

**BACTERIAL NOSOCOMIAL INFECTIONS AND THEIR
ANTIMICROBIAL SUSCEPTIBILITY PATTERNS IN
SURGICAL WARDS AND SURGICAL INTENSIVE CARE
UNIT (SICU) OF TIKUR ANBESSA UNIVERSITY HOSPITAL
ADDIS ABABA, ETHIOPIA.**



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August 2008

Bacterial Nosocomial infections and their antimicrobial susceptibility patterns in surgical wards and surgical Intensive care unit (SICU) of Tikur Anbessa University Hospital Addis Ababa, Ethiopia.

**A Thesis presented to the school of graduate studies of Addis Ababa University
In Partial Fulfillment of the Requirements for the Degree of
Masters of Science in Medical Microbiology**

August 2008

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ACKNOWLEDGMENTS

I am very much grateful to the persistent mentor, encouragement, support and patience of my advisors, Dr. Solomon Gebre-Selassie, Department of Microbiology, Immunology and Parasitology, and Dr. Berhanu Kotisso, Department of Surgery, Faculty of Medicine, Addis Ababa University without whom this research wouldn't have been successfully conducted. I extend my gratitude for their unreserved, constructive and invaluable commentary from the beginning of the research project till its conclusion. Also I would like to extend my best regards to Dr Endale Anberbir who supported me by identifying the target patient with careful clinical examination.

I wish to thank the staff of Surgical wards and SICU for their support in identifying the target patient and sample collection.

I am grateful to nurses at surgical wards for their cooperation during sample collection.

Words of appreciation must be extended to the staff of Microbiology and service Laboratory for their unreserved support in facilitating good working environment.

I greatly acknowledge the financial assistance of Addis Ababa University for covering the research.

Last but not least my everlasting appreciation is to my family. I am particularly indebted to my parents who inculcated in me at a very early age the love of education, the respect for study and hard work. In this regard, it would have been impossible for me to accomplish any of my academic pursuits without the love, inspiration, and continued support of my wife, Sr. Seniat Tsegaye.

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ABBREVIATIONS

API	Analytical profile index
BA	Blood Agar
CA	Chocolate Agar
BSI	Blood Stream Infection
CDC	Center for Disease Control
CVC	Central venous catheters
EDTA	Ethylene Diamine Tetraacetic Acid
EHNRI	Ethiopian Health and nutrition Research Institute
ESBLs	Extended Spectrum B-Lactamase
ICU	Intensive Care Unit
KIA	Kligler Iron Agar
MA	MacConkey agar
MIU	Motility Indole Urea
MRSA	Methicillin Resistance <i>Staphylococcus aureus</i>
NCCL	National Committee for Clinical Laboratory Standard
NI	Nosocomial infection
NLRTI	Nosocomial lower respiratory tract infection
NNIS	National Nosocomial Infections Surveillance
PICU	Pediatric Intensive Care Unit
SICU	Surgical Intensive Care Unit
SSI	Surgical site infection
UTI	Urinary Tract Infection
VRE	Vancomycin-resistant enterococci
WHO	World Health Organization

Abstract

Background: Nosocomial infections are defined as infections which are not present or not incubating when the patient is hospitalized and are acquired during hospital stay. Sign and symptoms of the infection may be evident during hospitalization or after discharge related to the length of the incubation period. It is usually defined as an infection that is identified at least 48-72 hours following admission to health institution. Infections acquired in hospital are likely to complicate illness, cause anxiety and discomfort, and can lead to death. Nosocomial infection is a global problem with multi facet out comes. The problem is well pronounced in developing countries. Epidemiological and etiological characteristics of nosocomial infections show variations among countries and even among different hospitals in the same country. Many of these infections are associated with micro-organisms that are resistant to multiple antibiotics and can easily spread on the hands of personnel. The most frequent types of nosocomial infections are urinary-tract infection, surgical-wound infection, pneumonia, and bloodstream infection. At present, the emergence of resistance to antimicrobial agents is a global public health problem, particularly in pathogens causing nosocomial infections. Antimicrobial resistance results in increased morbidity, mortality and health-care costs.

Objective: To determine the prevalence, etiological agents and drug susceptibility pattern of nosocomial infections at Tikur Anbessa University Hospital.

Methods: A cross-sectional study was conducted from June 2007 to April 2008 at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. During the study, all adult patients admitted to surgical wards and SICU with suspected of nosocomial infection were included. Among 854 patients admitted to surgical wards and SICU, 215 patients selected based on their clinical ground, after a careful clinical examination. Clinical samples were collected from the study subject and analyzed accordingly.

Results: Eight hundred fifty four patients admitted to surgical ward and SICU between June 2007 and April 2008 to Tikur Anbessa University Hospital in Addis Ababa were studied for prevalence of nosocomial infections. A total of 215(25.2%) patients, were selected based on

their clinical grounds from surgical wards (n=161) and SICU (n=54). The mean hospital stay from the date of admission until sample collection was 16.72 days with a range of 3 to 66 days. Of the 215 patients, 130(60.5%) were males and 85 (39.5%) were females. A total of nine percent (77/854) patients were confirmed to have nosocomial infections. Of the 77 patients, 51(66.2%) were males and 26(33.8%) females. The distribution of nosocomial infections among positive cases was surgical site infection 38(49.4%), urinary tract infections 23(29.8%) and blood stream infection 16(20.8%). The Gram-positive and negative bacteria accounted for 23/84(23.4%) and 61/84(72.6%) respectively. A total of 84 bacterial pathogens (strains) were isolated, *E. coli* accounted for 19.0% of the total isolates followed by *S. aureus* and *Klebsiella* spp. More than one bacteria etiologic agent was isolated from 7/77(9.1%) of the patients with nosocomial infection. Gram positive bacteria showed 100% resistance to penicillin, ampicillin, tetracycline, chloramphenicol, and trimethoprim-sulphamethoxazole; while gram negative bacteria showed 100% resistance to amoxicillin, Tetracycline and Trimethoprim-sulphamethoxazole.

Conclusion: The prevalence of nosocomial infection at Tikur Anbessa University Hospitals decreased from the previous study (16.4% and 13%) in the same hospital. Gram-positive bacteria isolated from nosocomial infection were 100% resistance to Ampicillin, Tetracycline, Trimethoprim-sulphamethoxazole and Chloramphenicol. In addition, gram-negative bacterial isolates were 100% resistance to Amoxicillin, Tetracycline and Trimethoprim-sulphamethoxazole. Due to the presence of high level drug resistance bacteria, empirical treatment to nosocomial infections may not be effective. Therefore, treatment should be based on the result of culture and sensitivity.

Keywords: Nosocomial infection, Surgical Site Infection, Blood Stream Infection, Urinary Tract Infection, Intensive Care Unit

Chapter I: INTRODUCTION

1.1. Introduction

Nosocomial infections (NIs), also called “hospital-acquired infections”, are defined as infections which are not present or not incubating when the patient is hospitalized and are acquired during the hospital stay. Sign and symptoms of the infection may be evident during hospitalization or after discharge related to the length of the incubation period. It is usually defined as an infection that is identified at least 48-72 hours following admission to health institution. Infections acquired in hospital are likely to complicate illness, cause anxiety and discomfort, and can lead to death (Saraçlı *et al*, 1999; WHO, 2002; Kasper *et al*, 2005; NINSS, 2002). These infections result in substantial morbidity, mortality, and increased financial burden. Nosocomial infections are also important public health problems in developing countries, as well as in developed countries. The socioeconomic impact, i.e., prolongation of hospitalization, mortality, and cost, of these infections adversely affects patients and nations’ economic well-being (Apostolopoulou and Katsaris, 2003; Celik *et al.*, 2005). Risk factors for the development of nosocomial infections in the Surgical Intensive Care Unit (SICU) setting include poor nutritional status, exposure to multiple antibiotics, indwelling central venous catheters, mechanical ventilation and length of ICU stay (Apostolopoulou *et al*, 2005).

Epidemiological and etiological characteristics of nosocomial infections show variations among countries and even among different hospitals in the same country. Many of these infections are associated with micro-organisms that are resistant to multiple antibiotics and can easily spread on the hands of personnel (Durmaz *et al*, 2000). They are important for both patient and public health problem in developing countries, as well as in some developed countries (Apostolopoulou and Katsaris, 2003). Now a days, over 1.4 million people worldwide suffer from infectious complications acquired in hospital. The highest frequencies of nosocomial infections were reported from hospitals in the Eastern Mediterranean and South-East Asia Regions (11.8 and 10.0%, respectively), with a

prevalence of 7.7 and 9.0%, respectively in the European and Western Pacific Regions (WHO, 2002).

The increased occurrence of nosocomial infection rates can be best summed up by three major contributing factors. The first is overuse of antimicrobials and long-term care facilities. The second contributing factor is many hospital personnel fail to follow the basic infection control procedures such as hand washing between patient contact and decontamination and sterilization precautions. Finally, patients in hospitals are becoming increasingly immuno-incompetent to control nosocomial infections (Nguyen, 2004; Kasper *et al*, 2005).

Twenty five to 50% of nosocomial infections are due to the combined effect of the patients own flora and invasive devices. Most infections acquired in hospital today are caused by micro organisms which are common in the general population, in whom they cause no or milder disease than among hospital patients (*Staphylococcus aureus*, coagulase-negative staphylococci, enterococci, Enterobacteriaceae) (WHO, 2002). Over the past several decades, the frequency of antimicrobial resistance and its association with serious infectious diseases have increased at alarming rates. The increasing resistance rate among nosocomial pathogens is a commonly encounter problem (Tullu *et al*, 1998; Jones, 2001). The most frequent types of infections are urinary-tract infection, surgical-wound infection, pneumonia, and bloodstream infection (Nguyen, 2004). Table 1.1 provides common nosocomial infection sites and Simplified criteria for each infection (WHO, 2002).

There are several reports on the prevalence of nosocomial infection in Africa from different countries in literatures; Algeria 4% (Atif *et al.*, 2006), Tunisian 13% (Dridi *et al.*, 2006), and Morocco 6.7% and 17.8% (El Rhazi *et al.*, 2007; Jroundi *et al.*, 2007) respectively. A study conducted in 1988 on 1006 surgical patients admitted to Tikur Anbessa hospital, in Addis Ababa revealed a prevalence of nosocomial infections of 16.4%. Of these, wound (59%), urinary tract (26%), and respiratory tract (6%) infections accounted for more than 90% of the infections (Habte-gabr *et al*, 1988). This study was done in Ethiopia at hospital in Addis about 20 years back. Therefore, the objective of this study was to determine recent

prevalence of nosocomial infection, bacteriological profile and their antibiotic resistance patterns in patients who were admitted in surgical ward and SICU at Tikur Anbessa University hospitals in Addis Ababa, Ethiopia.

Table 1.1. Simplified criteria and common nosocomial infection sites (adapted from WHO, 2002)

Type of nosocomial infection	Simplified criteria
Surgical site infection	Any purulent discharge, abscess, or spreading cellulitis at the surgical site during the month after the operation
Urinary infection	Positive urine culture (1 or 2 species) with at least 10^5 bacteria/ml, with or without clinical symptoms
Respiratory infection	Respiratory symptoms with at least two of the following signs appearing during hospitalization: <ul style="list-style-type: none"> — 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
Septicaemia	Fever or rigours and at least one positive blood culture

1.2. Literature Review

Nosocomial infections are frequent complications of hospitalizations (Inan *et al*, 2005). The issue has been recognized for more than a century as a critical problem affecting the quality of health care and a principal source of adverse outcomes. Today NI affects more than 2 million patients annually, at a cost of US 4.5 billion (Apostolopoulou and Veldekis, 2005).

The socioeconomic impact, i.e., prolongation of hospitalization, mortality, and cost of these infections adversely affects patients and nations' economic well-being (Inan *et al*, 2005). Hospital-acquired infections add to functional disability and emotional stress of the patient and in some cases, lead to disabling conditions that reduce the quality of life. Nosocomial infections are also one of the leading causes of death. The increased use of drugs, the need for isolation, and the use of additional laboratory and other diagnostic studies also contribute to costs (WHO, 2002).

Over 80% of nosocomial infections are related to device utilization needed for patient life support but responsible for such complications as ventilator-associated pneumonia, catheter-related bloodstream infections, surgical site infection and urinary tract infection (Corona and Raimondi, 2004). Intensive care units (ICUs) are where the most severely ill patients are treated and where the highest mortality rates occur. Nosocomial infection and mortality in ICUs are more prevalent than in other wards of the hospital. For ICU patients, the risk is as much as 5 to 10 times greater. Underlying diseases, multiple illnesses, malnutrition, extremes of age, impaired host defenses, invasive devices, immunosuppressive therapy, use of antibiotics and colonization with resistant micro organisms render patients highly susceptible to nosocomial infections in ICUs (Weber *et al*, 1999). According to the European Prevalence of Infection in Intensive Care study (EPIC), involving over 4500 patients, the nosocomial infection prevalence rate in ICU was 20.6%. On an average, a patient with hospital-acquired infection spends 2.5 times longer in hospital resulting in additional cost of 3000 Pounds more than an uninfected patient (Rizvi *et al*, 2007).

1.2.1. Site of nosocomial infections

There are different site of nosocomial infection, the most common are surgical site infections (SSIs), Urinary tract infections (UTIs), Bloodstream infections (BSIs) and lower respiratory tract infection as demonstrated in Figure 1.1.

1.2.1.1. Surgical Site infection (SSI)

Surgical site infections (SSIs) are the most common cause of nosocomial infection, resulting in considerable morbidity and mortality, because patients who develop SSIs have a longer hospital stay, are more likely to be readmitted, and are more likely to die. The delay in recovery and increased hospital stay also has economic consequences. It has been estimated that each patient with a surgical site infection requires an additional hospital stay of 6.5 days, and hospital costs are doubled (Fehr *et al*, 2006; Mannie`n *et al*, 2006; NINSS, 2002). In the late 19th century many surgical procedures were well developed in principle; however, their application for the treatment of surgical illnesses was limited mainly because of a mortality rate from postoperative infection alone of 50%. With better understanding of infection, implementation of effective prevention and the advent of modern antibiotics; wound infection rates have been considerably reduced in the last 80 years. However, even in this modern era, surgical wound infection is still a major public health problem (Taye *et al*, 2005). The incidence of surgical site infections varies from 0.5 to 15% depending on the type of operation and underlying patient status. These are significant problems which limit the potential benefits of surgical interventions. The impact on hospital costs and postoperative length of stay (between 3 and 20 additional days) is considerable (WHO, 2002). The most common bacterial pathogens are *S aureus*, *P aeruginosa*, *E coli*, *K pneumoniae*, *Enterobacter spp.*, *Enterococcus spp.*, CoNS and *Viribans streptococci* (Hsueh *et al*, 2002; Gelfand *et al*, 2005)

1. 2. 1. 2. Urinary tract infection (UTI)

Urinary tract infection (UTI) was defined as the growth of a single pathogen of $>10^5$ colony forming units/ml by properly collected mid-stream urine specimen (Prais *et al*, 2003). UTI is a cause of considerable morbidity in patients with indwelling urethral catheter (Kehinde *et al*, 2004). UTI continues to be the commonest nosocomial infection accounting for approximately 40 percent of the total number reported by acute-care hospitals and affecting an estimated 600,000 patients per year (Mohanty *et al*, 2003; CDC, 2002). The rate of nosocomial UTI is determined by the interactions of several factors such as primary disease and its severity, duration of hospitalization and treatment, and invasive interventions. Urinary tract infections are associated with urinary catheters in 66% to 86% of the cases.

Although not all catheter-associated urinary tract infections can be prevented, it is believed that a large number could be avoided by the proper management of the indwelling catheter (Savas *et al*, 2006; CDC, 2002). In the community and hospital settings the aetiology of UTIs and the antimicrobial susceptibility of urinary pathogens have been changing over the years. Factors such as the changing patient population, extensive use and misuse of antimicrobial agents, could all contribute to changes in the microbial profile of urinary tract isolates (Gales *et al*, 2000). More over, the incidence of nosocomial UTI has been increasing and its treatment has become more complicated because of the pathogens with increasing resistance to antimicrobial agents (Shingemure *et al*, 2005). It is known that between 5% and 25% of patients with indwelling urethral catheters develop asymptomatic UTI while only about 3% to 5% develop symptomatic UTI necessitating antimicrobial therapy or removal of the catheters (Prais *et al*, 2003). The most common bacterial pathogens are *P aeruginosa*, *E coli*, *S aureus*, *K pneumoniae*, *Enterobacter spp.*, *Enterococcus spp.*, *Proteus spp.* and *Citrobacter spp.* (Hsueh *et al*, 2002; Tullu *et al*, 1998).

1.2.1. 3. Bloodstream infections (BSI)

Bloodstream infections are among the most common hospital-acquired infections in pediatric patients, and are responsible for approximately 10% to 30% of the cases (Arnoni *et al*, 2007). Bloodstream infections usually are associated with intravascular catheters, particularly central venous catheters (CVCs). The highest BSI rates are in intensive care unit (ICU) patients, whose infections rates range from 2.4 to 30.0 per 1,000 CVC days (Apostolopoulou and Veldekis, 2005). BSI due to bacterial and fungal pathogens affect over 200,000 individuals annually in the United States alone and are a tremendously important cause of morbidity and mortality worldwide. The impact of specific etiologic agents on the outcome of BSI has been well documented and speaks to the need for a better understanding of the spectrum of pathogens causing both nosocomial and community-acquired BSI (Pfaller *et al*, 1998). Gram-negative bacteria were the most common causative agents in hospital-acquired bloodstream infections. However, Gram-positive bacteria became more predominant, probably correlated with the greater use of prophylactic antibiotics in at-risk patients, such as use of intravascular devices (Arnoni *et al.*, 2007). Nosocomial pathogens

have shifted away from easily treatable bacteria towards more resistant bacteria. These shifts continue to present challenges for nosocomial infection control and prevention (Jain *et al.*, 2007). In case of BSI, the common bacterial pathogens are *S aureus*, CoNS, *P aeruginosa*, *K pneumoniae*, *E coli*, *Enterobacter* spp., *Enterococcus* spp. and *Acinetobacter* spp.

1.2. 1. 4. Nosocomial bacterial pneumonia

Nosocomial bacterial pneumonia occurring after two days of mechanical ventilation is referred to as ventilator associated pneumonia, and is the most common nosocomial infection seen in the intensive care unit (Hunter, 2006). It causes the highest rates of morbidity and mortality. In the United States approximately 30 to 50% of deaths among patients with nosocomial pneumonia are directly attributable to the infection, with the highest mortality rates seen for patients with *Pseudomonas aeruginosa* or *Acinetobacter* infection or patients with concurrent bacteremia (Kashuba *et al.*, 1999). Intubation of the trachea and mechanical ventilation is associated with a 7-fold to 21-fold increase in the incidence of pneumonia and up to 28% of patients receiving mechanical ventilation will develop this complication (Hunter, 2006). Pathogenic mechanisms of NLRTI in ICU patients dominated by two processes: colonisation of the oropharynx and its contiguous structures, such as sinuses, dental plaque, trachea and gastric reservoir, and aspiration of the contaminated secretions into the lower airway (Nseir *et al.*, 2002). The predominant organisms responsible for infection are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Enterobacteriaceae, but etiologic agents widely differ according to the population of patients in an intensive care unit, duration of hospital stay, and prior antimicrobial therapy (Chastre and Fagon, 2002)

1.3. 1. 5. Other site of nosocomial infection

The above mentioned sites are the four most frequent and important nosocomial infections, but there are many other potential sites of infection. For example: skin and soft tissue infections: open sores (ulcers, burns and bedsores) encourage bacterial colonization and may lead to systemic infection; Nosocomial gastroenteritis are likely to be caused by hospital-acquired flora, which may include enteric organisms with acquired antibacterial resistance

or multi-resistant gram-negative bacteria, such as *P. aeruginosa*; sinusitis and other enteric infections, infections of the eye and conjunctiva and endometritis and other infections of the reproductive organs following childbirth (WHO, 2002; Tellado and Wilson, 2005; Asefzadeh, 2005; Grolman and Richards, 2005).

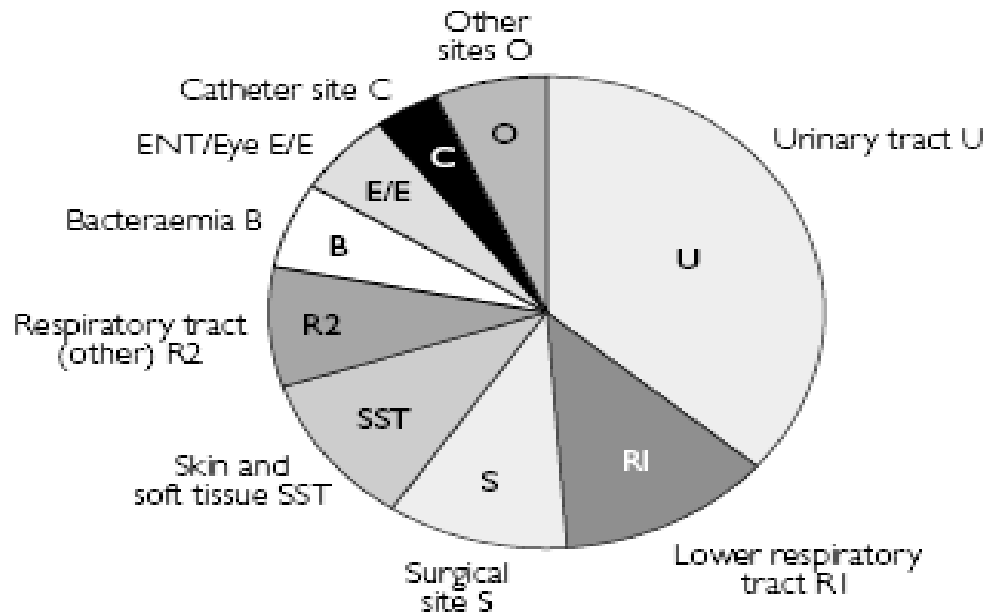


Figure 1.1. Sites of the most common nosocomial infections: distribution according to the French national prevalence survey (1996) (Adapted from WHO, 2002)

1. 2. 2. Pathophysiology and Risk factors of nosocomial infection

Within hours of admission, colonies of hospital strains of bacteria develop in the patient's skin, respiratory tract, and genitourinary tract (Nguyen, 2004). Many organisms gain entry to the body through breaches or evasion of "first line" body defences. Breaches in epithelial integrity (e.g., surgical wounds, intravascular cannulas and drain tubes), loss of the washing action of body fluids (e.g., because of a urinary catheter), and interference with first line respiratory defences (e.g., by anaesthesia and endotracheal intubation) are common precursors of hospital-acquired infections (Spelman, 2002).

Risk factors for the invasion of colonizing pathogens can be categorized into 3 areas: iatrogenic, organizational, and patient related. Iatrogenic risk factors include pathogens that are present on medical personnel hands, invasive procedures (eg, intubation, indwelling vascular lines, urinary tract catheterization), and antibiotic use and prophylaxis. Organizational risk factors include contaminated air-conditioning systems, contaminated water systems, and staffing and physical layout of the facility (eg, nurse-to-patient ratio, open beds close together). Patient risk factors include the severity of illness, underlying immuno compromised state, and length of stay (Nguyen, 2004; Asefzadeh, 2005).

1. 2. 3. Laboratory Diagnosis of nosocomial infection

A detailed physical examination and review of systems most likely reveal the involved organs or systems. Investigation should be focused on these abnormal areas such as; bloodstream, UTI, pneumonia and surgical-site infection. Laboratory test for nosocomial infection can be performed by taking samples from the sites of the infection (Nguyen, 2004). Laboratory analyses aim to identify the responsible infectious agent, evaluation of its susceptibility to anti-infectious treatments, typing of bacterial strains, etc (BioMérieux Clinical Diagnostics, 2008).

Stool examination: *Clostridium difficile* should be suspected in any patient with fever and diarrhea who received antibacterial agents or chemotherapy during the preceding three weeks. In such patients, stool specimens should be sent for evaluation. Stool cultures for other enteric pathogens are rarely necessary as these infections are rarely hospital-acquired (Gopalan, 2005).

Bloodstream infections can be diagnosis by blood cultures with samples from the intravenous line and peripheral vein are recommended to aid in differential diagnosis of line-associated bacteremia. Fungal cultures should be requested, if they are suspected. Nosocomial pneumonia can be diagnosis by Radiography, oxygenation, hemodynamic status determination and the sign and symptom of the patients. Examination of the sputum, endotracheal aspiration material, and pleural effusion fluid with Gram staining and culturing may be useful. Urinary tract infections are expected in patients who require an indwelling

urinary catheter. Efforts should be made to differentiate colonization, cystitis, and frank pyelonephritis by means of urinalysis, urine Gram staining, and culturing (Nguyen, 2004; Spelman, 2002). Surgical site infection can be diagnosed by culturing of wound swab from the infected sites (Fehr *et al*, 2006).

1. 2. 4. Antibiotic resistance pattern and Etiological agents

Antibiotic resistance has been a problem since the introduction of penicillin G and the sulphonamides in the 1940s (Norrby, 1995). There has been a race between the bacteria developing mechanisms for resistance and the scientists developing new antibiotics active against resistant organisms. Until now, that race has invariably been won by science. That is no longer the case. We have been outsmarted by the microbes and are in the position where we may lack effective antibiotics, while there are still several years before new drugs will be available for clinical use (Norrby, 1995).

One of the more alarming recent trends in infectious diseases has been the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial infection (Pfaller *et al.*, 1998). But, knowledge concerning the distribution of bacteria as causative agents of infections, together with their resistance patterns, is essential for selecting appropriate empirical antibiotic treatment. Although the microbial aetiologies of NIs differ among hospitals, or even among departments of the same hospital (Kohlenberg *et al.*, 2008).

Data reported by the National Nosocomial Infections Surveillance (NNIS) System for 1993-1997 compared with January-November 1998 show a continuing increase in antimicrobial-resistant pathogens; the increase is particularly marked for vancomycin-resistant enterococci (VRE) (55%), methicillin-resistant *Staphylococcus aureus* (MRSA) (31%), third-generation cephalosporin-resistant *Escherichia coli* (29%), imipenem-resistant *Pseudomonas aeruginosa* (32%), and quinolone-resistant *P. aeruginosa* (89%) (Hsueh *et al*, 2002). In PICU of a teaching hospital of India, 95 suspected cases of nosocomial infections were studied; the rate was 27.3% with an incidence of 16.2 per 100 patient days. The incidence of urinary, respiratory and intravascular catheter related infections was 56.5%, 34.8%, and

10.5% respectively. *Klebsiella* (33.3%) was the most common isolate with maximum sensitivity to amikacin (Deep *et al*, 2004).

In South Africa, fifty-six methicillin-resistant *Staphylococcus aureus* clinical isolates were collected over a two-month period from a large teaching hospital in Pretoria; and isolates were subjected to testing for vancomycin hetero-resistance. Thirty-three isolates were identified as possibly being hetero-resistant to vancomycin (Oosthuizen *et al.*, 2005). In South Africa, evaluating about 5000 adult ICU admissions revealed a median time between ICU admission and development of a blood stream infection (BSI) of 7.4 days. The most commonly isolated organism was *S. aureus* (18% of isolates), followed by coagulase-negative staphylococci (11%) and *Enterococcus faecalis* (8%). Antibiotic-resistant organisms were isolated in 12% and infections with more than one organism in 22% of cases (Mer, 2005). A study conducted in 1988, approximately 90% of the isolates were gram-negative bacteria, of which 84% were Enterobacteriaceae. More over, most of the isolates were resistant to the commonly used antibiotics. This study had clearly showed that the importance of the problem in this hospital and possibly in others in Addis Ababa (Habte-gabr *et al*, 1988). Another study conducted in about 384 clinical specimens (202 sputum, 164 urine and 18 pus) collected from patients admitted in different wards, a total of 57(15%) *Klebsiella* spp, were isolated with a distribution of 33(58%) from sputum, 18(31.5%) from urine and 6(10.5%) from pus. Of the 57 *Klebsiella* spp., 54(94.7%) were identified as *K. pneumoniae* and 3(5.3%) as *K. oxytoca*. In 19/57 (33.3%) of the *Klebsiella* isolates, extended Spectrum β -Lactamase (ESBL) production was detected (Seid and Asrat, 2005).

A study conducted at teaching hospital in Addis Ababa for nosocomial infection; of 2506 patients, 13% developed clinical infections. Wound infection was the most frequent type of nosocomial infection (49%) followed by urinary tract infection (25%). Gram-negative bacteria comprised 88% of all isolated strains Enterobacteriaceae; 75% of all isolates were found in over 60% of the infection, *Proteus* 25%, *Escherichia coli* 20% and *Klebsiella* 19%. The most widely used antibiotics were ineffective against 65 to 85% of the gram-negative strains (Gedebou *et al.*, 1987).

1. 2. 5. Prevention and control of nosocomial infection

Nosocomial infection is one major health threat. Each year, many people contract an infection during admission, adding unnecessary costs and burden to patients, families and the health care systems.

An effective infection control and prevention program can reduce the rate of nosocomial infection and its consequences (Juntaradee *et al.*, 2005). Prevention of nosocomial infections requires an integrated and monitored programme. Most of these infections can be prevented with readily available, relatively inexpensive strategies by: prevention of infection in staff members; protecting patients with appropriate use of prophylactic antimicrobials, nutrition, and vaccinations; controlling environmental risks for infection; limiting the risk of endogenous infections by minimizing invasive procedures, and promoting optimal antimicrobial use; surveillance of infections, identifying and controlling outbreaks; adhering to recommended infection prevention practices, especially hand hygiene and wearing gloves; paying attention to well-established processes for decontamination and cleaning of soiled instruments and other items, followed by either sterilization or high-level disinfection; and Improving safety in operating rooms and other high-risk areas where the most serious and frequent injuries and exposures to infectious agents occur (MOH, 2005; WHO, 2002). The two main arms of prevention are stopping the development of antibiotic resistance and preventing the spread of resistant organisms between patients (Spelman, 2002). Unfortunately, not all nosocomial infections are preventable. For example, some reflect the influence of advanced age, chronic diseases such as uncontrolled diabetes, end-stage kidney disease or advanced pulmonary emphysema, severe malnutrition, treatment with certain drugs (e.g., antimicrobials, corticosteroids and other agents that decrease immunity), the increasing impact of AIDS (e.g., opportunistic infections) and irradiation (MOH, 2005; WHO, 2002).

1. 3. Significance of the study

Nosocomial infection is a global problem with multi facet out comes. The problem is well pronounced in developing countries. In our country, effective nosocomial infection prevention programs such as the one established in the developed world intended to reduce the infection rates is not in place (Haley *et al.*, 1985; Mishriki *et al.*, 1990; Wenzel, 1995). Previous studies in Tikur Anbessa hospital showed a prevalence of 16.4% rate of nosocomial infection (Habte-gabr *et al.*, 1988). At present, the emergence of resistance to antimicrobial agents is a global public health problem, particularly in pathogens causing nosocomial infections. Antimicrobial resistance results in increased illness, deaths, and health-care costs. The distribution of pathogens causing nosocomial infections, especially antimicrobial-resistant pathogens, changes with time and varies among hospitals and among different locations in the same hospital (Hsueh *et al.*, 2002). Tikur Anbessa University Hospital is the biggest national tertiary referral and teaching hospital which is provides referral service for patients coming all over the country. In addition to that, many procedures which are risk factors for nosocomial infections are performed in this hospital regularly. Patients infected in this hospital, may act as carriers of infection else where in the community.

Because of the need of recent and up-to-date information on susceptibility pattern of microorganisms, which alter over time especially bacterial pathogens causing nosocomial infection. There come the need to re-asses the area. Therefore the primary intent of this study is to identify the prevalence of nosocomial infection and drug susceptibility pattern along associated risk factors of nosocomial infection.

1.4. Objective of the study

General Objective

To determine the prevalence of nosocomial infections and drug susceptibility pattern of isolates in surgical ward and surgical ICU at Tikur Anbessa University Hospital.

Specific Objectives

- To determine the frequency of nosocomial infections among patients admitted to surgical wards and surgical ICU.
- To isolate the bacterial pathogens responsible for the infection.
- To identify risk factors associated with nosocomial infections.
- To determine antimicrobial susceptibility pattern of isolates.

CHAPTER II: MATERIALS AND METHODS

2.1. Study design and study period

A cross-sectional study was conducted from June 2007 to April 2008 at Tikur Anbessa University Hospital.

2.2. Study area

The study was conducted at surgical wards and SICU, Tikur Anbessa University hospital, Addis Ababa, Ethiopia. Tikur Anbessa University Hospital is the largest hospital in the country compared to other hospitals, which has 560 beds and is located at the centre of the city in Lideta Sub city. The hospital receives referred patients from all parts of the country and provides local emergency service.

2.3. Study variables

Dependent variables: - UTI, surgical wound infection, septicemia and susceptibility pattern for antibiotics.

Independent variables: - age, sex, urinary catheter insertion, surgical procedure, mechanical ventilation, intravascular catheter, antibiotic usage and duration of admission.

2.4. Source Population

During the study, all adult (> 15 years) patients admitted to surgical wards and SICU with suspected nosocomial infection were included.

2.5. Selection and evaluation of study subjects

To select the target groups convenient sampling technique was employed. Thus, a careful clinical examination was conducted by physician to all adult patients who were admitted to surgical wards and SICU. Examination of wounds and catheter entry sites, review of

procedures that might lead to infection were made. This was done in order to exclude community-acquired infections and to determine any underlying risk factors.

2.6. Sample size determination

Relevant information including; demographic, clinical and other relevant data were obtained by physician/s and transferred to the questionnaire prepared for this study based on WHO criteria. (See appendix I).

The sample size (n) was calculated using the highest prevalence of nosocomial infection 16.4% (Habte-gabr *et al.*, 1988) in Addis Ababa hospital. The expected margin of error (d) taken was 0.05 with the confidence interval ($Z\alpha/2$) of 95%.

A minimum of 211 samples of patients was included for the study based on the following formula

$$\text{Total study subjects: } n = \frac{z^2 p (1-p)}{d^2} = 210.6$$

(Wayne, 1998).

Where P = prevalence of nosocomial infection (16.4%) (Habte-gabr *et al.*, 1988).

D = degree of accuracy desired (0.05)

$Z^2_{1-\alpha/2}$ = the standard normal deviation (1.96)

2.7. Eligibility and Exclusion Criteria

2.7.1. Inclusion criteria

Patients who developed urinary tract infection (UTI), primary bacteremia, central venous catheter-related infection, or surgical wound infection according to the definitions given subsequently (i.e. at least 48 h to 72h surgical wards and ICU admission) were eligible and included in study.

2.7.2. Exclusion criteria:

Patients with community-acquired infection were excluded from the study.

2.8. Data Collection and processing

Data on socio-demographic variables and associated risk factors were collected by pre-designed and pre-tested questionnaire.

2.8.1. Sample collection

Specimens were collected from patients admitted to the surgical wards and SICU and suspected of developing nosocomial infection based on their clinical findings. The specimens were collected using standard procedures and analyzed accordingly.

Blood sampling and processing: Blood sample were drawn aseptically when bloodstream infection was suspected (adult patients who had chills or fever). About 10ml of venous blood was collected and immediately inoculate directly in to a tube containing thioglycollate broth. Blood was incubated aerobically at 37°C for 10 days and checked for turbidity as an indication of growth (Annex I).

Urine specimens: Urine sample was collected for bacteriological examination by the mid stream method or catheterization into sterilized container and cultured before and after using catheter. Urine sample which did not show significant growth ($< 10^4$ CFU/ml of urine) before the insertion of catheter and urine culture with colony count $\geq 10^5$ CFU/ml of urine after catheterization were considered indicative of significant infection (Annex II).

Swab from wound infection: Wound infections occur as complications of surgery, trauma, or disease that interrupts a skin surface. Material from infected wounds was collected aseptically by using sterile swab and culture, gram stain and biochemical test done respectively to isolate the causative agents (Annex III).

2.8.2. Identification of organisms

2.8.2.1. Culture and gram staining

Urine, Swab and body fluid specimens were inoculated on blood agar and MacConkey agar; where as blood specimens were inoculated in thioglycollate broth (Oxoid, Ltd). The blood and MacConkey agar plates were incubated in aerobic atmosphere at 37°C for 24-48 hrs. Thioglycollate broth was incubated in aerobic atmosphere at 37°C for up to 2 weeks, examine daily up to 14 days for visible signs of bacterial growth such as turbidity above the red cell layer, colonies growing on top of the red cells ('cotton balls'), haemolysis gas bubbles and clots. Then, all positive specimens were sub cultured on Blood agar, Chocolate agar and MacConkey agar. Positive cultures were identified by their characteristic appearance on their respective media, gram-staining reaction (Annex IV) and confirmed by the pattern of biochemical reactions using the standard method (Cheesbrough, 2004).

2.8.2.2. Biochemical tests

Members of the family enterobacteriaceae were identified by indole production, H₂S production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization and other tests using API 20E identification kits (Biomerieux, France). For gram-positive bacteria coagulase, DNase, catalase, bacitracin and optochin susceptibility tests were used.

2.8.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for all isolates according to the criteria of Bauer *et al* (1966) and National Committee for Clinical Laboratory Standards (NCCLS, 2006; Villanova, 1993) by disk diffusion method.

From a pure culture, 3-5 colonies of bacteria were taken and transferred to tubes containing 5 ml of nutrient broth and mixed gently, to obtain a homogenous suspension and incubated at 37°C until the turbidity of the suspension became adjusted to 0.5 McFarland standard.

A sterile cotton swab was used and the excess suspension was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar and Mueller Hinton agar supplemented with 5% sheep blood only used for *S. pneumoniae* (Oxoid).

The inoculated plates were left at room temperature to dry for 3-5 minutes and a set of 16 antibiotic discs (Oxoid) were then delivered on the surface of a Muller-Hinton plate. The drugs for disc diffusion testing were in the following concentrations:

For gram negative bacteria; Ampicillin (AMP) (10 µg), Amoxicillin-Clavulanic acid (AMC) (30 µg), Chloramphenicol (C) (30 µg), Gentamicin (CN) (10 µg), Nalidixic acid (NA) (30 µg), Nitrofurantoin (FM) (300 µg), Amoxicillin (AML) (25 µg), Tetracycline (TTC) (30 µg), Trimethoprim-sulphamethoxazole (SXT) (25µg), Ceftriaxone (CRO) (30 µg), Doxycyclin (DO) (30 µg), Norfloxacin (NOR) (10 µg) and Ciprofloxacin (CIP) (5 µg).

For gram positive bacteria; Ampicillin (AMP) (10 µg), Amoxicillin-Clavulanic acid (AMC) (30 µg), Chloramphenicol (C) (30 µg), Gentamicin (CN) (10 µg), Cloxacillin (CX) (5 µg), Methicillin (MET) (5 µg), Penicillin G (P) (10 units), Amoxicillin (AML) (25 µg), Tetracycline (TTC) (30 µg), Trimethoprim-sulphamethoxazole (SXT) (25µg), Ceftriaxone (CRO) (30 µg), Doxycyclin (DO) (30 µg), Norfloxacin (NOR) (10 µg) and Ciprofloxacin (CIP) (5 µg).

The plates were then incubated at 37°C for 24-48 hours. Diameters of the zone of inhibition around the disc were measured to the nearest millimeter using a caliper, and the isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the NCCLS (NCCLS, 2006).

High, intermediate and low level of resistance is defined when the percentage of resistance is >80%, 60-80% and < 60%, respectively (Annex V).

2. 9. Reference Strains

Standard reference strain for *S. aureus* (ATCC-25923), *E. coli* (ATCC-25922) and *P. aeruginosa* (ATCC-27853) from the EHNRI laboratory stock were used as a quality control throughout the study for culture and antimicrobial susceptibility testing.

2.10. Data entry and analysis

Data entry and analysis was done using SPSS version computer 12.0 software. Comparisons were made using Chi-square test. A *p*-value of <0.05 was considered indicative of a statistically significant difference. Prevalence rate was calculated for the sum of the numbers of positive cases of examined subjects.

2.11. Ethical Considerations

This M.Sc research project was approved by the Department of Microbiology, Immunology and Parasitology (DMIP) of the Faculty of Medicine of Addis Ababa University, ethically cleared by the Faculty Research and Publications Committee (FRPC) and endorsed by the Faculty Academic commission. There was minimal risk associated with the process of sampling; it was the same as taking specimen for culture and sensitivity in the routine laboratory. Disposable syringes with needles and, sterile swabs were used to prevent HIV and other infectious agents. All the information contained within the questionnaire was kept confidential. Informed consent was obtained from all individuals who were diagnosed for nosocomial infections. If patients were not interested in the study they had the right to withdraw from the study. 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.

CHAPTER III: RESULTS

3.1. Description of the study subjects

Eight hundred fifty four patients admitted in surgical ward and SICU between June 2007 and April 2008 to Tikur Anbessa University Hospital in Addis Ababa were studied for prevalence of nosocomial infections. On admission, they were carefully examined clinically to exclude community-acquired infections and to determine any underlying risk factors. A total of 215(25.2%) patients, were selected based on their clinical ground from surgical wards (n=161) and SICU (n=54) of Tikur Anbessa University Hospital.

Of the 215 patients, 130(60.5%) were males and 85(39.5%) were females with an overall male to female ratio of 1.5:1. The age ranged from 17 to 79 years with a mean of 38.02 (\pm 14.82) years. The mean hospital stay from the date of admission until sample collection was 16.72 days with a range of 3 to 66 days. The age and sex distribution of patients investigated for bacterial nosocomial infections are presented in Table 3.1. A total of 88(40.9%) pus swabs from the surgical site, 84(39.1%) urine and 43(20.0%) blood samples were collected from the 215 patients. Concerning the wounds, 153(71.2%) were classified as clean, 42(19.5%) as clean contaminated and 20(9.3%) as contaminated. Antibiotic prophylaxis was given to 65.6% of the patients before sample collection. In this study, there were about 28 primary reasons for admission (diagnosis); of which benign prostatic hyperplasia (BPH) accounted 33(15.3%), car accident 25(11.6%), bullet injury 24(11.2%), head injury 20(9.3%), urethral stricture 13(6.0%), oesophageal cancer 13(6.0%), intestinal obstruction 12(5.6%), acute appendicitis 10 (4.7%) and 65(30.3%) accounted for other causes.

Table 3.1 Age and sex distribution of 215 patients investigated for bacterial nosocomial infections at Tikur Anbessa University Hospitals, Addis Ababa, Ethiopia (June 2007-April 2008)

Age Group	Female No (%)	Male No (%)	Total No (%)
< 26	23(10.7)	24 (11.2)	47 (21.9)
26-35	21(9.8)	41 (19.1)	62 (28.9)
36-45	28 (13.0)	28 (13.0)	56 (26.0)
46-55	6 (2.8)	14 (6.5)	20 (9.3)
56-65	6 (2.8)	10 (4.7)	16 (7.5)
> 65	1 (0.5)	13 (6)	14 (6.5)
Total	85 (39.5)	130 (60.5)	215 (100)

3.2. Patterns of nosocomial infection

Among 854 patients, 77 (9.0%) were confirmed to have nosocomial infection. Of the 77 patients, 51(66.2%) were males and 26(33.8%) females. The distribution of nosocomial infection among positive cases presented in Table 3.2 indicates that 38(49.4%) were surgical site infections, 23(29.8%) urinary tract infections and 16(20.8%) blood stream infections. Of the 38 patients with surgical site infection 11(14.3%) were females and 27(35.1%) were males; of the 23 patients with urinary tract infection 8 (10.4%) were females and 15(19.4%) were males; and of the 16 patients with blood stream infection 7(9.1%) were females and 9(11.7%) were males. Among different wards, BSI was predominantly observed in SICU as shown in (Table 3.2). Surgical procedures, insertion of urinary catheter, insertion of central venous catheter and mechanical ventilation were significantly associated with nosocomial infection ($p < 0.05$) in this study (Table 3.3.).

Table 3.2 Distribution of nosocomial infections by ward and gender observed in 77 patients at Tikur Anbessa University Hospitals Addis Ababa, Ethiopia (June 2007-April 2008)

Sites of Nosocomial infection	Female		Male		Total No
	Surgical wards	SICU	Surgical wards	SICU	
	No (%)	No (%)	No (%)	No (%)	
SSI	10 (13.0%)	1 (1.3%)	25 (32.5%)	2 (2.6%)	38 (49.4%)
UTI	5 (6.5%)	3 (3.9%)	14 (18.1%)	1 (1.3%)	23 (29.8%)
BSI	-	7 (9.1%)	3 (3.9%)	6 (7.8%)	16 (20.8%)
Total	15 (19.5%)	11 (14.3%)	42 (54.5%)	9 (11.7%)	77 (100%)

Table 3.3. Nosocomial infections and associated risk factors in 77 patients at Tikur Anbessa University Hospitals Addis Ababa, Ethiopia (June 2007-April 2008)

Associated risk factors		SSI	UTI	BSI	P value
Surgical procedure	Yes	38	23	16	0.000
Urinary catheter	Yes	17(44.7%)	23 (100.0%)	11 (68.8%)	0.000
	No	21 (55.3%)	0	5 (31.3%)	
Mechanical vent	Yes	3 (7.9%)	5 (21.7%)	11(68.8%)	0.000
	No	35 (92.1%)	18 (78.3%)	5 (31.3%)	
IV Catheter	Yes	10 (26.3%)	5 (21.7%)	9 (56.3%)	0.000
	No	28 (73.7%)	18 (78.3%)	7 (43.8%)	

3.3. Etiologic Agents

A total of 84 bacterial isolates were isolated from nosocomial infection (n=77) cases. *E. coli* accounted for 19.0% of the total isolates followed by *S. aureus* (16.7%), *Klebsiella* spp. (*K. pneumoniae* and *K. oxytoca*) (16.7%), *P. aeruginosa* (14.3%), coagulase negative staphylococcus (11.9%), *P. vulgaris* and *E. cloaceae* (7.1%), *S. pneumoniae* and *Citrobacter*

spp. (2.4%) and *Serratia* spp. and *Morgenella* spp (1.2%) (as shown in figure 3.1.) More than one bacteria etiologic agent was isolated from 7/77(9.1%) of the patients with nosocomial infection (data not shown). The Gram-positive and negative bacteria accounted for 23/84(23.4%) and 61/84(72.6%), respectively ($p < 0.05$).

Among surgical site infection ($n = 44$), *P. aeruginosa* (22.7%), *Klebsiella* spp. (*K. pneumoniae* and *K. oxytoca*) (20.4%) and *S. aureus* (15.9%) were isolated. Among UTI ($n=24$), *Escherichia coli* (45.8%), *E. cloacae* (20.8%) and *K. pneumoniae* (16.6%); and in BSI ($n=16$) *S. aureus* (37.5%), coagulase negative staphylococcus (37.5%) followed by *S. pneumoniae* (12.4%) were the commonest bacterial pathogens isolated (as shown in Table 3.4.).

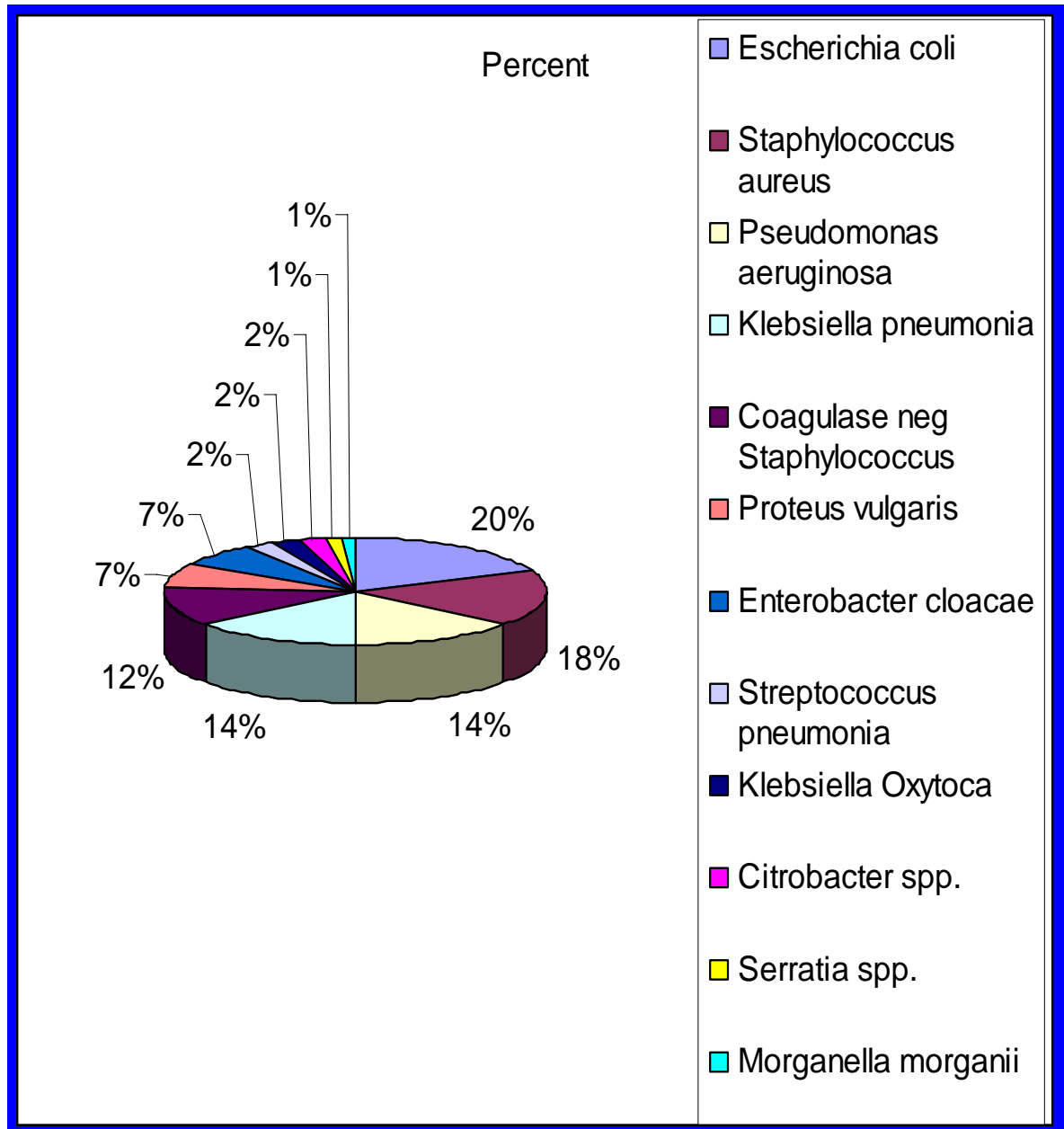


Figure 3.1. Bacterial Etiologic Agents of Nosocomial Infections isolated from patients who were admitted in surgical ward and SICU at Tikur Anbessa University Hospitals, Addis Ababa, (June 2007-April 2008)

Table 3.4. Sites of nosocomial infection and distribution of bacterial isolates from patients who were admitted in surgical ward and SICU at Tikur Anbessa University Hospitals, Addis Ababa, (June 2007-April 2008)

Sites of nosocomial infection	Bacterial isolates	Number	Percent
SSI (n= 44)	<i>Pseudomonas aeruginosa</i>	10	22.7
	<i>Klebsiella pneumoniae</i>	7	15.9
	<i>Staphylococcus aureus</i>	7	15.9
	<i>Proteus vulgaris</i>	6	13.6
	<i>Escherichia coli</i>	5	11.5
	CoNS	4	9.1
	<i>Citrobacter barakii</i>	2	4.5
	<i>Klebsiella oxytoca</i>	2	4.5
	<i>Enterobacter cloacae</i>	1	2.3
	UTI (n= 24)	<i>Escherichia coli</i>	11
<i>Enterobacter cloacae</i>		5	20.8
<i>Klebsiella pneumoniae</i>		4	16.6
<i>Pseudomonas aeruginosa</i>		1	4.2
<i>Morganella morganii</i>		1	4.2
<i>Staphylococcus aureus</i>		1	4.2
<i>Serratia marcescens</i>		1	4.2
BSI (n= 16)		CoNS	6
	<i>Staphylococcus aureus</i>	6	37.5
	<i>Streptococcus pneumoniae</i>	2	12.4
	<i>Klebsiella pneumoniae</i>	1	6.3
	<i>Pseudomonas aeruginosa</i>	1	6.3

CoNS: Coagulase negative staphylococcus

3.4. Antibiotics usage and Outcome

All of the patients included in these studies had received antibiotic/s, in the form of either prophylactic or therapeutic of whom, 77 patients had positive culture results.

3.5. Antimicrobial susceptibility testing

a) Gram positive bacteria

The susceptibility patterns of gram-positive bacteria (n=26) isolated from nosocomial infection against 14 antimicrobial agents are presented in Table 3.5. Almost all bacteria isolates showed multiple drug resistance (resistance to two or more drugs). Most isolates showed high level of resistance (100%) to penicillin, ampicillin, tetracycline, chloramphenicol, and trimethoprim-sulphamethoxazole; and >80% (high level of resistance) to amoxicillin-clavulanic acid, gentamicin, cloxacillin, methicillin, amoxicillin and doxycyclin. But ceftriaxone, norfloxacin and ciprofloxacin showed low level of resistance (<60%).

b) Gram negative bacteria

The susceptibility patterns of gram-negative bacteria (n=58) isolated from nosocomial infections against 13 antimicrobial agents are presented in Table 3.6. All isolates showed high level of resistance (100%) to Amoxicillin, Tetracycline and Trimethoprim-sulphamethoxazole; and > 80% (high level of resistance) to Ampicillin, Amoxicillin-Clavulanic, Chloramphenicol and Doxycyclin. Only Gentamicin showed intermediate level of resistance (60-80%). But Nalidixic acid, Nitrofurantoin, Ceftriaxone, Norfloxacin and Ciprofloxacin showed low level of resistance (<60%). Like Gram positive bacteria, almost all isolated Gram negative bacteria showed multi-drug resistance.

Table 3.5. Antimicrobial Susceptibility Patterns of Gram-Positive Bacteria Isolated from Nosocomial Infections from patients who were admitted to surgical ward and SICU at Tikur Anbessa University Hospitals, Addis Ababa, (June 2007-April 2008)

Organisms		Antimicrobial agents													
		AMP	AMC	CAF	CN	CX	MET	P	AML	TTC	SXT	CRO	DO	NOR	CIP
<i>S. aureus</i> (n=14)	S*	-	1	-	1	-	-	-	-	-	-	6	-	5	8
	I*	-	-	-	-	-	-	-	-	-	-	-	-	1	-
	R*	14	13	14	13	14	14	14	14	14	14	8	14	8	6
CoNS (n=10)	S	-	3	-	1	1	1	-	-	-	-	4	1	4	4
	I	-	-	-	-	-	-	-	1	-	-	-	-	1	-
	R	10	7	10	9	9	9	10	9	10	10	6	9	5	6
<i>S.pneumoniae</i> (n=2)	S	-	1	-	-	-	-	-	-	-	-	1	-	-	1
	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	2	1	2	2	2	2	2	2	2	2	1	2	2	1
Total (n=26)**	S	-	19%	-	8%	4%	4%	-	-	-	-	42%	4%	36%	50%
	I	-	-	-	-	-	-	-	4%	-	-	-	-	8%	-
	R	100%	81%	100%	92%	96%	96%	100%	96%	100%	100%	58%	96%	58%	50%

*S= Sensitive *I=Intermediate *R=Resistant

** Expressed in percent.

AMP: Ampicillin; AMC: Amoxicillin-Clavulanic acid; CAF: Chloramphenicol; CN: Gentamicin; CX: Cloxacillin; MET: Methicillin; P: Penicillin; AML: Amoxicillin; TTC: Tetracycline; SXT: Trimethoprim-sulphamethoxazole; CRO: Ceftriaxone; DO: Doxycyclin; NOR: Norfloxacin; CIP: Ciprofloxacin

Table 3.6. Antimicrobial Susceptibility Patterns of Gram-Negative Bacteria Isolated from Nosocomial Infections from patients who were admitted to surgical ward and SICU at Tikur Anbessa University Hospitals, Addis Ababa, (June 2007-April 2008)

Organisms		Antimicrobial agents												
		AMP	AMC	CAF	CN	NA	FM	AML	TTC	SXT	CRO	DO	NOR	CIP
<i>Escherichia coli</i> (n=16)	S*	-	1	3	7	6	12	-	-	-	6	2	8	10
	I*	-	1	2	1	3	1	-	-	-	1	-	-	-
	R*	16	14	11	8	7	3	16	16	16	9	14	8	6
<i>Klebsiella pneumoniae</i> (n=12)	S	-	1	-	1	1	4	-	-	-	6	-	4	3
	I	-	-	-	-	3	-	-	-	-	-	-	-	-
	R	12	11	12	11	8	8	12	12	12	6	12	8	9
<i>Pseudomonas aeruginosa</i> (n=12)	S	-	1	1	2	3	8	-	-	-	4	-	5	2
	I	-	-	-	-	3	1	-	-	-	1	-	1	1
	R	12	11	11	10	6	3	12	12	12	7	12	6	9
<i>Enterobacter cloacae</i> (n=6)	S	1	1	-	3	2	6	-	-	-	2	-	2	2
	I	-	-	-	-	2	-	-	-	-	-	-	-	-
	R	5	5	6	3	2	-	6	6	6	4	6	4	4
<i>Proteus vulgaris</i> (n=6)	S	-	-	-	2	2	4	-	-	-	3	2	3	3
	I	-	-	-	-	1	-	-	-	-	-	-	-	-
	R	6	6	6	4	3	2	6	6	6	3	4	3	3
<i>Citrobacter spp</i> (n=2)	S	-	-	1	1	2	2	-	-	-	1	-	1	2
	I	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	2	2	1	1	-	-	2	2	2	1	2	1	-
<i>Klebsiella oxytoca</i> (n=2)	S	-	1	-	-	-	-	-	-	-	2	1	2	1
	I	-	-	-	-	1	-	-	-	-	-	-	-	-
	R	2	1	2	2	1	2	2	2	2	-	1	-	1
<i>Morganella morganii</i> (n=1)	S	-	-	-	-	1	1	-	-	-	-	-	-	-
	I	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	1	1	1	1	-	-	1	1	1	1	1	1	1
<i>Serratia spp</i> (n=1)	S	-	-	-	-	-	-	-	-	-	1	-	-	-
	I	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	1	1	1	1	1	1	1	1	1	-	1	1	1
Total (n=58) **	S	2%	8%	9%	27%	29%	64%	-	-	-	43%	9%	43%	39%
	I	-	2%	3%	2%	23%	3%	-	-	-	4%	-	2%	2%
	R	98%	90%	88%	71%	48%	33%	100%	100%	100%	53%	91%	55%	59%

*S= Sensitive *I=Intermediate *R=Resistant

** Expressed in percent

AMP: Ampicillin; AMC: Amoxicillin-Clavulanic acid; CAF: Chloramphenicol; CN: Gentamicin; NA: Nalidixic acid; FM: Nitrofurantoin; AML: Amoxicillin; TTC: Tetracycline; SXT:

Trimethoprim-sulphamethoxazole; CRO: Ceftriaxone; DO: Doxycyclin; NOR: Norfloxacin; CIP:
Ciprofloxacin

CHAPTER IV: DISCUSSION

Nosocomial infections (NIs) are becoming increasing problems for hospitalized patients (Tullu *et al.*, 1998). They are major causes of death and disability worldwide. According to estimates reported by the World Health Organization, up to 15% of hospitalized patients suffer from infections associated with health care (Siempos *et al.*, 2007). More over, hospitals worldwide are continuing to face the crisis of the upsurge and dissemination of antimicrobial-resistant bacteria, particularly those causing nosocomial infections in ICU patients (Hsueh *et al.*, 2002).

In this study, the overall prevalence of nosocomial infection in Tikur Anbessa University Hospital is (9.0%), which is lower than the previous studies in the same hospital, which were 16.4% (Habte-gabr *et al.*, 1988) and 17% (Gedebou *et al.*, 1988) and in Addis Ababa teaching hospitals 13% (Gedebou *et al.*, 1987). This slight decrease in the prevalence of nosocomial infections in this hospital may paralleled to paying attention to well-established processes for decontamination and cleaning of soiled instruments and other items, followed by sterilization and high-level disinfection processes and improving safety in operating rooms and other high-risk areas where the most serious and frequent injuries and exposures to infectious agents occur. Another factor that can account for the lower infection rate may be due to the availability of high number of health personnel and setup of the hospital. On the other hand, the previous report included all departments of the hospital unlike the present study. Other possible reason may be during the study period there was a new method for elected cases from the emergence operated immediately with in two days. This short period of exposure before operation may contribute to low prevalence rate of nosocomial infection in surgical wards.

Besides these, the current study showed lower prevalence of nosocomial infection than reports from different countries like Tunisia which was 13% (Dridi *et al.*, 2006), Kosova 17.4% (Raka *et al.*, 2006) and Morocco 17.8% (Jroundi *et al.*, 2007). One reason for this lower prevalence may due to the method of sample analysis, which was dependent mainly on bacteriological agents. Anaerobic bacterial infections are not included since they are also

other causative agents of nosocomial infection. Viral and fungal agents were not assessed due to the unavailability of technology and laboratory facilities in this study, even though they could cause nosocomial infections. On the other hand most of the study in the above mentioned countries included anaerobic bacteria, fungi and viruses in their study.

In this study, the most nosocomial infection was found to be surgical site infection. Since all patients were exposed for surgical procedures, there is an increase risk of getting an infection in the hospital by direct invading of the patient's body, gives bacteria a way in to normal sterile parts of the body. This infection can be acquired from contaminated surgical equipment or from health care workers. And also the susceptibility to surgical wound infections were enhanced by poor wound care and prolonged hospitalization. Due to the above reason, surgical wound can colonized by micro-organisms and showed the highest nosocomial infection site. Urinary tract infection was the second infection site in the present study. Since, all of the patients with nosocomial UTI had urinary catheters and catheterization increased the rate of infection. There may be bacteria in or around the urethra but they normally can not enter to the bladder. A catheter can pick up bacteria from the urethra and allow them into the bladder, causing an infection. The other infection site was BSI predominantly occurs with a frequency of 4.3 times in SICU than the other surgical wards. This is because; patients in the SICU were critically ill and patients who can not cough or gag very well are most likely to inhale colonized microorganisms into their lungs. Some respiratory procedures can keep patients from gagging or coughing. Patients who are sedated or who lose consciousness may also be unable to cough or gag. Therefore, the inhaled microorganism grows in the lungs and causes an infection that can lead to blood stream infection. More over, many interventions measures which were risk factors for BSI frequently done in this ward like use of invasive-devices (e.g. venous catheterization, respiratory intubation, urinary or nasogastric tubes); mechanical ventilation; suctioning of material from the throat and mouth; the utilization of drugs such as sedatives; or the influence of surgical procedures. The distribution of nosocomial infection in different sites are in agreement with previous studies done in Ethiopia by Habte-gabr *et al.* (1988) and Gedebeu *et al.* (1987), as well as in Latvia by Dumpis *et al.*(2003), in Turkey by Durmaz *et*

al. (2000), in Taiwan by Wu *et al.* (2006) and Chang *et al.* (1990), in Tunisia by Dridi *et al.* (2006) and Cuba by Izquierdo-Cubas *et al.* (2008).

The common interventions done in the hospitals are mechanical ventilation, urinary catheterization, surgical procedures and insertion of central venous lines and not surprisingly, these are responsible for the nosocomial infections. Such invasive procedures are routinely done in the surgical wards and these wards are becoming reservoirs of multiple drug resistance bacteria. In the present study, these interventions have significant relationship with nosocomial infection (Table 3.3.). They can also introduce infectious agents to the sites where instruments are placed. This can be favoured for bacterial colonization, which if left unchecked, can become full-blown infections. Considering that these determinants can be altered, efforts to reduce nosocomial infection should be directed along this line. These are in line with other studies in Turkey (Savas *et al.*, 2006, Inan *et al.*, 2005), Kuwait (Kehinde *et al.*, 2004), India (Tullu *et al.*, 1998) and Latvia (Dumpis *et al.*, 2003). In order to alleviate this problem, interventions like urinary catheters should only be used when required, should be inserted under aseptic conditions and cleaned daily, should not be left in place for a long time, closed drainage systems should be used when possible and suprapubic catheters should be used in selected cases. Hand washing, a simple but proven practice that reduces nosocomial infection spread should not be forgotten.

Among the total bacteria isolated gram-negative bacteria (72.6%) were the predominant isolates while gram-positive bacteria constitute for only 23.4%. Similar findings have been observed in Ethiopia (Gedebou *et al.*, 1987; Habte-gabr *et al.*, 1988; Gedebou *et al.*, 1988), Algeria (Atif *et al.*, 2006), Tunisia (Ben Jaballah *et al.*, 2006), Philippines (Alora *et al.*, 1990) and French (Garrabé *et al.*, 2000).

Data presented in this study indicate that most frequent bacterial isolates from surgical site infections were *P. aeruginosa* (22.7%), *Klebsiella* spp. (20.4%) and *S. aureus* (15.9%). Similar finding have been seen in Philippines (Alora *et al.*, 1990) and Turkey (Bayram and Balci, 2006). Among urinary tract nosocomial infections *E. coli* is the most frequent bacterial isolate. This finding also similar with other finding in different publications,

Ethiopia (Moges *et al.*, 2002; Wolday and Erge, 1997), Taiwan (Hsueh *et al.*, 2002), India (Mohanty *et al.*, 2003; Tullu *et al.*, 1998), Turkey (Akbas *et al.*, 1998; Savas *et al.*, 2006; Saraçlı *et al.*, 1999), Poland (Hryniewicz *et al.*, 2001), Philippines (Alora *et al.*, 1990), USA (Jarvis and Martone, 1992) and Brazil (Dias Neto *et al.*, 2003). Coagulase negative staphylococcus (37.5%) and *Staphylococcus aureus* (37.5%) were the predominant organism in blood stream nosocomial infections. Similar findings have been observed in Ethiopia (Asrat and W. Amanuel, 2001), United States (Karlowsky *et al.*, 2004), Taiwan (Wu *et al.*, 2006), USA (Jarvis and Martone, 1992) and United States and Canada (Pfaller *et al.*, 1998).

Currently many micro organisms have become resistant to different antimicrobial agents and in some cases to nearly all agents. Resistance to antimicrobial agents is a problem in health care facilities, but in hospitals, transmission of bacteria is amplified because of the highly susceptible population (WHO, 2002). The antibiotic sensitivity of our study confirmed the alarming percentage of resistance exhibited by pathogens to the common antibiotics in use. In this particular study, both Gram-positive and Gram-negative bacterial isolates showed high resistance level to Ampicillin, Amoxicillin-Clavulanic acid, Chloramphenicol, Amoxicillin, Tetracycline, Trimethoprim-sulphamethoxazole and Doxycyclin. But only Gram-positive bacteria showed high resistance level for Gentamicin and Gram-negative bacteria showed intermediate level of resistance to Gentamicin. The result has also indicated that Gram-positive and Gram-negative bacteria isolated from nosocomial infections had low resistance rates (<60%) to ciprofloxacin, Norfloxacin, and Ceftriaxone (Tables 3.5 and 3.6). Gram-negative bacteria showed low-level resistance to Nitrofurantoin and Nalidixic acid. The Rate of resistance for gram positive range from 50% to 100%, and for gram negatives range from 33% to 100%. Nitrofurantoin, Nalidixic acid, Ciprofloxacin, Norfloxacin, and Ceftriaxone were relatively effective antibiotics for the treatment of pathogens which are responsible to cause nosocomial infections. This is perhaps so because these agents are not commonly used and newly introduced. However, the uses of drugs like Nalidixic acid, Norfloxacin, and Nitrofurantoin are limited in practice because of their high cost. Due to this fact, they showed low level of resistance. According to this study, it appears that the clinician is left with very few choices of drugs for the treatment of nosocomial infections.

In general, rates of resistance to all antibiotics tested for gram negatives were low as compared to gram positives bacterial isolates. This is in agreement with previous study done in Ethiopia where most of the isolates from NIs were resistant to commonly used antibiotics (Habte-gabr *et al*, 1988; Gedebeu *et al.*, 1987; Asrat and W. Amanuel, 2001) and Thailand (Danchaivijitr *et al.*, 2005). However, the present study showed a high prevalence of resistance to the commonly prescribed antimicrobial agents. This may be because of the intense use of antimicrobial agent in the hospital, easy availability and indiscriminate use of these drugs out side the hospitals, and many antibiotics are available over the counter for self-medication. These problems, coupled with the increase chance of cross infection among inpatients, are known to account for circulating resistance strains.

LIMITATIONS OF THE STUDY

- The study does not include all wards in Tikur Anbessa University Hospitals like Medical ward, Medical ICU, Paediatric ward and Gynaecology and Obstetrics ward in which high nosocomial infections are suspected.
- It was not possible to include anaerobic bacteria due to budget and laboratory facilities constraints.
- The design of the study did not include fungi and other pathogens that are important causes of nosocomial infections as well.
- Patients who develop nosocomial infections after being discharged were not included in this study.

CONCLUSION

The prevalence of nosocomial infection at Tikur Anbessa University Hospitals decreased from the previous study in the same hospital. In SSIs and UTIs gram-negative bacteria (75% and 95.8%), respectively were the predominant isolates, where as in BSIs gram-positive bacteria (87.4%) were the predominant organisms. Ceftriaxone and Ciprofloxacin were relatively effective drugs for gram-positive bacteria, where as Norfloxacin, Ceftriaxone and Nitrofurantoin were relatively effective drugs for gram-negative bacteria. However, gram-positive bacteria isolated from nosocomial infection were 100% resistance to Ampicillin, Tetracycline, Trimethoprim-sulphamethoxazole and Chloramphenicol. In addition, gram-negative bacterial isolates were 100% resistance to Amoxicillin, Tetracycline and Trimethoprim-sulphamethoxazole.

RECOMMENDATIONS

Based on these findings the following recommendations are made: -

- The prevalence and drug susceptibility pattern of Nosocomial infections should be done by including anaerobic bacteria, fungus and other micro-organism in all wards in Tikur Anbessa University hospital.

- Empirical treatment to nosocomial infections provoke drug resistance, therefore treatment should be based on the result of culture and sensitivity. In order to achieve this, the capacity of microbiology laboratory should be strengthened with trained manpower, budget and necessary laboratory equipments.
- There is a need for a continuous surveillance for resistant bacteria to provide the basis of alternative treatment.
- Nosocomial infection control should be directed to the hospital and strength with different staff, supplies, budget, etc.
- This study suggests that if one could not wait the culture results in Nosocomial infection, Ampicillin, Amoxicillin-Clavulanic acid, Amoxicillin, Methicillin, Tetracycline, Trimethoprim-sulphamethoxazole, Doxycyclin, Chloramphenicol and Penicillin are quite ineffective to treat these infections

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Appendix I

Questionnaire

The purpose of this questionnaire is to study the demography and nosocomial related infections (prevalence with their associated risk factors) in patients who are admitted to surgical wards and intensive care unit (ICU) of Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. Your responses will not be used for other purposes than research. So you are kindly requested to give your responses honestly.

A data collection form for Prevalence of Nosocomial infections

1. Patient identification

Ward -----

Age (years) _____

Gender Male Female

Date of admission in the hospital (dd/mm/yy) _____

2. Patient exposure

Surgical procedure (during the last month) _ _ Yes No

Urinary catheter Yes No

Mechanical ventilation Yes No

Intravascular catheter Yes No

Antibiotic Yes No

If yes, prescription for

Prophylaxis Therapy Other/unknown

3. ***Nosocomial Infection,*** Yes No

If yes, fill the following items

Surgical site infection Yes No

Urinary tract infection Yes No

Bloodstream infection Yes No

Pneumonia Yes No

Other respiratory infection Yes No

Line-related infection Yes No

Other nosocomial infection Yes No

A data collection form for surgical site infection

Hospital _____

Unit _____

1. Patient

Patient identification _____

Age (years) _____

Gender Male Female

Cause of admission (diagnosis) _____

Date of admission (in the hospital)(dd/mm/yy)_____

Date of discharge (from the unit)(dd/mm/yy)_____

2. Operation

Date of operation (dd/mm/yy)_____ Main procedure (code)

Wound class Clean Contaminated

Clean-contaminated Dirty/infected

ASA score 1. 2. 3. 4. 5.

Duration of operation (minutes)_____

Urgent Yes No

Prosthesis/implant Yes No

Multiple procedures Yes No

Coeliosurgery Yes No

3. Antibiotics

Antimicrobial prophylaxis __ Yes No

Starting date (dd/mm/yy)_____

Duration (days)_____

4. Surgical site infection

Surgical site infection Yes No

Date of infection (dd/mm/yy) _____

Infection site _ _ Superficial Deep Organ/Space

Microorganism 1 _____

Microorganism 2 _____

5. Any risks

Age -----

Underlying diseases _____

Invasive devices _____

Appendix II

Consent form

The objective of this study is to investigate the prevalence of nosocomial infections, their antibiotic susceptibility patterns and associated risk factors which lead to nosocomial infections among patients who are admitted in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

Complications in nosocomial infections cause devastating consequences in the socio-economics of our community in general and the patients in particular. Patients will be assessed for nosocomial infections and their associated risk factors during the study period. This Cross sectional study has the potential to point out measures to reduce the incidence of cases in nosocomial infections, significantly increased health care cost, and the possibility of longer period of hospitalization with final goal of improving patient outcomes.

Therefore, here we request your kindly participation in this study which requires your willingness to give samples for laboratory examination, to respond to an interview, to allow physical examination and follow-up needed. You have full right and free choice to either participate or not in this study and it will never affect your right of getting appropriate treatment. Results will be confidential and reported to the requesting physician for appropriate treatment and management.

For adult patients who are able to respond:

I _____, after being fully informed about the purpose of this study, hereby give my consent to participate in this study as the doctors find best for me.

Signature _____ Date _____

For families or attendants of patients unable to respond:

I _____ parent/guardian/attendant, after being fully informed about the purpose of this study, here by give my consent on the patient's participation in this study as the doctors find best for the patient.

Signature: _____ Date _____

የስምምነት መግለጫ ቅጽ

(ትርጉም በአማራኛ)

የዚህ ጥናት ዋና ግብ ለሕመምን ሆስፒታል ውስጥ ተኝተው በሚታከሙበት ወቅት ተኝተው ከሚታከሙበት በሽታ ሌላ ሌሎች ሕመም ሊያስከትሉ የሚችሉ ተህዋስያንን ለይቶ ማውጣትና እንዲሁም ተያያዥነት ያላቸውን ችግሮች ማወቅ ሲሆን

ሕመምን በሆስፒታል ቆይታቸው ወቅት በሽታ ሊያስከትሉ በሚችሉ ተህዋስያን መመረዛቸው ለተለያዩ ወስብስብ ችግሮች ሲዳረጉ ይታያሉ። ስለዚህ በጥቅሉ የዚህ ጥናት አላማ እነዚህን ችግሮች አውቆ የመፍትሔ እርምጃ እንዲወስድ ለማመልከት ነው። በዚህ ጥናት የሚዳሰሱ ነገሮች ሐሳባቸውን መግለጥ ለሚችሉ አዋቂች፡

እኔ _____, የዚህን ጥናት ግብ በግልፅ

ስለተረዳሁ በጥናቱ ለመሳተፍና ለሕመሙ ያመነበትን አካሄድ ለመከተል መስማማቴን በፊርማዬ አረጋግጣለሁ።

ፊርማ _____ ቀን _____

ለሕጻናትና ሐሳባቸውን መግለጽ ለማይችሉ አዋቂች አስታማሚች፡

እኔ _____, የበሽተኛው አስታማኝ ስሆን የዚህን ጥናት ዓላማ በወል በመገንዘብ በሽተኛው ቢሳተፍበትና ሐኪሙ ያመነበትን አካሄድ ቢከተል የምስማማ መሆኔን በፊርማዬ አረጋግጣለሁ።

ፊርማ _____ ቀን _____

Annex I

Laboratory procedure for Collection and Culturing of blood specimen:

Blood should be collected aseptically at the time when the patient's temperature is beginning to rise. If, however, infective endocarditis is suspected, blood can be collected at any time.

Blood for culture must be collected as aseptically as possible. The following technique is recommended.

1. Using a pressure cuff, locate a suitable vein in the arm.
2. Cleanse thoroughly the skin over the vein using tincture of iodine followed by ethanol-ether. Use a circular action to swab the area beginning at the point where the needle will enter the vein.

3. Lift back the tape or remove the protective caps from the tops of the culture bottles, and cleanse the top of each bottle using an ethanol-ether swab. Do not use a bottle of culture medium if it shows signs of contamination, i.e. turbid broth.
4. Using a sterile syringe and size 21-gauge needle, withdraw 10-12ml of blood.
5. With care, remove the needle from the syringe (return it to its holder), and replace with another sterile needle of similar size.
6. Insert the needle through the rubber liver of the bottle cap and dispense 5ml of blood into each culture bottle. Dispense the remaining blood into a tube or bottle containing ethylene diamine tetra acetic acid (EDTA).
7. Using an ethanol-ether swab, wipe the top of each culture broth and replace the tape or protective caps. Gently mix the blood with the broth mix the blood in the EDTA container.

N.B. The blood must not be allowed to clot in the culture media because any bacteria will become trapped in the clot.

8. Using a lead pencil, label each bottle with the name and number of the patient, and the date and time of collection.
9. As soon as possible, incubate the inoculated media.

Thioglycollate broth

- Incubate at 35-37 °C for up to 2 weeks, examine daily up to 14days look for visible signs of bacterial growth such as turbidity above the red cell layer, colonies growing on top of the red cells ('cotton balls'), haemolysis gas bubbles and clots.
- Subculturing a blood culture broth in a strict aseptic technique to avoid contaminating and inoculate the broth on Blood agar, Chocolate agar and MacConkey agar.
- If growth is present, subculture on blood agar, chocolate agar, and MacConkey agar.
- Examine a gram stained smear of the colonies depending on the bacteria seen, test the colonies further e.g. for coagulate, catalase, oxidase, urease and motility.

- If large gram-positive rods resembling *C. perfringens* are seen, subculture also on lactose egg yolk milk agar and incubate the plate anaerobically.

If motile, urease and oxidase negative, gram-negative rods are isolated, subculture the colonies on Klinger iron agar or perform a rapid APIZ screening test for salmonella.

- If catalase positive gram-negative coccobacilli are isolated from a diphasic culture that has been incubated in a carbon dioxide atmosphere, suspect *Brucella* species and send the culture to a reference laboratory for identification.
- If no growth is seen on the slope of the diphasic culture, wash the broth over the slope before reincubating the culture (do not allow the broth to flow into the neck of the bottle) (Bauer *et al.*, 1966).

Annex II

Laboratory procedure for collection and culturing of urine sample:

Mid- stream urine specimen: - a specimen obtained from the middle part of urine flow:

Clean catch urine specimen

The best method is properly collected “clean catch” urine which is collected as follow:

- ↳ The genital area should be cleaned with soap and water and rinsed well. This is to keep off bacteria on the skin from contaminating the urine specimen.
- ↳ The patient should urinate a small amount and this is discarded.
- ↳ The urine that comes next, the mid-stream specimen, should be collected into a sterile container of 30 to 50ml.
- ↳ After obtaining the specimen the patient continues to urinate and this is discarded

Urine Culture is required if the urine contains bacteria, cells, protein, nitrite, or has a markedly alkaline or acid reaction.

Blood agar and MacConkey agar: Mix the urine well by inverting the container several times. Using a sterile calibrated wire loop, inoculate a loopful of urine on blood agar and MacConkey agar.

The loop must be held vertical and only the loop must be dipped in the urine. If the stem is also immersed, more than the required volume of urine will be inoculated.

The use of blood agar is recommended in addition to MacConkey agar because it assists in the rapid identification of pathogens, and enables haemolytic streptococci and other Gram positive organisms to be isolated which grow poorly or not at all on MacConkey agar.

Incubate the inoculated plates aerobically at 35-37 °C over night.

Estimating bacterial numbers

It is necessary to estimate the approximate number of bacteria in urine because normal specimens may contain small numbers of contaminating organisms, usually less than 10,000(10^4) per ml of urine. Urine from a person with an untreated urinary infection usually contains 100,000(10^5) or more bacteria per ml (Cheesbrough, 2004).

Annex III

Laboratory procedure for collection and culturing of wound swab from surgical site:

- Be aware that most wounds need some form of preparation to reduce the risk for introducing extraneous organisms into the collected specimen. In the presence of

moderate to heavy pus or drainage, irrigate the wound with sterile saline until all visible debris has been washed away. When culturing chronically present wounds (pressure sores), remove the wound surface of any loose necrotic, sloughed material before culturing. Cultures of the surface alone may be misleading; biopsies of deeper tissue are recommended.

- Disinfect the surface of the wound with 70% alcohol or an iodine solution.
- Apply sterile gauze pads to absorb excess saline and to expose the culture site. Always culture highly vascular areas of granulation tissue. Wearing sterile gloves, separate margins of deep wounds with thumb and forefinger to permit insertion of the swab deep into the wound cavity. Press and rotate the swab several times over the clean wound surfaces to extract tissue fluid containing the potential pathogen. Avoid touching the swab to intact skin at the wound edges.
- Immediately place the swab into the appropriate transport container (Fischbach, 2004).

Annex IV

Laboratory procedure for Gram staining technique:

Gram staining technique

1. Labeling the slides clearly with the date and patient's name and number.
2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.
4. Fix the dried smear by using heat or chemicals (methanol).
5. Cover the fixed smear with crystal violet stain for 30-60 seconds.
6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.
7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with clean water.
10. Cover the smear with neutral red or safranin stain for 2 minutes.
11. Wash off the stain with clean water.
12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.
13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

- Gram positive bacteria -----dark purple
- Gram-negative bacteria -----pale to dark red (Bauer *et al.*, 1966).

Annex V

Laboratory procedure for disc diffusion sensitivity testing:

1. Emulsify several colonies of similar appearance of test organism a small volume of sterile peptone water, nutrient broth, or quarter strength ringer solution.
2. Match the turbidity of the suspension against the turbidity standard which has a similar appearance to an over night broth culture. When comparing turbidity it is easier to view against a printed card or sheet of paper.

Note: If preferred, the test organism can be subculture in sterile peptone water or nutrient broth to give a growth which matches that of the turbidity standard.

3. Using a sterile loop of about 4mm diameter, apply a loopful of the test organism suspension (subculture) to the centre of the sensitivity testing plate use a sterile dry cotton wool swab to spread the inoculum evenly across the third of the plate.
4. Using a similar inoculation technique, inoculate an over night broth culture of the control organism evenly across the upper and lower third of the plate, leaving a distance of no more than 5 mm on each side of the test organism.
5. Allow the inoculum to dry for a few minutes with the Petri dish lid in place.
6. Using sterile forceps or a needle mounted in a holder, place the antimicrobial discs (previously warmed to room temperature) between the test and control inoculum.
7. Within 30 minutes of applying the disks incubate the plate aerobically at 35-37°C over night.
8. Read the tests after checking that the bacterial growth of the test and control organism is neither too heavy nor too light and the control inhibition zone, from the edge of the disc to the edge of the zone the end point of inhibition is growth starts.

Interpretation of results

Report the reaction of the test organism to each antibiotic as 'sensitivity', 'intermediate', or 'resistant', as follows:

Sensitivity (S): Zone of radius is wider than, equal to, or not more than 3mm smaller than the control.

A pathogen reported as sensitivity suggests that the infection it has caused is likely to respond to treatment of the drug to which it is susceptible is used in normal recommended dose.

Intermediate (I): Zone radius is more than 3mm smaller than the control but not less than 3mm.

A pathogen reported as being intermediately sensitive suggests that the infection it has caused is likely to respond to treatment if the drug to which it is susceptible is used in larger doses than normal.

Resistant (R): No zone of inhibition or zone radius measure 2mm or less.

A pathogen reported as resistant implies that the infection it has caused will not respond to treatment with the drug to which it is resistant irrespective of dose or site (Cheesbrough, 2004).

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the candidate _____

Signature _____

Place _____

Date of submission ____/____/____

This thesis has been submitted for examination with my approval as university advisor.

Name of advisor _____

Signature _____

Place _____

Date of submission ____/____/____

Name of examiner _____

Signature _____

Place _____

Date of submission ____/____/____