተጨማ ሪም ከጥናቱ ባልሳተፍ ሆነ አቋርጬ ብወጣ ከጤና ተቋሙ በማገኘዉ የህክምና አገልግሎት ምንም አይነት ችግር እንደማይደርስብኝ ተነግሮኛል፡፡ የሚደረገዉን ጥናት አስፈላጊ መ ሆኑን ስለተስማማሁበት በጥናቱ ለመሳተፍ ሙሉ ፈቃደኛ መሆኔን በፊርማዬ እገልጻለሁ፡፡

የተሳታፊ ስምና ፊርማ _____ ቀን _____

የተመ ራማ ሪስም ና ፊርማ _____ ቀን ___

የልጆች ስምምነት መጠየቅ ያቅጽ

የጥናቱ ተሳታፊ መለያ ቁጥር፡_____

በአዲስ አበባ ዩኒቨርሲቲ፤ የጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ትምህርት ክፍል በሁለተኛ ዲግሪ ተማሪ የመመረቂያ ጥናት ላይ ልጆትን እንዲያሳትፉ ተጋብዘዋል፡፡

እባክዎ በዚህ ጥናት ላይ ከመሳተፍዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡ / ይመልሱ ፤ ግልፅ ያልሆነ ነገር ካጋጠመዎት ይጠይቁ፡፡

የዚህ ጥናት ርእስ "በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል በሚገኙት""ሕሙማ ን ሆስፒታል ውስጥ ተኝተው በሚ ታከሙበት ወቅት ተኝተዉ ከሚታከሙበት በሽታ ሌላ ሌሎች ህመም ሊያሰክትሉ የሚ ችሉ ተህዋሲያንን ለይቶ ማውጣትና ተህዋሲያኑ ለመድሃኒት የሚሰጡት ምላሽ " ጥናቱ የሚካሄደው በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል ይሆናል። እባክዎትን ከዚህ በታች የተዘረዘሩ ነጥቦች በጥሞና ያንቡቡ እና በመጨረሻ በተሰጠው ክፍት ቦታ ይፈርሙ::

1. በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል በሚገኙትበ""ሕሙማ ን ሆስፒታል ውስጥ ተኝተው በሚ ታከሙበት ወቅት ተኝተዉ ከሚታከሙበት በሽታ ሌላ ሌሎች ህመም ሊያሰክትሉ የሚ ችሉ ተህዋሲያንን ለይቶ ማውጣትና ተህዋሲያኑ ለመድሃኒት የሚሰጡት ምላሽ ለማወቅ የሚካየሄደውን ጥናት ዓላማ ተረድቻለሁ።
2. ልጀ የሚሰጠው ናሙና ለዚህ ጥናት ብቻ እንደሚዉል አውቂያለሁ።
3. ለጥናቱ የሚሰጠው ናሙና እንዲሁም ውጤቱ በሚስጥር እንደሚያዝ ተረድቻለሁ።
4. ልጀ ጥናቱ በመሳተፉ የሚከፈለኝ ክፍያ እንደሌለ አውቂያለሁ።
5. ሁሉም የሚያስፈልገው ነገር በተመራመረሪው ይብራራልኛል።

ስለዚህ ከላይ የተጠቀሱትን ነጥቦች በመረዳት የልጀን ናሙና (ደም ቁስል አክታ ሽንት ወዘተ) ለመስጠት ተስማምቻለሁ።

የተሳታፊ ፌርማ፡_____

ቀን፡_____

Annex III. - Laboratory procedures (CLIS guidelines)

1. blood specimen collection and culturing

Blood specimen must be collected from appropriate vein when the patient temperature is beginning rise.

Procedure

1. Select the appropriate patient vein and use Tourniquet to get visible vein

2. Clean the vein using tincture of iodine and Alcohol (Ethanol ether) wait until dry.

3. using a sterile syringe (size 21-gauge needle,) take 5-10ml of blood.

4. Remove the needle from the syringe safely, and replace with another sterile needle of similar size.

5. Insert the needle through the rubber liver of the bottle cap and dispense 5ml -10ml of blood toeach culture bottle.

6. Using a lead pencil, label each bottle with the name and number of the patient, and the date and time of collection.

7. Incubate the inoculated media immediately

N.B Prevent the blood clot in the culture media because any bacteria will

Become trapped in the clot.

Trypto soya broth

Procedure

This media important for culturing of Blood Incubate at 35-37 ^{o}C for up to 2 weeks (to 14 days). Examine daily for visible colony (growth) such as turbidity hemolysis, gas production (bubble formation), and clot formation.

- Whether there is visible growth or not sub culture in to Blood agar (BAP), Chocolate agar (CAP) and MacConkey agar aseptically.
- Depending on the bacteria characteristic examine a gram stained smear further test examined such as coagulase, catalase, oxidase, urease and motility etc.

2. **Urine sample collection and culturing**

Mid stream urine is important urine specimen for microbiological tests (Clean catch urine specimen). The following procedure use to collect appropriate mid stream urine specimen:

- In ordered to avoid contamination clean the genital area with soap and water and rinse appropriately.
- The first small amount voids urine discarded.
- The urine that comes next, the mid-stream specimen, should be collected into a sterile container of 30 to 50ml.
- After the specimen collecting the patient continues to urinate and discarded.

If the collected urine contains bacteria, cells, protein, nitrite the urine sample must be culture in Blood agar (BAP) and MacConkey agar. Mix the urine appropriately take using a sterile calibrated wire loop, inoculate a loopful of urine into CLED (Cystine lysine electrolyte deficiency), Blood agar plate (BAP) and MacConkey agar incubate over night at 35-37 ^{o}C aerobically. The use of blood agar is to isolate fastidious organisms not grow in MacConkey agar. Bacteria Urine from a person with an untreated urinary infection usually contains 100,000(10^5) or more bacteria per ml less than this it is not clinically treatable may be due to contamination (44).

3. Wound swab collection and culturing from surgical site infection

Appropriate collection of wound swab from surgical in fiction collected safely in order to avoid contamination with commensal organisms from the skin. The specimen will be collected by an experienced nurse and special care will be taken (44).

Procedure

1. Disinfect the surface of the wound with 70% alcohol or an iodine solution.

2. With sterile cotton tipped applicator stick moistened with normal saline collect sample from the infected site.

3. Label the sample correctly with the patient code number (Identification Number).

4. Inoculate in to Blood agar plate, MacConkey agar and Manitol salt agar appropriately.

5. Incubate the plate aerobically at 35-37 ^{0}C for 18-24 hours.

6. Examine the culture look for colony characteristics and perform biochemical test.

7. Determine drug susceptibility pattern of the isolated organism.

4. **Gram staining technique**

Procedure

1. Labeling the slides correctly with the date and patient's name and number

2. Prepare the smear from the culture or specimen covering an area about 15-20mm diameter on a slide

3. Allow to air-dry and fix the smear by heat or alcohol.

4. Cover the fixed smear with crystal violet for 1 min.

5. Rinse with clean water and tip off all the water.

6. Cover the smear with Lugol's iodine for 1 min.

7. Wash off the iodine with clean water.

8. Add acetone-alcohol for 30 sec.

9. Wash the smear immediately with clean water.

10. Cover the smear with saffranin for 1-2 minutes.

11. Rinse with clean water.

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

13. Examine microscopically, first with the 40x objective and then with the oil immersion objective for white cells, bacteria and other.

Results Look like microscopically as follows:

Gram- positive bacteria ----------Dark purple

Gram- negative bacteria ----------Pale to dark red

Control: Always check new batches of stain and reagents for correct staining reactions using a smear containing known Gram positive and Gram negative organisms

5. **Biochemical testing**

Gram-positive cocci will be identified based on the test catalase and coagulase

Catalase test: This test will be used to differentiate *staphylococci* from *streptococci*

Procedure

- Add 2-3 ml of 3% hydrogen peroxide to a test tube.
- Using a sterile wooden stick take the test organism and immerse into the hydrogen peroxide solution and mix.
- Look for immediate bubbling.

Interpretation: Active bubbling positive test (*staphylococci)* and No resulting of bubbles-negative test (streptococci*).*

Coagulase test: This test is used to differentiate *Staphylococcus aureus* from other *Staphylococcus* species.

Procedure

- Place a drop of physiological saline on two separate slides
- Emulsify the test organism in each of the drop to make thick suspension.
- Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the organism within 10 seconds.

Interpretation

Clumping within 10 seconds ----------*S.aureus*

No clumping within 10 seconds ----------other staphylococcus species

Identification of Gram negative bacteria: will be based on their test result with a series of biochemical tests.

Procedure

1. Prepare a suspension of the test organism with nutrient broth 3-4 colony of test organism in 5 ml nutrient broth.

2. A loop full of the bacterial suspension is inoculated in to indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, manitol, urea agar and motility medium.

3. Incubate at 35-37 OC for 18-24 hours.

4. Look for color change (turbidity for motility) of the medium.

5. Identify the test organism by considering the result of the six biochemical tests

A. **Indole test:** Few colonies of the culture will be inoculated into peptone water and incubated at 37^{o}C for 24 hours. Few drops of indicator (Kovac's reagent) will be added and gently shake to mix well. Color change will be then observed. If the layer of indicator reagent turns to red within 1 minute, it is Indole positive (positive result). If the layer of indicator reagent remains yellow within 1 minute, it is indole negative (negative result).

B. **B. Urease test (Christensen's (modified) urea broth):** Urea agars will be inoculated heavily over the entire surfaces of the slants in bijou bottles. The cap will be loosened and then incubated at 37 ^{o}C for 3-12 hours. A urease-positive culture produces an alkaline reaction in the medium, evidenced by pinkish red color of the Medium. Urease-negative organisms do not change the color of the medium, which is pale yellow-pink.

C. **Triple Sugar Iron (TSI) Agar Slant:** Using a sterile inoculating needle, stab the butt of the LIA slant twice then streak back and forth along the surface of the agar with the organism. Incubate at 37^{o}c for 18 to 24 h. If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented. If alkaline slant–acid butt (red–yellow): glucose fermented only. If alkaline slant–alkaline butt (red–red): glucose not fermented. The

presence of black precipitate (butt) indicates hydrogen sulfide production, and presence of splits or cracks with air bubbles indicates gas production.

D. Citrate utilization test using Simmon's citrate agar: Simmon's citrate slopes will be prepared in bijou bottles as recommended by the manufacturer (stored at 2-8^{o}C). And the slopes will be then stabbed and incubated at 37^{o}C aerobically for 48 hours. Blue colour indicates a positive reaction and if Simmon's citrate agar slopes remained as green in colour indicate negative reaction.

E. Motility Test (using motility agars): Motility agar will be prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility will be examined after 35-37^{0}C for 24 hour. Motility will be indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation.

6. Antimicrobial susceptibility testing

Procedure

1. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth.

2. Much the turbidity of suspension with turbidity standard.

3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).

4. Spread the inoculums evenly over the Muller-Hinton agar plate with the swab.

5. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate.

6. Incubate the plate aerobically at 35-37$_{o}$C for 18-24 hours.

7. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the radius of the inhibition zone.

8. Interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate or resistance, using the standard.

9. Sensitive – zone of radius is wider or equal to the control.

Intermediate –zone of radius is more than three mm smaller than the control.

Resistance – no zone of inhibition

Isolated bacteria	Antibiotics		AM	AM	SXT	FOX	C	CTX	GE	TE	NO	FN	OX	IMI	ME
	Drug Susceptibility	S													
		I													
		R													

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7. Phenotypic Extended spectrumBeta lactamase detection (ESBL) by DDST

PROCEDURE

Detection of Extended Spectrum Beta Lactamases (ESBL) was done by the double disc synergy test (DDST) method according to CLSI guidelines as follow:

- Standardized inoculums (0.5 McFarland) of the test organisms were inoculated on Muellar Hinton Agar (MHA) using sterile swab stick.
- Amoxicillin/clavulanic acid disc (30ug,) was placed at the center of the inoculated MHA.Aztreonam(30ug), Ceftrixone(30ug),Ceftazidime (30ug,) Cefepime(30ug),Cefotaxime (30ug) and Cefpodoxime (10ug) were placed 15mm center to center from the Amoxycillin/clavulanic acid discs.
- The plates were incubated at 37oC for 24 hours.
- After incubation, enhancement of zone of inhibition of any one of the antibiotics the Aztreonam, Ceftriaxone, Ceftazidime ,Cefepime,Cefotaxime and Cefpodoxime discs towards the Amoxycillin/Clavulanic acid discs is indicative of ESBL production, often showing shape zone referred to as keyhole.The enhancement is due to inhibition of ESBL by clavulanic acid and subsequent action of the extended spectrum cephalosporins

8. Laboratory data collection form

1. Code no_____

2. Age _____Sex _____ Date_____

3. Types of specimen swab, urine, blood, tracheal aspirates

4. Media used

_____

5. Organism isolated

_____

Culture and biochemical tests identification

_____

_____

7. Gram stain from specimen_____

8. Result of Gram stain from culture_____

9. Antimicrobial test

Sensitive to _____

Intermediate to_____

Resistance to_____

Comments

Name of principal investigator _____

Signature _____ Date_____

Annex IV .Declaration

Declaration

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

M.Sc. candidate: Jemal Mohammed(B.Sc.)

Signature:_____

Date of submission:_____

This thesis has been submitted with our approval as advisors.

Advisor: Dr Adane Bitew (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Gebreab teklebrahan (Msc, PhD Fellow)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.