LONGITUDINAL BACTERIOLOGY OF BURN PATIENTS AT YEKATIT 12 HOSPITAL BURN CENTER, ADDIS ABABA, ETHIOPIA

BY

AWOKE DERIBIE (BSc.)

A THESIS SUMMITED TO GRADUATE STUDIES, ADDIS ABABA UNIVERSITY, FACULTY OF MEDICINE, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL MICROBIOLOGY

APRIL, 2011

ADDIS ABABA-ETHIOPIA

Addis Ababa University
School of Graduate Studies Faculty of Medicine
Department of Medical Microbiology, Immunology and Parasitology

MSc. thesis

LONGITUDINAL BACTERIOLOGY OF BURN PATIENTS AT YEKATIT 12 HOSPITAL BURN CENTER, ADDIS ABABA, ETHIOPIA

Principal investigator: **Awoke Deribie (BSc.)**

Advisors:

1. **Tamrat Abebe** (BSc., MSc., PhD. candidate)
   Faculty of Medicine Department of Medical Microbiology, Immunology and Parasitology, AAU

2. **Adane Mihret** (DVM, MSc., PhD. candidate)
   Faculty of Medicine Department of Medical Microbiology, Immunology and Parasitology, AAU

3. **Yohannes Demisie** (MD, Chief Plastic Surgeon)
   Faculty of Medicine, Department of surgery, AAU. Head, Burn unit at Yekatit 12 hospital

ACKNOWLEDGEMENTS

I would like to pass on my heartfelt gratitude to my advisors Mr. Tamrat Abebe, Dr. Adane Mihret and Dr. Yohanes Demisie for their wholehearted valuable professional support in advising and commenting me on this thesis development starting from the very beginning.

I would like also thank all the staff of clinical laboratory team at Yekatit 12 hospital for their assistance and support. My special thanks is goes to Mr. Ayelign Derebe and other members of the bacteriology unit who were with me until the end of data collection period with their technical assistance.

I am glad to thank all staff of the Burn center at Yekatit 12 hospital for their kind cooperation during sample collection.

This research was funded by the core budget of School of Graduate Studies, Addis Ababa University. My host institution was Wollega University.

Lastly, my acknowledgment is extends to my families, friends and those who put their hands on directly or indirectly for accomplishment of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

AAU	Addis Ababa University
BA	Blood Agar
BU	Burn Unit
CoNS	Coagulase Negative Staphylococci
DMIP	Department of Medical Microbiology, Immunology and Parasitology
KIA	Kligler Iron Agar
MDR	Multi Drug Resistant
MOH	Ministry of Health
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSA	Manitol Salt Agar
NNIS	National Nosocomial Infection Surveillance
NCCLS	National Committee for Clinical Laboratory Standards.
OPD	Outpatient Department
QC	Quality Control
SOPs	Standard Operating Procedures
Spp	Species
SPSS	Statistical Package for Social Sciences
TBSA	Total Burned Surface Area
TSY	Tryptone Soya agar
WHO	World Health Organization

ABSTRACT

Introduction: Burn is one of the most common, devastating and a very painful form of trauma. Significant burn injuries induce a state of immunosuppression that predisposes burn patients to infectious complications. Sepsis and the resultant multi-organ failure are the leading causes of death in intensive burn care units. Rapidly emerging nosocomial and community acquired pathogens and the problem of multidrug resistance necessitates periodic review of isolation outline and their antibiotic susceptibility pattern in burn centers.

Objective: The aim of this study was to determine longitudinal bacteriology of burn patients at Yekatit 12 hospital burn center, Addis Ababa.

Methods and materials: A prospective hospital based study was carried out from Dec 2010 to Feb 2011. Wound swab and blood samples were collected on 0, 7th, and 14th, days of hospital stay and processed by conventional culture and biochemical identification procedures. Once identified, isolates were tested against commonly used antibiotics in the hospital by Modified Kirby-Bauer disc diffusion method. Data was analyzed by SPSS version 17.0 for Windows.

Results: Forty one patients were enrolled in the study from both burn unit (BU) and outpatient (OPD). The mean total burned surface area (TBSA) was 11.9%. From 104 pus cultures 101 isolates were identified during the study period. In the first pus culture *S. aureus* was predominated, 15(46.9%) but later on *Pseudomonas* spp was increasing. Both spp together accounted 87.1% of the total isolates. Regarding blood culture isolates there was no significant change on time. Of 92 blood cultures 15 gram positive isolates were recognized. *Coagulase negative Staphylococci* (CoNS) noted with the largest percentage, 8 (53.3%) followed by *S. aureus* 6 (40%). *Staphylococcus aureus* showed moderate degree of resistance to the commonly used antibiotics. Majority of isolates were susceptible for clindamycin and vancomycin. But *Pseudomonas* spp were resistant for most of antibiotics used in the hospital. However, it was found more sensitive to norfloxacine as it was evidenced by only 15% resistance.

Conclusion: Every treatment facility has microorganisms unique to it and these might change with time. It is therefore of paramount importance to have an in-depth knowledge of the resident organisms and their antibiotic sensitivity pattern so that infection related morbidity and mortality will improved. The nature of microbial wound colonization and flora changes should be taken into consideration in empirical antimicrobial therapy of burned patients.

Key words/phrases: ***Burn, Sepsis, Time related bacterial colonization, Antibiotic resistance***

CHAPTER ONE
INTRODUCTION

1.1. Background

Skin is one of the largest organs in the human body in terms of size (1.5 to 2.0 m^2) and weight (6 to 10 kg for average adult). An intact human skin surface is vital to the preservation of body fluid homeostasis, thermoregulation, and the host's protection against infection. The skin also has immunological, neurosensory, and metabolic functions such as vitamin D metabolism (Chalise *et al*., 2008)**.** The skin is an essential component of the nonspecific immune system, protecting the host from potential pathogens in the environment. Breaches in this protective barrier thus represent a form of immunocompromised that predisposes the patient to infection.

Thermal burns are burns to the skin caused by any external heat source. This may be in the form of a naked flame from an open fireplace or house fire, a scald from steam, hot or molten liquid, or via direct contact with a hot object such as a hot oven rack or hot cooking pan. Other types of burns include radiation burns (from the sun's ultraviolet rays), chemical burns and electrical burns. (Chalise *et al.*, 2008; Lawrence, 2008)

Developing countries have a high incidence of burn injuries, creating a formidable public health problem. Moreover, high population density, illiteracy and poverty are the main demographic factors associated with a high risk of burn (Khajuria *et al*., 2009). Despite major advances in the care of burned patients, infectious complications remain an important cause of morbidity and death. Furthermore, wound invasion still represents a major cause of infection in burn intensive care units (Santucci *et al.*, 2003).

The burn injury causes devitalization of tissues and produces extensive raw areas. The wound is moist owing to flow of serous exudate at temperatures approaching 37 ^{0}C or above. The dead and denatured burn eschar and the inmost wound environment favor the colonization and proliferation of a variety of microorganisms. Burn causes immune suppression. The dysfunction of the immune system, large cutaneous bacterial load, the possibility of gastrointestinal bacterial translocation, prolonged hospitalization, and invasive diagnostic and therapeutic procedures all contribute to sepsis. The burn wound has a much higher incidence of infections compared with other forms of trauma because of extensive skin barrier destruction as well as alteration of the cellular and humoral immune responses (Sanyal, 1998).

Initially, the burnt area is considered free of microbial contamination. But gram-positive bacteria in the depth of sweat glands and hair follicles heavily colonize the wounds within 48 h of the injury from the patient's own normal flora, exogenous sources in the environment, and from healthcare personnel (Vindenes and Jerknes, 1995). Following colonization, these organisms start penetrating the viable tissue depending on their invasive capacity, local wound factors and the degree of the patient's immunosuppression. If sub-eschar tissue is invaded, disseminated infection is likely to occur. (Srinivasan and Vartak, 2009). Infection with systemic sepsis remains the major cause of death in burn patients. Between 63 and 75% of deaths from burn injuries in different centers were due to sepsis. This led to the indiscriminate use of broad spectrum antibiotics which transformed burn units into sites of multidrug resistant virulent microbial flora (Sanyal, 1998).

The ultimate goal of burn-wound management is closure and healing of the wound. Early surgical excision of burned tissue, with extensive debridement of necrotic tissue and grafting of skin or skin substitutes, greatly decreases mortality rates associated with severe burns. In addition, topical antimicrobial agents dramatically decrease the bacterial burden of burn wounds and reduce the incidence of burn-wound infection (Lawrence, 2008).

1.2. Statement of the problem

Burn injury is a major problem in many parts of the world. It has been estimated that 75% of all deaths following burns are related to infection. Burn injury destroys the skin barrier that normally prevents invasion by microorganisms. This makes the burn wound the most frequent origin of sepsis in these patients. Multi-organ failure and infection complications are the major causes of morbidity and death in serious burn injury and as many as 10,000 patients in United States die of burn-related infections each year (Lawrence, 2008; Wolf and Herndon, 1999).

The causative infective microorganisms in any burn facility change with time. Individual organisms are brought into the burns ward on the wounds of new patients. These organisms then persist in the resident flora of the burn treatment facility for a variable period of time, only to be replaced by newly arriving microorganisms. Introduction of new topical agents and systemic antibiotics influence the flora of the wound. Thus, it is just not sufficient to be aware of the microorganisms that pose a problem for burn patients. To have an in-depth knowledge of the organisms that are predominant in that particular treatment facility during the particular period along with their sensitivity pattern is vital as many septic burn patients need to be treated with antibiotics before the results of microbiological cultures are available. This would be crucial to reduce the overall infection-related morbidity and mortality (Srinivasan and Vartak, 2009).

Burn injuries are very common throughout the world and especially so in the underdeveloped country like ours where people are depend on traditional way of cooking and handling fire. Burns cause great suffering to individual patients and are a great cost to one of the poorest countries in the world where access to burn care facilities and thus proper treatment are compromised (Khajuria *et al*., 2009).

Rapidly emerging nosocomial and community acquired pathogens and the problem of multi-drug resistance necessitates periodic review of isolation patterns and antibiogram in the burn ward. The burn center at Yekatit 12 hospital is no more than of its kind in the country. Isolated burn units were absent in Ethiopia prior to the establishment of this hospital burn center. No studies have been done on longitudinal bacteriology profile of burn patients in Ethiopia even though some investigations have been done on general microbiology of burn patients. This study was carried out to determine time related burn wound colonization and septicaemia caused by bacterial pathogens isolated from burned patients at Yekatit 12 hospital burn center, also the susceptibility of these isolates to various antibiotics was assessed.

CHAPTER TWO

LITERATURE REVIEW

2.1. Common bacterial pathogens associated with burn wound infection

Bacteria rapidly colonize open skin wounds after burn injury. Microorganisms colonizing the burn wound originate from the patient's endogenous skin and gastrointestinal and respiratory flora. Microorganisms may also be transferred to a patient's skin surface via contact with contaminated external environmental surfaces, water, fomites, air, and the soiled hands of health care workers. Immediately following injury, gram-positive bacteria from the patient's endogenous skin flora or the external environment predominantly colonize the burn wound. Endogenous gram-negative bacteria from the patient's gastrointestinal flora also rapidly colonize the burn wound surface in the first few days after injury. Wound colonization by yeasts and fungi usually occurs later due to the use of broad-spectrum antibiotic therapy. Microorganisms transmitted from the hospital environment tend to be more resistant to antimicrobial agents than those originating from the patient's normal flora (Church *et al*., 2006).

Staphylococcus aureus became the principal etiological agent of burn wound infections shortly after the introduction of penicillin G in the early 1950s, which resulted in the virtual elimination of *Streptococcus pyogenes* as a cause of infection in thermally injured patients. Although *Staphylococcus aureus* remains a common cause of early burn wound infection, *Pseudomonas aeruginosa* from the patient's endogenous gastrointestinal flora and/or an environmental source is the most common cause of burn wound infections in many centers. The incidence of infections due to less commonly encountered microbes, including other gram-positive and gram-negative bacteria, fungi, and viruses, has also increased steadily in subsequent decades (Church *et al*., 2006; Lawrence, 2008).

In a study conducted to assess Changes of microbial flora and wound colonization in Turkey; from Periodic swabs taken on admission and 7th, 14th, and 21st days of hospitalization. The most prevalent isolates were *Coagulase-negative staphylococci* (CoNS, 63.0%) and *Staphylococcus aureus* (19.7%). There was a gradual decrease in the number of isolates of CoNS and a marked increase in the numbers of *S. aureus* and *P. aeruginosa* from admission to 21st day. At the 21st day, the most frequent organisms were *S. aureus* (37.6%), CoNS (34.7%), and

P. aeruginosa (16.2%). Methicillin resistance staphylococcus (MRSA) strains were increased constantly in study period. While 35.3% of burn wounds were sterile on admission, microbial colonization reached 86.3% within the first week (Erol *et al*., 2004).

In another study designed to assess Bacterial isolates from burn wound infections and their antibiograms at Government Medical College Hospital, Chandigarh: during the period from 2002 to 2005 *Pseudomonas* species was the commonest pathogen isolated (51.5%) followed by *Acinetobacter* species (14.28%), *S. aureus* (11.15%), *Klebsiella* species (9.23%) and *Proteus* species (2.3%). *Pseudomonas* species was resistant to amikacin (85.18%), gentamicin (89.22%), ciprofloxacin (78.81%), and ceftazidmine (79.09%) (Mehta *et al.*, 2007).

There was a study at Yekatit 12 Hospital conducted by Negeri in (2005) to assess Microbiology of BU. The investigator took periodic swab sample to observe the time related change of colonizing pathogens. In this study from the whole study population (n=52), a total number of 171 wound swabs from 38 infected burn wounds were processed. Fifty five isolates were recovered from the swabs of infected wounds, of which *S. aureus* accounted for 40.0% (22/55), and *P. aeruginosa* for 27.3 %(15/55). On the other hand, 32 isolates were recovered from the 33 patients who presented with no infection at admission of which the most common isolates were *S. aureus* 12 (37.5%) *P. aeruginosa*, 12 (37.5%) and *Citrobacter spp*.2 (6.3%). From the study, all of the isolates (n=26) of *S. aureus* were sensitive to methicillin, clindamycin and vancomycin. Twelve isolates (46.2%) were sensitive to chloramphenicol, 22(84.6%) to cephalothin, 24(92.3%) to amoxicillin-clavulanic acid, and 8(30.8%) to tetracycline. On the other hand, 24(92.3%) and 25(96.2%) were resistant to ampicillin and penicillin G respectively. A high level of drug resistance was seen among the Gram negative isolates, particularly *P. aeruginosa*. Ninety percent of the isolates (18/20) were resistant to amoxicillin-clavulanic acid, 50% (10/20) to ceftriaxone, 60 %(12/20) to gentamicin, and 70 %(14/20) to kanamycin whereas 95 %(19/20) of the isolates were sensitive to ceftazidime (Negeri, 2005).

In another study study by Sewnet, (2010) among a total of 50 burn patients in Yekati 12 burn center 21(42%) were found bacteremic. Five different bacteria were isolated from blood which in accordance with their frequency were *Coagulase negative Staphylococci* 9(42.8%), *S.aureus* 8(38.2%), *Bacillus spps* 2(9.52%), *Klebsella pneumoniae* 1(4.8%) and *Pseudomonas aeruginosa* 1(4.8%). The wound swab showed *S.aureus* (34.04%) and *Pseudomonas aeruginosa* (31.8%)

predominantly. The bacterial isolates identified at this particular center were resistant to the commonly used drugs. High resistance was observed for Ampicillin (77.4%), Doxycycline (74.0), Naldixic acid (70.5%), Penicillin G (68.2%), tetracycline (67.5%), Augumentin (37.5%), Methicillin (29.5%), Gentamycin (19.1%) and Ceftriaxone (18.5%). (Sewnet, 2010)

2.2. Epidemiology

Burn wound infections are one of serious complications that occur in the acute period following injury. Very young children and the elderly have an increased risk of being burned and worse clinical outcomes than patients in other age groups (Church *et al.,* 2006).

The highest numbers of nosocomial infections occur in surgical and medicine services. High rates also are observed with burn (15%) (Baron, 2000). This high rate of nosocomial infection in burn patients is due to various factors like nature of the burn injury itself, immunocompromised status of the patient, invasive diagnostic and therapeutic procedures in prolonged ICU stay. Complicating this high rate of infection is the fact that spectrum of bacterial isolates varies with time and geographical area (Mehta *et al.,* 2007).

Over the past decade, the estimated incidence of burn injuries in the United States has steadily declined; still, however, >1 million burn injuries are brought to medical attention each year. While many burn injuries are minor and require little or no intervention, 50,000 persons are hospitalized for these injuries, and 20,000 have major burns involving at least 25% of the total body surface area (Lawrence, 2008). From this it is estimated that 5,000 burn patients die each year from complications related to the burn (Wolf and Herndon, 2009).

Developing countries have a high incidence of burn injuries, creating alarming public health problem. Moreover, high population density, illiteracy and poverty are the main demographic factors associated with a high risk of burn. The high incidence makes burns an endemic health hazard. Social, economic, and cultural factors interact to complicate the management, reporting, and prevention of burns (Khajuria *et al.,* 2009).

2.3. Pathophysiology

An intact human skin surface is vital to the preservation of body fluid homeostasis, thermoregulation, and the host's protection against infection (Chalise *et al.*, 2008). Loss of the cutaneous barrier facilitates entry of the patient's own flora and of organisms from the hospital environment into the burn wound. Initially, the wound is colonized with gram-positive bacteria from the surrounding tissue. The avascularity of the eschar, along with the impairment of local immune responses, favors further bacterial colonization and proliferation (Church *et al.,* 2006; Lawrence, 2008). Burns provide a highly nutritious medium for bacteria. Colonization may be limited to the eschar (nonviable skin debris on the surface), invade deeper tissues, or spread systemically through lymph and blood (septicemia). In burn patients, septicemia is often polymicrobic. Normal neutrophil function is a key determinant to limiting the severity of the infection (http://findarticles.com/p/articles/mi_qa3964/is_199911/ai_n8855297/).

The cascade of events that follow a severe burn injury and that lead to multi-organ system failure and death are thought to represent a two step process: The burn injury itself, with ensuing hypovolemia and tissue hypoxia, is followed by invasive infection arising from large amounts of devitalized tissue. The frequency of infection parallels the extent and severity of the burn injury. Severe burn injuries cause a state of immunosuppression that affects innate and adaptive immune responses. The substantial impact of immunocompromise on infection is due to effects on both the cellular and the humoral arms of the immune system. For example, decreases in the number and activity of circulating helper T cells, increases in suppressor T cells, decreases in production and release of monocytes and macrophages, and diminution in levels of immunoglobulin follow major burns. Neutrophil and complement functions have also been shown to be impaired after burns. The increased levels of multiple cytokines detected in burn patients are compatible with the widely held belief that the inflammatory response becomes dysregulated in these individuals; bacterial cell products play a potent role in inducing proinflammatory mediators that contribute to this uncontrolled systemic inflammatory response. Another contributor to secondary immunosuppression after burn injuries is the endocrine system; increasing levels of vasopressin, aldosterone, cortisol, glucagon, growth hormone, catecholamines, and other hormones that directly affect lymphocyte proliferation, secretion of proinflammatory cytokines, natural killer cell activity, and suppressive T cells are seen (Church *et al.*, 2006; Lawrence, 2008).

2.4. Clinical Manifestations

Since clinical indications of wound infection are difficult to interpret, wounds must be monitored carefully for changes that may reflect infection. A margin of erythema frequently surrounds the sites of burns and by itself is not usually indicative of infection. Signs of infection include the conversion of a partial-thickness to a full-thickness burn, color changes (e.g, the appearance of a dark brown or black discoloration of the wound), the new appearance of erythema or violaceous edema in normal tissue at the wound margins, the sudden separation of the eschar from subcutaneous tissues, and the degeneration of the wound with the appearance of a new eschar (Lawrence, 2008)

Early surgical excision of devitalized tissue is now widely used, and burn-wound infections can be classified in relation to the excision site as burn-wound impetigo (infection characterized by loss of epithelium from a previously reepithelialized surface, as seen in a partial thickness burn that is allowed to close by secondary intention, a grafted burn, or a healed skin donor site); burn-related surgical wound infection (purulent infection of excised burn and donor sites that have not yet epithelialized, accompanied by positive cultures); burn wound cellulitis (extension of infection to surrounding healthy tissue and invasive infection in unexcised burn wounds) infection that is secondary to a partial- or full-thickness burn wound and is manifested by separation of the eschar or by violaceous, dark brown, or black discoloration of the eschar (Khajuria *et al.,* 2009; Lawrence, 2008; Chalise *et al.,* 2008; Santucci *et al.,* 2003).

Changes in body temperature, hypotension, tachycardia, neutropenia or neutrophilia, thrombocytopenia, and renal failure may result from invasive burn wounds and sepsis. Given the difficulty of evaluating burn wounds solely on the basis of clinical observation and laboratory data, wound biopsies are necessary for definitive diagnosis of infection. The biopsy specimen is examined for histologic evidence of bacterial invasion, and quantitative microbiologic cultures are performed. The presence of $>10^5$ viable bacteria per gram of tissue is highly suggestive of invasive infection and of a dramatically increased risk of sepsis (Church *et al.,* 2006; Lawrence, 2008).

In addition to infection of the burn wound itself, a number of other infections due to the immunosuppression caused by extensive burns and the manipulations necessary for clinical care put burn patients at risk. Pneumonia, now the most common infectious complication among hospitalized burn patients, is most often nosocomially acquired via the respiratory route; among

the risk factors associated with secondary pneumonia are inhalation injury, intubation, full-thickness chest wall burns, immobility, and uncontrolled wound sepsis with hematogenous spread. Septic pulmonary emboli may also occur. Suppurative thrombophlebitis may complicate the vascular catheterization necessary for fluid and nutritional support in burns. Endocarditis, rinary tract infection, bacterial chondritis (particularly in patients with burned ears) and intra abdominal infection also complicate serious burn injury (Lawrence, 2008).

Based on the magnitude (depth) burn injuries could be first-, second-, or third-degree burns (Church *et al.,* 2006; Lawrence, 2008).

2.5. Diagnosis and Management

The diagnosis and management of burn wound infection is based on early identification of an infected wound. Clinically, burn wound infection is most often recognized based on gross appearance or conversion of a partial thickness to a full thickness wound. Once there is clinical suspicion of invasive burn wound sepsis, it is imperative to obtain quantitative wound cultures. Generally, at greater than 10^5 organisms/gm of tissue are considered indicative of a wound at significant risk for invasive sepsis. It is important to realize, however, that histologic confirmation of actual tissue invasion by the microbial pathogens is the only way to clearly establish the diagnosis of invasive burn wound sepsis. The most common pathogens include methicillin-sensitive and methicillin-resistant *Staphylococcus* and *P. aeruginosa* (Wolf and Herndon, 1999).

A blood culture positive for the same organism seen in large quantities in biopsied tissue is a reliable indicator of burn sepsis. Burn induced inflammatory response are indistinguishable from those of infection and the local signs of infection may be absent, minimal or late in developing. Hence the diagnosis shall be generally on the basis of a combination of clinical features and the result of continuous and periodic surveillance of blood cultures.

Surface cultures (e.g., over the ears, eyes, or digits) may provide some indication of the microorganisms present in the hospital environment but are not indicative of the etiology of infection (David *et al*., 2007; Lawrence, 2008).

The survival rates for burn patients have improved substantially in the past few decades due to advances in resuscitation, nutritional support, pulmonary care, wound care, and infection control practices in specialized burn units (Chalise *et al.,* 2008). Topical antimicrobial agents play an important role in decreasing the incidence of burn wound infection. The astute clinician must be cognizant; however, that antimicrobial therapy is not a substitute for aggressive debridement of grossly infected and devitalized tissue. Excision of all infected tissue continues to be the mainstay of treatment. Grafting of skin or skin substitutes greatly decreases mortality rates associated with severe burns. Nonetheless, topical antimicrobial agents are useful and it is important to be familiar with them. There are several commonly used agents which include silver sulfadiazine cream, mafenide acetate cream, silver nitrate cream, and nanocrystalline silver dressings—dramatically decrease the bacterial burden of burn wounds and reduce the incidence of burn-wound infection; these agents are routinely applied to partial- and full-thickness burns (Lawrence, 2008; Wolf and Herndon, 1999).

Treatment of infections caused by emerging resistant pathogens remains a challenge in the care of burn patients. MRSA, enterococci, multidrug-resistant gram-negative rods, and Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBL) have been associated with burn-wound infections and identified in BU outbreaks. Strict infection-control practices (including microbiologic surveillance in burn units) and appropriate antimicrobial therapy remain important measures in reducing rates of infection due to resistant organisms. All burn patients should undergo tetanus booster immunization if they have completed primary immunization but have not received a booster dose in the past 5 years. Patients without prior immunization should receive tetanus immune globulin and undergo primary immunization (Church *et al.,* 2006; Lawrence, 2008).

2.6. Antimicrobial resistance in burn centers

The numbers of antibiotic-resistant bacteria have increased in recent years and such resistance can compromise the efficacy of antimicrobial therapy. Antibiotic-resistant bacteria can be associated with infections with higher mortality than those caused by antibiotic-susceptible strains (Laura *et al.,* 2009).

The therapy of both nosocomial and community-acquired infections is affected by the continuing evolution of and challenges presented by antimicrobial resistance. This increasing emergence

and spread of multi drug resistant bacteria in hospitals in general and burn centers in particular is of great concern and continues to challenge infection control and hospital epidemiology practice worldwide (Lorian, 2005). Most of the antimicrobial resistance which is now making it difficult to treat some infectious diseases is due to the extensive use and misuse of antimicrobial drugs which have favored the emergence and survival of resistant strains of micro-organisms (Cheesbrough, 2006).

Microbes have genetic plasticity, which means that they have the capacity to evolve in response to their environment. The major impetus for developing resistance is selective pressure resulting from antibiotic use. The bacteria that survive are those that develop mechanisms to avoid being killed by antibiotics. The treatment of several pathogens is becoming problematic. For instance in 1944, most staphylococci were susceptible to penicillin G, though a few resistant strains had been observed. Presently, penicillin-resistant staphylococci include not only those acquired in hospitals, like in burn units, but also 80–90% of those isolated in the community. These organisms also tend to be resistant to other drugs, eg, tetracyclines. Nafcillin-resistant staphylococci are common in tertiary hospitals. Vancomycin has been the major drug used for treatment of nafcillin-resistant *S aureus* infections, but recovery of isolates with intermediate resistance and the reports of several cases of high-level resistance to vancomycin have spurred the search for newer agents (Melnick, 2007).

On a study in Iran on the Bacterial infection of burn patients at a burn hospital, the microorganisms causing infections were *P. aeruginosa* (37.5%), *S. aureus* (20.2%), and *Acinetobacter baumanni* (10.4%). Among these isolates *P. aeruginosa* was found to be 100 per cent resistant to amikacin, gentamicin , carbenicillin, ciprofloxacin, tobramycin and ceftazidime; 58% of *S. aureus* and 60% of coagulase negative *Staphylococcus* were methicillin resistant (Alireza and Enayat, 2007). Other studies on drug resistance profile are presented above.

Multidrug resistant bacteria have frequently been reported as the cause of nosocomial outbreaks of infection in burn units or as colonizers of the wounds of burn patient. Similarly, antimicrobial resistance in some of the most frequent bacterial species isolated from burn patients include *S.aureus, P. aeruginosa*, and other Gram negative bacilli has reached to a worrying level. Based on National Nosocomial Infection Surveillance System (NNIS) criteria, all the burn patients are required to follow the distribution of bacterial species among burn isolates, and the antimicrobial

susceptibility of the pathogens in order to adapt empirical antibiotic strategies (Alireza and Enayat, 2007).

New solutions are needed to preserve the activity of our current antibiotic armamentarium, to lower the overall risk of bacterial resistance and to successfully treat patients with resistant bacterial infections. Options include: development of new antibiotics to treat resistant organisms; vaccination to prevent infections; and improved use of antibiotics. Because bacteria will eventually develop means to avoid being killed by antibiotics, judicious use of antibiotics by all clinicians is imperative. Appropriate antibiotic use involves selection of a "targeted spectrum" antibiotic, as well as an appropriate dose and duration. This entails updated databases on the antibiotic susceptibility of such databases to new as well as traditional antibiotics (Manal *et al.,* 2006).

2.7. Prevention and Control of Infections in Burn centers

Advances in treating initial burn shock, infection control, early wound closure, and modulation of the hypermetabolic response have decreased morbidity and mortality in the last two decades. Specialized burn care centers, using a multidisciplinary approach, not only successfully treat large burns and their complications, but provide the necessary rehabilitation and psychological support required for readjustment back into society (Thuan *et al.,* 1996).

Modern burn centers have a contained perimeter that is designed to minimize the unnecessary traffic of health care workers and visitors alike through the unit. Cross-contamination is further diminished within the unit by housing burn patients in individual nursing units composed of individual isolation rooms, each with its own laminar airflow (Church *et al.,* 2006).

2.7.1. Topical Antimicrobial Therapy

Several studies have demonstrated the role of topical antimicrobials in decreasing morbidity and mortality in patients with major burn injuries (partial- or full-thickness skin involvement), particularly before early excision. The efficacy of various topical antimicrobials in common use in modern burn centers is dynamic due to the ability of microorganisms to develop resistance rapidly (Church *et al.,* 2006). The four widely used topical antimicrobial agents are silver sulfadiazine cream, mafenide acetate cream, silver nitrate cream, and nanocrystalline silver dressings dramatically decrease the bacterial burden of burn wounds and reduce the incidence of

burn-wound infection (Lawrence, 2008). Selection of topical antimicrobial therapy should be based on the agent's ability to inhibit the microorganisms recovered from burn wound surveillance cultures and monitoring of the nosocomial infections acquired in the burn unit (Church *et al.,* 2006).

2.7.2. Prophylactic Systemic Antibiotics

The use of systemic antimicrobial chemoprophylaxis in severely burned patients is a subject of much controversy. Conventional wisdom holds that topical antimicrobial therapy and aggressive wound care are sufficient for severely burned patients in the absence of significant signs of infection. Proponents of this philosophy maintain that only after clinical suspicion of an infection exists, should systemic antimicrobial therapy be initiated (Lawrence, 2008; Wolf and Herndon. 1999).

Studies of the clinical benefit of prophylactic courses of systemic antibiotics in burn patients in decreasing the occurrence of burn wound infections have not demonstrated improved outcome compared to the use of topical therapy along with surgical excision. An exception involves cases requiring burn-wound manipulation. Since procedures such as debridement, excision, or grafting frequently result in bacteremia, prophylactic systemic antibiotics are administered at the time of wound manipulation; the specific agents used should be chosen on the basis of data obtained by wound culture or data on the hospital's resident flora (Lawrence, 2008).

2.7.3. Selective Bowel Decontamination/Selective decontamination of the digestive tract (SDD)

The relationship between the translocation of bowel micro flora due to increased intestinal permeability and the subsequent colonization of the burn wound by enteric gram-negative microorganisms has been well described and several studies compared the ability of oral prophylactic antibiotic regimens to selectively decontaminate the normal bowel flora in burn patients, thereby reducing burn wound colonization and infection. A study showed that burn wound colonization was delayed and the rate of infection was reduced in patients who received an oral bowel flora-suppressive antibiotic regimen (neomycin, erythromycin, and nystatin). However, this broad-spectrum decontamination regimen not only suppressed aerobic gram-

negative bacteria but also destroyed the anaerobic flora that is important in host “colonization resistance (Church *et al.,* 2006).

In general, two practices have revolutionized burn care to improve outcomes by decreasing invasive wound infections. Early excision and closure of the burn wound prevents infection by eliminating the eschar that harbors microorganisms and providing a barrier to microorganism growth and invasion. The other is the timely and effective use of antimicrobials both topical and systemic (Cunha, 2010).

Modern burn unit designs should allow all intensive and burn care procedures, including ventilation and operative procedures, to be done within the burn center itself, or, as a minimum, the facility design should minimize the need to transfer patients out of the burn unit for different aspects of their care. The infection control program for burn centers requires strict compliance with a number of environmental control measures that include strictly enforced hand washing and the universal use of personal protective equipment (i.e., gowns, gloves, and masks). Health care personnel must be gowned (including use of disposable or reusable gowns and disposable plastic aprons to prevent soiling of health care workers’ clothing during wound care procedures) and gloved at each entry to the burn patient’s isolation room. Monitoring and diagnostic equipment is housed in each burn patient’s room to prevent cross-contamination between patients (Cunha, 2010; Church *et al.,* 2006; Wolf and Herndon, 1999).

CHAPTER THREE
SIGNIFICANCE OF THE STUDY

Burn injury remains an important cause of morbidity and mortality worldwide. Infectious complications, including sepsis, septic shock and sepsis-related organ failure, are common among patients with moderate to severe burn injuries (Robert *et al.,* 2006).

Burn care in low- and middle-income countries is very dependent on the availability of financial resources, equipment, and expertise. An additional important problem is that burn care in most developing countries is usually delayed. In rural areas, access to burn care facilities and thus proper treatment are compromised by factors such as long distances to travel, inaccessible roads, and unavailable transportation means (Atiyeh et *al.,* 2010).

To establish any gains in infection control measures, it requires a brief understanding of wound bacteriology. It is very crucial for every burn institution to determine the specific pattern of burn wound microbial colonization, the time related changes in the dominant flora and the antimicrobial sensitivity profiles (Sanjay *et al.,* 2007).

Based on NNIS criteria, all the burn patients are required to follow the distribution of bacterial species among burn isolates, and the antimicrobial susceptibility of the pathogens in order to adapt empirical antibiotic strategies (Alireza *et al.,* 2007).

Antibiotic usage patterns effective in one centre may not be effective in another or at another time in the same unit. Each individual unit varies in its baseline population of microorganisms over time, and generalizations drawn on the basis of results in one unit may have little applicability to others. In the developing world like ours, a wide gap between the number of burn patients and available resources exists. There is only one functional specialized burn unit in our country. So, even severe burn patients, who require specialized care, are forced to be managed in general wards in the hands of general practitioners or surgeons who do not have specialized training in managing burn patients. As a result, we have not been able to lower the burn related deaths as compared to western world. So, this study was designed in view of assessing the longitudinal burn wound bacteriological colonization and septicaemia status in our set up at Yekatit 12 hospital. It is also essential for the burn center at Yekatit 12 Hospital to determine time-related changes in predominant flora, and antimicrobial resistance profiles. This would allow early management of septic complications with proper systemic antibiotics.

CHAPTER FOUR
OBJECTIVES

1.1. General objective

- To assess longitudinal bacteriology of burn patients at Yekatit 12 Hospital burn center, Addis Ababa.

1.2. Specific Objectives

- ✓ To assess the pattern of burn wound bacterial colonization and to evaluate the time-related changes of predominant bacteria.
- ✓ To compare the outline of bacterial isolate and their drug susceptibility profile between burn OPD and burn unit patients.
- ✓ To identify etiologies of septicaemia in burn patients

CHAPTER FIVE
METHODS AND MATERIALS

5.1. Study setting and period

The study was carried out among burn patients attending Yekatit 12 hospital burn center, Addis Ababa, Ethiopia. Yekatit 12 hospital is one of the hospitals under Addis Ababa city administration health bureau that has been giving routine health services for the city community and other referral cases from different regional states of Ethiopia.

The study was conducted prospectively from December 2010 to February 2011.

5.2. Study design

Hospital based prospective longitudinal study was employed.

5.3. Study Population

Patients with open burn wound visiting Yekatit 12 hospital burn center.

5.4. Sample size determination and sampling technique

All 41 burn patients visiting the burn center (burn OPD and burn unit) of Yekatit 12 hospital within the data collection period were included in the study; for this reason the sampling technique was convenient.

5.5. Inclusion and exclusion criteria

All burn patients attending medical care at burn outpatient department (OPD) and burn unit (BU) of Yekatit 12 burn center during the study period were included in the study. Those seriously ill burn patients who had been in the burn center before the study started were excluded.

5.6. Definition of Terms

- ✓ **Burn**: burns are injuries caused by dry heat or scalds by moist heat. Severe burns are also caused by contact with electric wires, and by the action of acids and other chemicals.
- ✓ **Longitudinal study**: A study repeated over time i.e. process of sampling and studding at different points in time from the same study subjects.
- ✓ **Nosocomial infection**: an infection acquired as a result of hospitalization or treatment received in hospital after 48 hour of admission to hospital or before 30 days of discharge from hospital.

- ✓ **Sepsis**: the condition or syndrome caused by the presence of microorganisms or their toxins in the tissue or the bloodstream (Poisoning by the products of the growth of micro-organisms in the body).
- ✓ **TBSA:** an estimate of the percentage of total body surface area involved in burn exposure and injury.
- ✓ **Wound**: a wound is any breach suddenly produced in the tissues of the body by direct violence. An extensive injury of the deeper parts without corresponding injury of the surface is known as a bruise or contusion.
- ✓ **Wound infection**: Bacteria present in the wound and wound eschar at high concentrations. No invasive infection. Pathologic diagnosis: $>10^5$ bacteria/g tissue.

5.7. Variables

5.7.1 Dependant variables: bacteriology of burn patients

5.7.2. Independent variables: degree of burn, hospitalization, resident hospital flora

5.8. Specimen collection and handling

Periodic wound swab and blood samples were collected at 0, 7th and 14th days of hospital stay at Yekatit 12 hospital Burn center**.**

5.8.1 Wound swab collection

A variety of different approaches have been described for assessing the nature and extent of microbial involvement in burn wounds, although the optimal sampling technique continues to be debated. Infection surveillance of the burn wound requires taking samples on a regular basis, by either biopsying tissue or collecting surface swabs. Burn wound surface swabs immersed with sterile 0.9% NaCl (normal saline) are a convenient and effective method (Deirdre *et al.,* 2006). In the present study wound swabs were collected with a swab immersed with normal saline on 0, 7th and 14th days of hospital stay before dressing changes and administration of antibiotics.

5.8.2. Blood sample collection

About 10 ml of blood from an adult or about 2-5 ml from a young child was collected using standard venous blood collection technique to assess septicaemia status of burn patients. It was collected periodically on 0, 7th and 14th days of hospital stay before dressing changes and administration of antibiotics. Prior to blood sample collection the area (about 50 mm in diameter) was cleaned using 70% ethanol.

All samples collected were processed immediately (within~10-15minitues) in bacteriology laboratory unit of Yekatit 12 hospital.

5.9. Processing of specimens and laboratory identification

5.9.1. Analysis of wound swabs

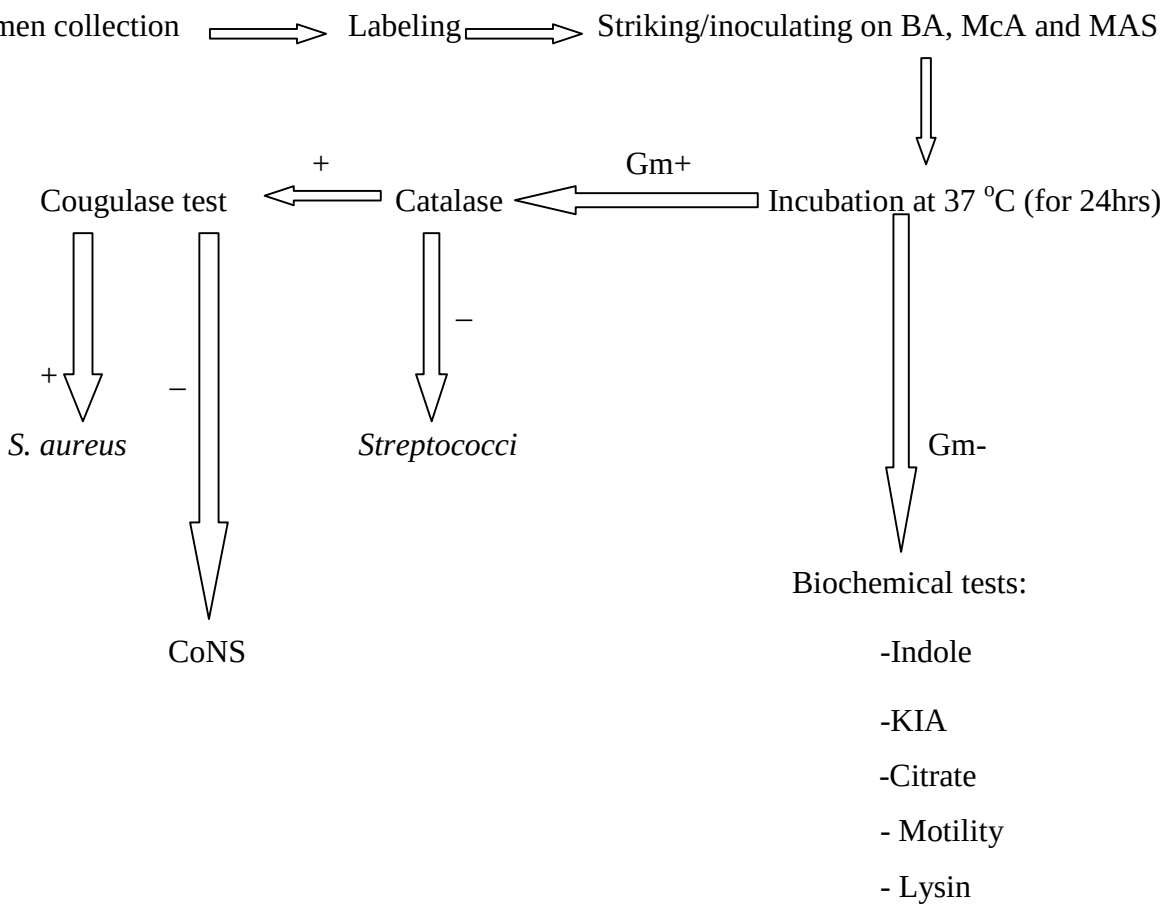
All wound swab specimens were inoculated on Blood Agar (BA) plate, Manitol Salt Agar (MSA) and Mac Conkey agar (McA), and incubated at 37^{o}C for 18-24 hours (Benson, 2001; Cheesbrough M, 2006). Preliminary identification of bacterial isolates were done using colony morphology and characteristics (like pigmentation, haemolysis pattern on blood agar) and also by Gram-staining whenever necessary. Conventional biochemical tests from nutrient broth suspensions were performed on colonies from primary cultures for final identification of the isolates. In brief, Gram-negative rods were identified by performing of a series of biochemical tests (Oxoid, LTD), namely: indole, Simon's citrate agar, kligler iron agar (KIA), lysine iron agar, and motility. Gram-positive cocci were identified based on their preference of growth on BA plates and MSA followed by catalase and Coagulase test. *Streptococci* spp were tested for bacitracin sensitivity and when the colonies were difficult to recognize on BA plate, gram staining was done (Benson, 2001; Cheesbrough, 2006).

5.9.2. Analysis of blood samples

Collected blood samples (10ml from adult and 2-5ml from young child) were inoculated in to Tryptone soya broth (50 and 25ml respectively) and incubated aerobically for up to a week. All bottles (with and without growth) were sub-cultured on BA plates, MSA and McA and identification was done as for wound swabs. No attempt was made to cultivate anaerobes as it is not common to find them in burn patients.

The identification procedure of the bacteria from both pus and blood culture is summarized below in fig 1.

Fig 1: flow chart for bacterial identification from wound swab at Yekatit 12 burn center. Addis Ababa, 2011



For blood culture

- Blood sample collection / aseptically/
- Inoculation on TSY ⇩
- Incubation aerobically at 37 °C ⇩
- Examine the bottle for growth each day for a week ⇩
- After a week; subculture on BA, McA and MSA ⇩
- Presumptively colonies checked for hemolysis and pigmentation ⇩
- Then the identification continues as mentioned above

➢ Antibiotic susceptibility pattern was done on Muller Hinton Agar after preparation of bacterial suspension on sterile nutrient broth as described below.

5.10. Antimicrobial Susceptibility Testing

Disc diffusion technique was performed to evaluate antimicrobial susceptibility pattern of isolates for most commonly used antibiotics in Yekatit 12 hospital burn center which mentioned below. A suspension of each isolate was made so that the turbidity becomes equal to 0.5 McFarland standards and then plated onto Muller-Hinton agar plate (Oxoid, LTD). Sterile commercially available antibiotic filter paper disks onto which a definite amount of antibiotic has been absorbed (Oxoid, LTD) were applied to each plate. After incubation at 37^0C for 24 h, zone size was measured (Cheesbrough, 2006).

To sum up; the suspension of the test organisms were prepared by picking parts of similar colony with a sterile wire loop. These were suspended in nutrient broth. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5. A sterile swab dipped into the suspension of the isolate in broth, squeezed free from excess fluid against the side of bottle and then spreaded over the agar plate so as to get a matt growth. Sterile antibiotic discs (Oxoid) were equidistantly placed on these plates and gently pressed onto the medium with the help of sterile forceps to ensure complete contact with the agar surface. A zone of inhibition was measured in millimetres and the organisms classified as *sensitive*, *intermediate* or *resistant* according to the zone size interpretation chart (Benson, 2001; Cheesbrough, 2006).

The drugs that were tested includes, for gram negatives: Agumentin (Amoxicillin-Clavulanic acid=30µg), Amoxacillin(10µg), Ampicillin(10µg), Ceftriaxone(30ug), Ceftazidime(30ug), Chloroamphenicol(30µg), Doxicyclin, Norfloxacin and Naldicsic acid(30µg). For gram positives: Agumentin(30µg), Amikacin, Chloroamphenicol(30µg), Clindamycin(2µg), Cephalothin(30µg), Kanamycin(30µg), Methicillin(5µg), Penicillin G(10 IU) and Vancomycin(30µg).

5.11. Quality control

The reliability of the study findings were guaranteed by implementing QC measures throughout the whole process of the laboratory work. Staining reagents, culture medias and antibiotic discs were checked for their normal shelf life before use. All culture plates were stored at recommended refrigeration temperature (2-8^0C) after prepared and sterilized by autoclaving at 121 ^{0}C for 15 minutes. Antibiotic discs were also stored at the same temperature range before use. The standard reference strains *Staphylococcus aureus* ATCC 25923 and *Pseudomonas*

aeruginosa ATCC 27853 were tested as a control on the biochemical tests and agar plates including Mueller Hinton with antibiotic discs. Proper Sample collection and handling were done by experienced nurses who were working at the burn center.

In general all laboratory procedures were done based on recommended standard laboratory procedures by strictly following Pre-analytical, analytical and post-analytical stages of quality assurance that are incorporated in SOPs of the microbiology laboratory of Yekatit 12 hospital.

5.12. Data analysis

Generated data was compiled by frequency tables and charts and other statistical summary measures after analyzing by SPSS version 17.0 for Windows.

5.13. Ethical considerations

- ✓ This M.Sc thesis proposal was approved by Microbiology, Immunology and Parasitology, Department ethical clearance committee of, Addis Ababa University.
- ✓ Official Letter from the department was written to Yekatit 12 hospital and, other concerning bodies.
- ✓ Written and informed consent of the study participants/ or from their guardians was got to take part in the study voluntarily after adequate explanation about the purpose, importance and potential discomforts and benefit of the study.
- ✓ Patients were directly benefited for better management by communicating the lab result with physicians.
- ✓ The procedure of specimen collection was also explained for all participants. Patients were not subjected for unnecessary sample collection unless to benefit them.
- ✓ All information from participants was kept confidential.

CHAPTER SIX
RESULTS

6.1. Socio-demographic and clinical characteristics

A total of 41 patients were included in the study. Of the total patients, 16 (39%) of them were hospitalized in the burn unit and 25 (61%) of them were burn OPD patients at Yekatit 12 hospital burn center. The mean age of the patients was 25.7 years (range: 02-65 years). Fifteen (36.6%) of patients were in the age group of 21-30 years. There were 24 (58.5%) male and 17(41.4%) female patients, with male to female ratio of 1.4:1. Three of them died before data collection was completed (two from burn unit and one from OPD). Much greater than half of the patients, 32 (78.0%), came to the hospital from urban, while the rest were from rural environment. The most common cause of burn was exposure to hot liquid or steam scald 27 (65.9%) followed by flame 10 (24.4%) and electrical injuries 4(9.8%). Most of burn injuries were at home 30 (73.2%). Anatomically the majority of the burns were confined to the extremities 21(51.2%) followed by on the extremities, head and trunk 7(17.1%) and extremities and head 4(9.8%). The total burned surface area (TBSA) ranged from 2.0 % to 59.0 % with a mean of 11.9% and standard deviation of 11.7. Most of the patients (65%) had less than 10% TBSA. The reported depth of the burn was full thickness in 8 (19.5 %) patients and partial thickness in 33(80.5%) patients as shown below in table 1and 2.

Table 1: Socio-demographic and clinical characteristics of burn patients at Yekatit 12 burn center. Addis Ababa,2011

Variables	Categories	Frequency (%)
Age	Mean	25.7
	Range	2-65
Sex	Male	24 (58.5)
	Female	17 (41.5)
Address	Urban	32 (78)
	Rural	9 (22)
Cause of burn	Scald	27 (65.9)
	Flame	10 (24.4)
	Electricity	4 (9.7)
Place of injury	Home	30 (73.2)
	Restaurant	3 (7.3)
	Factory	1 (2.4)
	Other different sites*	7 (17.1)
Anatomical location of the burn	Extremities	21 (51.2)
	Trunk	1 (2.4)
	Perineum	1 (2.4)
	Extremities, head and neck	8 (19.5)
	Extremities and trunk	4 (9.6)
	Extremities and perineum	3 (7.3)
	Extremities, trunk, head and neck	2 (4.9)
Depth of the burn	Partial thickness	33 (80.5)
	Full thickness	8 (19.5)
%TBSA	Mean	11.9
	Range	2-59
Ward of the patient	In patient (BU)	16 (39)
	Out patient	25 (61)

M to F ratio= 1.4:1

*On church ceremony, road, hotel, construction site, at work.

TBSA= Total body (burned) Surface Area

BU= Burn Unit

Table 2: Age and percent TBSA distribution of burn patients at Yekatit 12 burn center. Addis Ababa, 2011

Age		%TBSA		
Range	Frequency (%)	Range	Frequency (%)	
1-10	4 (9.8)	1-10	26 (65)	Mean age= 25.7
11-20	11(26.8)	11-20	8 (20)	
21-30	15 (36.6)	21-30	2 (5)	Mean %TBSA of all patients= 11.9
31-40	7 (17.1)	31-40	3 (7.5)	Mean %TBSA of BU patients= 15.3
41-50	2 (4.9)	41-50	0	Mean %TBSA of OPD patients=9.7
>50	2 (4.9)	>50	1 (2.5)	
Total	41 (100)	Total	40	

Most numbers of patients 15 (36.6%) were in the age group of 21-30 years. About (65%) of patients had less than 10% TBSA. Those patients who get admission in burn unit were relatively with larger %TBSA as the mean indicates (%TBSA had marginal statistical association with whether the patients were in OPD or burn unit ($p<0.56$)). Since the burn unit has limited beds (about 19), most of burn patients especially those with low level of burn were subjected to attend medical care in burn OPD. Whenever there is free beds, there is a possibility that a patient with relatively sever burn to be admitted in burn unit.

6.2. Pattern of bacterial colonization of burn wound

Periodic swabs were collected from burn wound on 0, 7th, and 14th, day. A total of 101 microbial isolates were identified from 104 wound swabs processed during the study period. About 19 (18.3%) wound swabs were observed with mixed growth; of which much proportion, 15 (78.9%), accounted for *Pseudomonas* spp and *S. aureus* co-growth. *Staphylococcus aureus* 15(46.9%) and *Pseudomonas* spp 9(28.1%) were the most prevalent isolates on day 0 cultures (pus 1). There was a gradual increase in the number of *S. aureus* and *Pseudomonas* spp from day 0 to 14th day. At the 14th day (pus 3), the most frequent organisms isolated were *S. aureus* (50%), and *Pseudomonas* spp (45.8%). While 15 (36.6%) burn wound swabs were sterile on day 0, microbial colonization reached 94.7% within the first week. Level of bacterial isolation from burn wound culture is found to be significantly associated with degree of TBSA ($P<0.02$). Forty percent (n=25) of wound swabs collected at the 14th day were without growth. Among the total isolates from burn wound swab; *Klebsiella* spp, *E. coli* and *Citrobacter* spp noted with equal

frequency (two isolates for each). Similarly *Providencia* spp and *S. pyogens* were isolated with the same amount, one isolate each. (Table 3 and figure 2)

Table 3: Isolation pattern of bacteria from burn wound swabs of patients at Yekatit 12 hospital burn center. Addis Ababa, 2011.

Bacteria isolated	Pus 1 isolates f (%)	Pus 2 isolates f (%)	Pus 3 isolates f (%)	Total
S. aureus	15 (46.9)	21 (46.7)	12 (50)	48 (47.5%)
Pseudomonas spp	9 (28.1)	20 (44.4)	11 (45.8)	40 (39.6%)
Proteus spp	3 (9.3)	1 (2.2)	1 (4.2)	5 (5%)
Klebsiella spp	2(6.3)	-	-	2 (2)
E. colli	1(3.1)	1 (2.2)	-	2 (2)
Citrobacter spp	1(3.1)	1(2.2)	-	2 (2)
S. pyogens	1(1.1)	-	-	1
Providencia spp	-	1(2.2)	-	1
No growth	15 (36.6)	2 (5.3)	10 (40)	27
Total isolates	32 (100)	45 (100)	24	*101*

Of the total 101 isolates *Staphylococcus aureus* and *Pseudomonas* spp together accounted (87.1%). Maximum growth was recorded at the end of first week. (Figure 1 and 2)

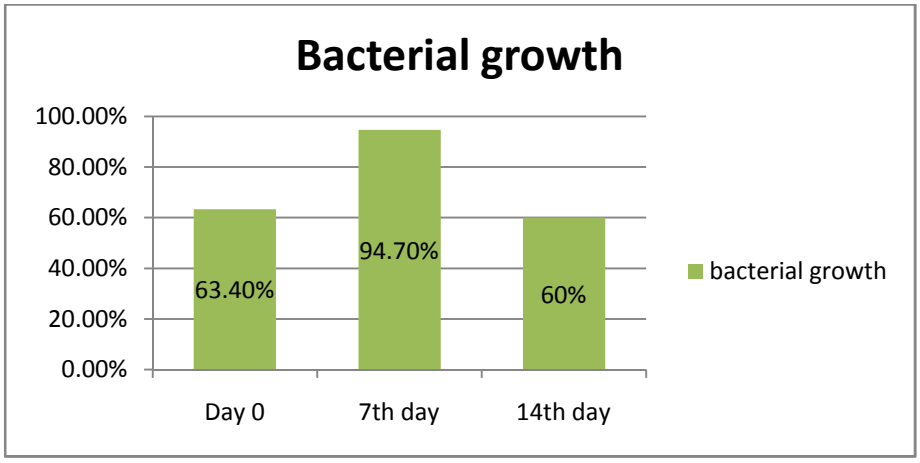


Fig 2: Rate of bacteria growth from periodic pus culture of patients at Yekatit 12 hospital burn center. Addis Ababa, 2011.

The number of isolates in third wound swab were decreased as some of the patients were with healing wound and some of the patients were get discharged from the burn center or otherwise transferred to other health centers near to their place of residence when they get better prognosis.

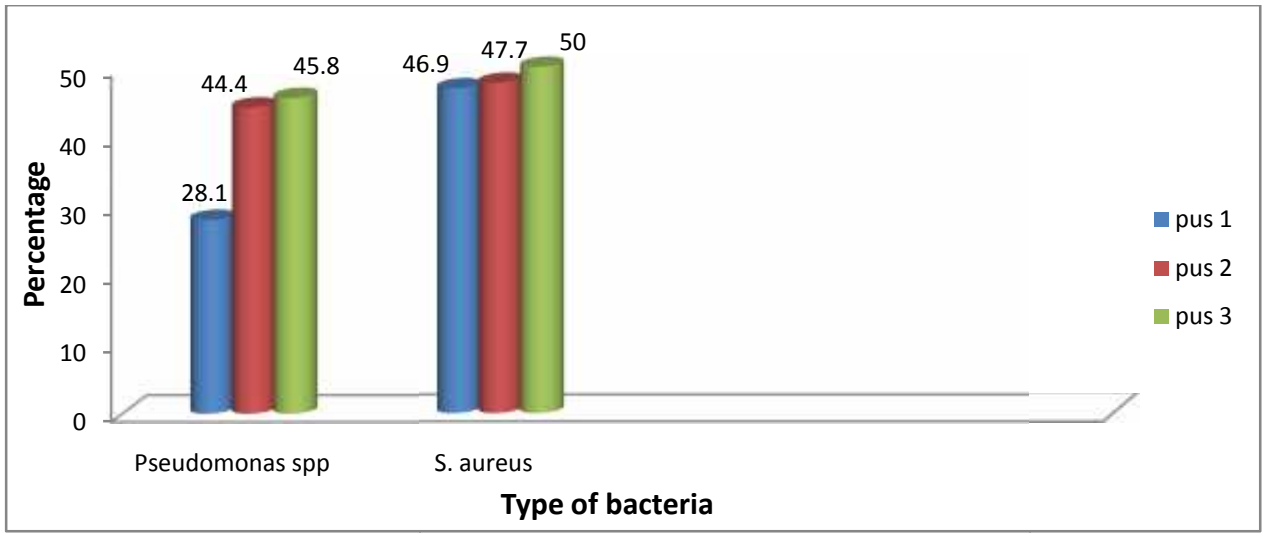


Fig 3: Isolation pattern of *Pseudomonas* spp and *S. aureus* from pus, at Yekatit 12 hospital burn center. Addis Ababa, 2011.

Regarding the type of isolates between OPD and burn unit patients there was no significant difference. However, mixed growth of *Pseudomonas* spp and *S. aureus* in burn unit patients 11(73.3%) is by far larger than that of OPD patients 4(26.7%) although the number of patients enrolled in burn OPD is higher. Similarly among pus culture with no growth, 21 (77.8%) were from burn OPD patients who were relatively with low burn percent of TBSA. (Table 4)

Table 4: Bacterial colonization pattern of burn OPD and burn unit patients at Yekatit 12 hospital burn center. Addis Ababa, 2011.

Ward	Bacterial isolates from burn wound										
	No of pts	*Pse*	P+S	*S. au*	*Cit*	*Kle*	*Pro*	*Prov*	*E. co*	*S. py*	No growth
Burn OPD	25	18	4	19	1	2	4	1	0	0	21
Burn unit	16	7	11	14	1	0	1	0	2	1	6
Total	41	25	15	33	2	2	5	1	2	1	27

Pse: *Pseudomonas* species, **P+S**: *Pseudomonas* and *S. aureus* mixed growth ***S. au****: S. aureus*, ***Cit****: Citrobacter* species, ***Kle***: *Klebsiella* species, ***Pro***: *Proteus* species, ***Prov***: *Providentia* species, ***E.col***: *E. coli*, ***S.py***: *S. pyogenes.*

Pts= patients

6.3. Pattern of blood culture isolates from burn patients

Periodic blood cultures were also performed, starting from time of first day of hospital visit to 14th day of patient's hospital stay to establish the etiologies of septicaemia and to perform antimicrobial susceptibility tests for selection of empiric therapy. The number of blood cultures done was decreasing starting from the first blood sample as those with growth on the first blood sample were excluded in the later consecutive blood culture tests and some of the patients were get discharged from the burn center or otherwise transferred to other health centers near to their place of residence when they get better prognosis. Out of 92 blood cultures done in aerobic condition only 15(16.3%) yielded 15 isolates. There was no much significant change on the isolation pattern from each periodic blood samples. All of the isolates were gram positive and CoNS was with the largest recovery percentage 8 (53.3%) closely followed by *S. aureus* 6 (40%). One *S. pyogenes* isolate was also identified. There was no mixed growth observed in blood culture.

Almost all blood cultures, 77 (83.7%) were without bacterial growth, i.e they were sterile during the study period as table 7 shows below. Percent TBSA is marginally associated with degree of bacterial isolation from blood ($P<0.057$) but no association was observed with depth of the burn ($P<0.17$). It is important to note that this study focused exclusively on the microbiological profile and no attempt has been made to correlate this with other clinical data, i.e blood culture was done periodically regardless of the patient's clinical septicaemic status.

Table 5: Pattern of colonizing bacteria from blood culture of burned patients at Yekatit 12 hospital burn center. Addis Ababa,2011.

Bacteria isolated	Blood 1, f (%)	Blood 2, f (%)	Blood 3, f (%)	Total
S. aureus	4	1	1	6
CoNS	3	3	2	8
S. pyogens	1	-	-	1
Total isolates	8	4	3	15 (16.3%)
No growth	33 (80.5)	25 (86.2)	19 (86.4)	77 (83.7%))
Total no of pts	41	29	22	92 (100)

Of 41 patients enrolled in the study, 3 (7.3%) of them died before data collection was finished. Among the dead, one of them was with positive blood culture for *S. aureus*.

6.4. Antibiotic susceptibility pattern of bacterial isolates from burn wound

As described earlier the predominant isolates from burn wound culture were *S. aureus* 48(47.5%) and *Pseudomonas* spp 40(39.6%). Each isolate was tested for their susceptibility pattern against commonly used antibiotics in Yekatit 12 hospital. A high level of drug resistance was seen among gram negative isolates especially *Pseudomonas* species. It was moderately resistant to Ceftriaxone (37.5%) whereas resistance was more marked with other antimicrobials. All isolates of *Pseudomonas* spp were completely resistant for ampicillin, agumentin, amoxicillin and ceftazidime and almost all (97.5% and 95%) isolates were also resistant for doxycyclin and nalidixic acid respectively. On the other hand, *Pseudomonas* species were found to be more sensitive to Norfloxacine as it is evident by only 15% resistance. Thus it is considered as multidrug resistant to the commonly used drugs in the burn unit and the country at large.

The antibiotic susceptibility pattern of *S. aureus* showed that most isolates were susceptible for commonly used antibiotics. However, all isolates were observed resistant for penicillin G where as only 31.3% resistance was seen for methicillin. Majority of the isolates were recognized as susceptible for clindamycin and vancomycin as only 4.2% and 8.3% resistance was seen respectively. Both isolates of *Citrobacter* spp were markedly resistant for most of antibiotics tested for. The two isolates of *Klebsiella* spp and *Proteus* spp were found to be completely susceptible for norfloxacine and nalidixic acid as shown in Table 6.

Table 6: Antibiotic susceptibility pattern of bacterial isolates (101) from burn wound at Yekatit 12 hospital burn center. Addis Ababa, 2011.

Antibiotic	bacterial isolates from burn wound								
		Pse f (%)	*S. au* f (%)	*Pro*	*Cit*	*Kle*	*E. co*	*Prov*	*S. py*
Ampicillin	**Resistant**	40 (100)		5	2	2	1	1	
Agumentin		40 (100)	10 (20.8)	1	2	2	0	1	0
Amoxycillin		40 (100)		5	2	2	1	1	
CAF		35 (87.5)	19 (39.6)	2	2	1	0	1	1
Ceftazidime		40 (100)		3	2	2	2	1	
Ceftriaxone		15 (37.5)		1	2	1	0	0	
Doxycycline		39 (97.5)		5	2	2	1	1	
NA		38 (95)		0	1	0	0	0	
Norfloxacine		6 (15)		0	1	0	0	0	
Cepalothin			18(37.5)						0
Methicillin			15 (31.3)						0
Penicillin G			48 (100)						0
Amikacin			9 (18.8)						0
Clindamycine			2 (4.2)						1
Vancomycin			4 (8.3)						0
Kanamycine			12 (25)						0
Total isolate		40	48	5	2	2	2	1	1

Pse: *Pseudomonas* species, ***S. au***: *S. aureus*, ***Pro***: *Proteus* species, ***Cit***: *Citrobacter* species, ***Kle:*** *Klebsiella* species, ***E. co***: *E. coli*, ***Prov***: *Providentia* species, ***S. py***: *S. pyogenes*

-Agumentin = *amoxicillin-clavulanic acid*.

6.5. Antibiotic susceptibility profile between burn OPD and burn unit isolates

Roughly equal numbers of *Pseudomonas* spp isolates were identified in burn unit and burn OPD patients. All burn unit isolates of *Pseudomonas* spp were found to be completely resistant for Chloramphenicol and Doxycycline as compared with 77.3% and 95.5% resistance respectively in burn OPD. Similarly only two isolates (9.5%) of *Pseudomonas* spp were resistant for norfloxacine but it were four isolates (22.2%) resistance in burn unit. (Table 6)

In contrast to *Pseudomonas* spp, low level of resistance pattern was observed on burn unit isolates of *S. aureus* than burn OPD isolates. The number of isolates in burn unit and OPD was almost comparable. As it is depicted above, there was no *S. aureus* isolate in burn unit that was resistant for clindamycin and vancomycin but there was 8.7% and 17.4% resistance respectively on burn OPD isolates. One (4%), 6(24%) and 1(4%) isolate of *S. aureus* in burn unit had resistance for agumentin, Chloramphenicol and amikacin respectively. However, the respective resistance of the isolates in burn OPD were 9 (39.1%), 13 (56.5%) and 8 (34.8%). Seven isolates (30.4%) in burn OPD showed relatively lower resistance level for Cephalotine and methicillin than burn unit isolates of *S. aureus* .(Table 7).

Table 7: Antibiotic susceptibility pattern of bacterial isolates (88) from burn wound of OPD and burn unit patients at Yekatit 12 hospital burn center. Addis Ababa, 2011.

Antibiotic	Bacterial isolates from burn wound				
	Burn OPD			Burn unit	
		Pseudomonas spp f (%)	*S. aureus* f (%)	*Pseudomonas* spp f (%)	*S. aureus* f (%)
Agumentin	**Resistant**	22 (100)	9 (39.1)	18 (100)	1 (4)
CAF		17 (77.3)	13 (56.5)	18 (100%)	6 (24)
Ceftriaxone		9 (40.9)		6 (33.3)	
Doxycycline		21 (95.5%)		18 (100%)	
NA		21 (95.5%)		17 (94.4)	
Norfloxacine		2 (9.5)		4 (22.2)	
Cepalothin			7 (30.4)		11(44)
Methicillin			7 (30.4)		8 (32)
Amikacin			8 (34.8)		1(4)
Clindamycine			2 (8.7)		0
Vancomycine			4 (17.4)		0
Kanamycine			7 (30.4)		5 (20)
Total isolate		22	23	18	25

6.6. Antibiotic susceptibility pattern of bacterial isolates from blood culture

A surface wound is the likely source of entry to the blood stream. The kind of bacteria and its dominant sensitivity pattern was assessed on blood cultures of burn patients from both OPD and burn unit. Five (83.3%) of six isolates of *S. aures* were found to be resistant for penicillin G. No *S. aureus* was found with resistance to amikacin, clindamycin and vancomycin. Only two isolates were resistant for methicillin.

Regarding CoNS two of eight isolates were resistant for penicillin G and kanamycin. The remaining isolates were susceptible for the rest of antibiotics except one that was found resistant for methicillin as shown in table 8.

Table 8: Antibiotic susceptibility pattern of the isolates (15) from blood culture at Yekatit 12 hospital burn center. Addis Ababa, 2011.

Antibiotic	Bacterial isolates from blood culture			
		S. aureus	CoNS	*S. pyogenes*
Agumentin	**Resistant**	4 (66.7%)	0	0
CAF		2	0	0
Cepalothin		2	0	0
Methicillin		2	1	0
Penicillin G		5 (83.3)	2	0
Amikacin		0	0	1
Clindamycine		0	0	0
Vancomycin		0	0	0
Kanamycine		2	2	1
Total isolate		6	8	1

CHAPTER SEVEN
DISCUSSION

Thermal injury destroys the barrier function of skin, allowing microbial colonization of wounds and even with the use of topical antimicrobials, contamination of wounds is unavoidable (Shankar *et al*., 2009). The dysfunction of the immune System, a large cutaneous bacterial load, the possibility of gastrointestinal bacterial translocation, prolonged hospitalization, and invasive diagnostic and therapeutic procedures all contribute to sepsis (Deirdre *et al*., 2006). Burn induced inflammatory response are indistinguishable from those of infection and the local signs of infection may be absent, minimal or late in developing. Thus, knowledge of the burn ward microbial flora and the current antibiotic sensitivities at any time is important for the better treatment of burn patients.

In this study a total of 41 (24 male and 17 female) patients enrolled from both OPD (25 in number) and 16 from burn unit. Three (7.3%) of them were died during the study period (Table 1). Among the dead, one of them was died within the first week of admission in burn unit. The remaining two deaths were recorded in the second week. One of the patients who died had TBSA of 59% and colonized by three different kinds of bacteria on his wound. The isolates were *S. aureus*, *Klebsiella* spp and *Proteus* spp. His blood culture was also positive for growth of *S. aureus* that has almost the same susceptibility pattern with the one isolated from his pus. In most of the cases the organisms isolated from blood were the same as isolated from pus. This indicates that the organism could enter the bloodstream through the wound and is a potential threat for disseminated infection which can be life threatening. (Kaur *et al.,* 2006; Shankar *et al.,* 2009).

The rest two of the dead were negative for blood culture but their wounds were colonized by bacteria (*S. aureus* and *Pseudomonas* spp). It has been estimated that up to 75% of all deaths following burns are related to infection (Sanyal, 1998; Shankar *et al.,* 2009). On Chalise *et al.,* (2008) study there were 14% death in Nepal among a total of 50 patients whose mean TBSA was 33.9%. But in this study the low mortality rate (7.3%) was probably due to factors such as low %TBSA (mean=11.9), continuous clinical and microbiological surveillance leading to quick detection of etiology, antibiotic therapy and care for regular wound cover. It is also stated that the death rates of burn patients have improved substantially in the past few decades due to advances in resuscitation, nutritional support, pulmonary care, wound care, and infection control practices in specialized burn units (Chalise *et al.,* 2008).

Burn biopsy examination is better tool to determine microbiological colonization and invasion and for quantitative evaluation. It is also less fallacious. Many centers however, in our country and all over the world, continue to rely on swab culture reports to initiate treatment as the specimens are easier to obtain and takes comparatively lesser processing time (Church *et al.,* 2006; Shankar *et al.,* 2009).

In this study a total of 101 isolates were identified from 104 swab (pus) cultures during the study period. *Pseudomonas* spp and *S. aureus* together accounted 87.1% and this finding is more or less similar with a study by Sewnet, 2010 (unpublished) in Yekatit 12 hospital burn center. It has been observed that when patients were brought to the hospital with exposed burnt areas, 15 (36.6%) of the initial swabs revealed no growth. It is stated that initially, the burnt area is considered free of microbial contamination (Vindenes and Jerknes, 1995). However, in our set up being the people are under poor socio-economic status and low health awareness patients may not seek treatment as early as possible so that their wound may get exposed for microbes from the environment. After applying a closed dressing in the hospital, periodic swabs from the same patient reveal presence of different medically important bacteria. High percentage growth immediately after burn injury is natural as open burn wound is favorable environment for growth of bacteria. It is also stated that immediate colonization could be from the patient's endogenous origin, or via contact with contaminated external environmental surface es, water, fomites, air, and from hands of health care workers (Church *et al.,* 2006; Kyros *et al.,* 2007; Manal *et al.,* 2006). Out of 27 wound swabs without growth during the study period in general, 21(77.8%) were from burn OPD patients **(**Table 4**)** which probably because these patients had relatively low (mean=9.7) percent of TBSA than BU patients (mean=15.3) and also it was found that %TBSA had significant association with level of isolation. This is also demonstrated by another study, (Negeri, 2004).

Staphylococcus aureus 15(46.9%) and *Pseudomonas* spp 9 (28.1%) were the most prevalent isolates in day 0 cultures. There was a gradual increase in the number of isolates of *S. aureus* and *Pseudomonas* spp from day 0 to 14th day. At the 14th day, the most frequent organism isolated was *S. aureus* (50%), closely followed by *Pseudomonas* spp (45.8%). This finding is comparable with the studies done in Turkey and Nepal (Erol *et al.,* 2004; Chalise *et al*., 2008). It has been shown that gram positive isolates, *S. aureus,* was dominant (46.9%) on day 0 pus culture (Church *et al*., 2006; Negeri, 2004 (unpublished)). Later on the numbers of gram negative

isolates were increased as the percentage of *Pseudomonas* spp was seen with 45.8% recovery rate at the 14th day (Erol *et al.,* 2004, Srinivasan *et al.,* 2009*).* Five (4.95%) isolates of Proteus spp were identified in the present study which is comparable in other study done in India (Mehta *et al.,* 2007) with 2.3% degree of isolation**.** There were also two isolates of *Citrobacter* spp during the study period and the same number of the bacteria was also identified by a study in Yekatit 12 hospital burn unit (Negeri, 2004).

In the present study, the predominant isolates from burn wound culture were *S. aureus* 48(47.5%) and 40(39.6%) *Pseudomonas* spp. In another study which was conducted a year ago demonstrated a slightly lower number of *S. aureus* (34.04%) and *Pseudomonas* spp (31.8%) in the same study area (Sewnet, 2010). The difference may be because of the disparity in sampling procedure i.e. in this study there was periodic sampling but that was a cross sectional.

Fifteen mixed growths of *Pseudomonas* and *S. aureus* were observed from patients in the study (Table 4). Among these 11(73.3%) of them were from burn unit patients and this may be because patients in burn unit had relatively larger mean %TBSA (15.9) than OPD patients (9.7%). It was found that bacterial isolation from burn wound was statistically significant with percent TBSA ($P<0.02$). Similarly among no growth reports from pus culture 21 (77.8%) were from burn OPD patients who were relatively with low burned percent of TBSA. This could complement the above statement. It was also reported in another study that in case of a severe burn, the probability of wound colonization increases as the patients spends more time in the burn unit and undergo more frequent dressings (Chalise *et al.,* 2008).

Each isolate was tested by disc diffusion technique for their susceptibility pattern against commonly used antibiotics in Yekatit 12 hospital particularly in the burn center.

It was observed that a high level of drug resistance among gram negative isolates especially *Pseudomonas* species. It was moderately resistant to Ceftriaxone (37.5%) whereas resistance was more marked for other antibiotics. All isolates of *Pseudomonas* spp were completely resistant for ampicillin, agumentin, amoxicillin and ceftazidime. Almost all i.e. 97.5% and 95% isolates were also resistant for doxycyclin and nalidixic acid respectively. High resistance for these drugs also demonstrated by other study (Sewnet, 2010). On the other hand, it was found to be more sensitive to Norfloxacine as it is evident by only 15% resistance. This is comparable with study

by Sewnet, (2010) in the same study area. Thus, it is considered as multidrug resistant to the commonly used drugs in the burn unit and the country at large.

Negeri, (2004) also demonstrated that 90% (18/20), 60% and 95% of *Pseudomonas* spp were resistant for agumentin, ceftriaxone and ceftazidime respectively. Resistance for ceftazidime is slightly comparable with the present study. Resistance for agumentin was increased in this study this could be because of the difference in the number of isolates in which in case of Negeri study isolates were small enough. This increasing emergence and spread of multi drug resistant bacteria in hospitals in general and burn centers in particular is of great concern and continues to challenge infection control and hospital epidemiology practice worldwide. (Laura *et al*., 2010, Cheesbrough, 2006)

This study also revealed that 17(77.3%), 21(95.5%) and 2(9.5%) isolates of *Pseudomonas* spp from burn OPD patients were resistant for Chloramphenicol, doxycycllin and norfloxacine respectively. The respective percentage for burn unit isolates was 18(100%), for the 1st two drugs and 4(22.2%) for the 3rd drug. This difference showed that relatively burn unit isolates were more resistant than OPD isolates for the same drug. This might be because of continual exposure of the bacteria for common antibiotics used in hospital environment that makes them to become more resistant (Lorian**,** 2005). Resistance pattern for the rest of antibiotics was comparable (Table 7).

On the other hand both isolates of *Citrobacter* spp were found markedly resistant for most of antibiotics tested for. The two isolates of *Klebsiella* spp and *proteus* spp were found to be completely susceptible for norfloxacine and nalidixic acid. This is comparable with a study by Negeri, (2004).

Observed decline in susceptibility of these common pathogens for frequently used antibiotics calls for increased efforts to ensure more rational use of these drugs. The main forces driving the increase in antimicrobial resistant bacteria are poor infection control practices and inappropriate use of antibiotics (Kavitha *et al.,* 2010). Specific antibiotic utilization strategies like antibiotic restriction, combination therapy and antibiotic cycling may help to decrease or prevent the emergence of resistance.

The antibiotic susceptibility pattern of *S. aureus* showed that most isolates were susceptible for commonly used antibiotics. However, all isolates were resistant for penicillin G where as for

methicillin only 31.3% resistance was observed. The later was comparable with Sewnet, study (29.5%) (2010). But higher than another study done by Negeri, (2004) where all of the isolates (n=26) of *S. aureus* were sensitive to methicillin and 96.2% were resistant to penicillin G. This showed that methicillin and penicillin resistance has been increasing from time to time as it was also established by Erol S et al., (2004). Majority of the isolates were susceptible for clindamycin and vancomycin as only 4.2% and 8.3% resistance was seen for each drug respectively and this finding was consistent with (Negeri, 2004) study in Yekatit 12 burn center.

The occurrence of Methicillin resistant *S. aureus* (MRSA) is more common because of indiscriminate use of higher antibiotics as an emergency empirical therapy. Clindamycin and vancomycin remains the drug of choice for the gram positive isolates in the burn center.

Drug susceptibility pattern of burn unit and OPD isolates of *S. aureus* were compared. The number of isolates in burn unit and OPD was almost equal. There was no *S. aureus* isolate in burn unit that was resistant for clindamycin and vancomycin but there was 8.7% and 17.4% resistance on burn OPD isolates respectively. One (4%), 6(24%) and 1(4%) isolate of *S. aureus* in burn unit had resistance for agumentin, Chloramphenicol and amikacin respectively. However, the respective resistance of the isolates in burn OPD were 9 (39.1%), 13 (56.5%) and 8 (34.8%). Seven isolates (30.4%) in burn OPD showed relatively lower resistance for Cephalotine and methicillin than burn unit isolates of *S. aureus* (table 7).

Along with pus culture periodic blood culture was also performed to demonstrate etiologies of septicaemia (if any) on burn patients. Most of blood samples 77 (83.7%) studied for blood culture noted without growth. No attempt was made to grow anaerobes as it is not common to isolate them in burn patients (David *et al.*, 2007). Similarly another study by Sewnet, (2010) in the same study area found no anaerobe isolates from blood culture.

Only 15 gram positive isolates were identified. *Coagulase negative Staphylococci* renowned with the largest percentage, 8 (53.3%), closely followed by *S. aureus* 6 (40%). One *S. pyogenes* isolate was also identified. There was no mixed growth. The presence or absence of these isolates was unnoticed by the clinicians who were in charge of following the patients. Hence, regards to blood stream infection in burn patients the diagnosis shall be generally on the basis of a combination of clinical features and the result of continuous and periodic surveillance of blood

cultures. Sewnet T, also showed that CoNS 9(42.8%) and *S.aureus* 8(38.2%) dominantly from blood culture. In most of the cases the organisms isolated from blood were the same as isolated from pus. This indicates that the organism has entered the bloodstream through the wound and is a potential threat for disseminated infection which can be life threatening. (Kaur *et al.,* 2006)

Percent TBSA was marginally associated with degree of bacterial isolation from blood ($P<0.057$) but no association was observed with depth of the burn ($P<0.17$). Majority of patients (65%) had less than 10 percent TBSA. This may contribute for low level of bacterial isolation from blood. A false negative result is also more likely if there are few microorganisms in the bloodstream. Number of blood samples drawn at a time also matter the isolation rate (Cheesbrough, 2006; David *et al.,* 2007). In this study at a time only one blood sample had collected because of the availability of limited laboratory resource. But it is recommended at least two, but not more than four blood specimens, should be drawn to detect most common organisms in the blood (David *et al.,* 2007). Because of limited resource this study focuses exclusively on the microbiological profile and no attempt has been made to correlate with other clinical data, i.e blood culture was done periodically regardless of the patient's clinical septicaemic status. This may also slightly contribute for lesser degree of isolation as the fact that blood culture gives best result when blood is drawn while the patient is under indicative sign and symptoms of blood infection (Cheesbrough, 2006) although it may not be always possible to expect exact sign and symptoms from burn patients (Church *et al.,* 2006; David *et al.,* 2007).

Sensitivity pattern of isolates from blood cultures was assessed and two of eight CoNS isolates were found resistant for penicillin G and kanamycin. The remaining isolates were susceptible for the rest of antibiotics except one that was found resistant for methicillin (Table 8).

LIMITATION OF THE STUDY

- The study sampling technique was conventional i.e. all patients visiting the burn center during the study period were included. This might affect the inference power.
- The study subjects were only 41 which are quite small; hence it might weaken the conclusion power of the study.
- Absence of some biochemical reagents and antibiotic discs might influence the appreciation of complete profile of isolates.
- The researcher was unable to collect complete three times periodic samples from some patients who enrolled in the study due to some reasons like being the patients got discharged or transferred to other health centers.
- Regular clinical assessment was not done. The study was exclusively on laboratory profile. This might influenced the bacterial isolation rate from blood samples.
- Because of shortage of laboratory facilities, repeated blood samples were not collected at a time as the fact that a single set should not be used to evaluate any patient with suspected bacterial blood infection. The optimal yield is obtained with two or three sets of blood cultures.
- Serotyping and genotyping of bacterial isolates especially multidrug resistant organisms was not possible due to limited laboratory facility.

CHAPTER EIGHT
CONCLUSION AND RECOMMENDATIONS

8.1. CONCLUSIONS

Summary of major findings: a total of 41 patients were included in the study. The most common cause of burn was exposure to scald (65.9%) followed by flame (24.4%). The mean TBSA was 11.9%. From periodic pus and blood cultures 101 and 15 isolates were recognized respectively. In the first pus culture *S. aureus* was predominated but later on *Pseudomonas* spp showed with increasing percentage. However, regarding blood culture isolates there was no significant change on time. Among total isolates from pus culture, *S. aureus* and *Pseudomonas* spp together accounted (87.1%). Of blood culture isolates, CoNS was with the largest percentage followed by *S. aureus*. It should be noted that the presence or absence of these isolates was unnoticed by the clinicians who were in charge of following the patients. Majority of patients (65%) had less than 10% TBSA. This may contribute for low level of bacterial isolation from blood and low mortality rate. Relating to their antibiotic susceptibility profile, isolates of *S. aureus* from both pus and blood culture showed a moderate degree of resistance to the commonly used antibiotics. But *Pseudomonas* spp were found resistant for most of commonly used antibiotics.

It may be concluded that the composition of bacterial flora in burns is dependent on different factors like the depth and extent of the burn. Repeated swab cultures and antibiotic susceptibility patterns are advised for proper selection of antibiotics to control sepsis which remains the major cause of death in burn patients (Sanyal *et al.*, 1998; Srinivasan *et al.*, 2009). The development of resistance to a particular antibiotic is dependent on the use of that antibiotic in society at large. Indiscriminate and overuse of broad-spectrum antibiotics predisposes burn centers in to sites of multi drug resistant virulent micro flora. Yekatit 12 hospital burn center gets patients from all over the country. Due to this huge diversity, there is a particular microorganism predominant at a particular point in time, but then, it is also difficult to comment on the source of the changing trends.

Finally, burn injuries are very common throughout the world and especially so in the underdeveloped country like ours where people are depend on traditional way of cooking and handling fire. Burns cause great suffering to individual patients and are a great cost to one of the poorest countries in the world where access to burn care facilities and thus proper treatment are compromised.

8.2. RECOMMENDATIONS

Based on this study the following recommendations are drawn:

- ✓ Public awareness has to be created to decrease burn injuries as most burns are preventable.
- ✓ Majority of patients in the burn center were from areas other than Addis Ababa. Therefore, for the sake of ease access specialized medical facilities to give burn related care should be expanded in the country at least a center in each regional state.
- ✓ Every treatment facility has microorganisms unique to it and these change with time. It is therefore of paramount importance to have an in-depth knowledge of the resident organisms and their antibiotic sensitivity pattern so that infection-related complications will improved.
- ✓ The development of resistance to a particular antibiotic is dependent on the use of that antibiotic in society at large. Indiscriminate and overuse of broad-spectrum antibiotics predisposes to multi drug resistance. Hence, the nature of microbial wound colonization and flora changes should be taken into consideration in empirical antimicrobial therapy of burned patients.
- ✓ Similarly, the pattern of bacterial sensitivities is subject to frequent changes. Its regular assessment is important for clinical and epidemiological purposes.
- ✓ Microbiological and drug susceptibility surveillance should cover for all kinds of medically important microbes found on burn wound, hospital environment and on medical staffs to decrease infection and death of burn patients who are markedly immunocompromised.
- ✓ It was demonstrated that a high degree of resistance by the isolates to commonly available antibiotics in Yekatit 12 hospital. The most efficient antibiotics in Yekatit 12 hospital burn center were Norfloxacin, Clindamycin and Vancomycin which can be used empirically pending reports of culture and sensitivity if systemic antibiotics need to be started.
- ✓ Serotyping and genotyping of bacterial isolates especially multidrug resistant organisms was not done in the present study. Therefore, serotyping and genotyping analysis of the isolates using suitable techniques is essential in the future.

REFERENCES

Alireza Ekrami & Enayat Kalantar. (2007). Bacterial infections in burn patients at a burn hospital in Iran. Indian J Med Res; 126: 541-544.

Atiyeh B, Masellis A, Conte F. (2010). Optimizing Burn Treatment In Developing Low- And Middle-Income Countries With Limited Health Care Resources. Annals of Burns and Fire Disasters; 23:1

Baron's. (2000). Medical Microbiology, 4th ed. McGraw-Hill, P: 458-459

Benson. (2001). Microbiological Applications Lab Manual, 8th ed. McGraw-Hill, P: 50-70

Camberlain N. (2009). Medical microbiology. McGraw-Hill, P: 74-75

Chalise PR, Shrestha S, Sherpa K, Nepal U, Bhattachan CL and Bhattacharya SK. (2008). Epidemiological and bacteriological profile of burn patients at Nepal Medical College Teaching Hospital. Nepal Med Coll J; 10(4): 233-237.

Cheesbrough M. (2006).District Laboratory Practice in Tropical Countries. Part II 2nded.P: 80-84

Church D, Sameer Elsayed, Owen Reid, Brent Winston, and Robert Lindsay. (2006). Burn Wound Infections. Clinical Microbiology Reviews; 19(2): 403-434

Cunha B. (2010). Infectious Diseases in Critical Care Medicine, 3rd ed. P: 359-376

David G. Greenhalgh, Jeffrey R. Saffle, James et al. (2007). American Burn Association Consensus Conference to Define Sepsis and Infection in Burns. J Burn Care Res; 28:776–790

Ebrahim Salehifar, Ghasemali Khorasani, Shahram Ala. (2009). Time-related Concordance Between Swab and Biopsy Samples in the Microbiological Assessment of Burn Wounds. Wounds. 21(3) Health Management Publications, Inc.

Erol S, Altoparlak U, Akcay MN, Celebi F, Parlak M.A. (2004). Changes of microbial flora and wound colonization in burned patients. Burns; 30(4):357-61

Herjinder Kaur, Jyothi Bhat, Anup R. Anvikar, Savinder Rao, Vijay Gadge. (2006). Bacterial Profile of Blood and Burn Wound Infections in Burn Patients. Burns; 34: 89-95

Kavitha Prabhu, Sevitha Bhat and Sunil Rao. (2010). Bacteriologic profile and antibiogram of blood culture isolates in a pediatric care unit. J of laboratory Physicians; 2(2): 85-88

Khajuria B, Sharma R, Verma A. (2009). The mortality profile of burn cases in jammu. J of Clinical and Diagnostic Research; 3:1608-1610.

Kyros Ipaktchi, Aladdein Mattar, Andreas D, Andreas D. Niederbichler, Laszlo M. et al. (2007). Topical p38 MAPK Inhibition Reduces Bacterial Growth on Burn Wounds in an in vivo Burn Model. Surgery; 142(1): 86–93.

Laura J, Piddock V, Mark I, Garvey, Mukhlesur M, Rahman2 and Simon Gibbons. (2010). Natural and synthetic compounds such as trimethoprim behave as inhibitors of efflux in Gram-negative bacteria. J Antimicrob Chemother; 65: 1215–1223

Lawrence C. Madoff, Florencia Pereyra. (2008). In: Principle of Internal medicine, Harrison,s. 17th ed. McGraw-Hill, P: 835-836

Lorian, V. (2005). Antibiotics in Laboratory Medicine, 5th Edition. Lippincott Williams & Wilkins, P: 156

Manal M, Baddour, Abuelkheir and Amal J Fatani. (2006). Trends in antibiotic susceptibility patterns and epidemiology of MRSA isolates from several hospitals in Riyadh, Saudi Arabia. Annals of Clinical Microbiology and Antimicrobials; 5:30

Mehta Manjula, Priya Dutta, Varsha Gupta. (2007). Bacterial isolates from burn wound infections and their antibiograms: A 8-year study. Indian Journal of Plastic Surgery; 40: 25-28

Melnick (2007). In: Jawetz, Melnick, & Adelberg's. Medical Microbiology, 24th ed. McGraw-Hill, P: 270

Negeri C. (2005). Microbiology of Burn Unit at Yekatit 12 Hospital, Addis Ababa. (Unpublished) found in Addis Abeba University electronic library.

Robert C, Barber, Ling-Yu E. Chang, Brett D. Arnoldo, Gary F. Purdue, John L. Hunt, Jureta W. Horton, and Corinne C. Aragaki. (2006). Innate Immunity SNPs are Associated with Risk for Severe Sepsis after Burn Injury. Clinical Medicine & Research; 4(4):250-255

Sanjay Dhar, Rakesh Saraf, Kailash Singh, Bhavani Raina. (2007). Microbiological Profile of Chronic Burn Wounds among Patients Admitted in Burn Unit. JK SCIENCE; 9:4

Santucci SG, Gobara S, Santos CR. (2003). Infections in a burn intensive care unit: experience of seven years. J Hosp Infect.53:6 –13.

Sanyal S.C., Mokaddas E.M., Gang RX, Bang R.L. (1998). Microbiology of Septicaemia In Burn Patients. Annals of Burns and Fire Disasters; 11:1

Sewnet T. (2010). Determination of magnitude of bacteremia in burn patients at Yekatit 12 hospital, Addis Abeba, Ethiopia. (Unpublished)

Shankar Srinivasan, Arvind M. Vartak, Aakanksha Patil, and Jovita Saldanha. (2009). Bacteriology of the burn wound at the Bai Jerbai Wadia Hospital for children. Indian J Plast Surg; 42(2): 213–218.

Sleigh D and Timbury.(1994). Notes on Medical Bacteriology. 4th ed. P: 352

Srinivasan S, Vartak. (2009). Bacteriology of the burn wound at the Bai Jerbai Wadia Hospital for children, Mumbai, India-A 13-year study, Part I-Bacteriological profile. Indian J Plast Surg 42:213-8

Thuan T. Nguyen, David A. Gilpin, Nicholas A. Meyer,and David N. Herndon. (1996). Current Treatment of Severely Burned Patients, Annals of Surgery; 223 (1): 14-25

Vandepitte J, Verhaegen J, Rohner P, Piot P, Heuck C. (2003). Basic Laboratory Procedures in Clinical Bacteriology, WHO manual. P: 86-97

Vindenes, Jerknes.(1995). Microbial colonization of large wounds. Burns 21:575-9

Wolf S and Herndon D. (1999). Burn Care. P: 1, 72-79

http://findarticles.com/p/articles/mi_qa3964/is_199911/ai_n8855297/: accessed on 26th Aug – 2010

ANNEXES

Annex I: Investigation form for patients in Yekatit 12 Hospital burn center

I. Socio-demographic data

1. Serial number_____ 2. Card number_____

3. Age_____ 4. Sex_____ 5. Address_____

6. Occupation_____

II. Clinical data

1. Cause of burn trauma (encircle one);

   a. Scald b. Flame c. electricity d. chemical agents e. others (specify)

2. Place of injury (encircle one): a. home b. factory c. others (specify)
3. Anatomic location of the burn (encircle as many):

   a. extremities b. trunk c. head & neck d. perineum

4. Depth of the burn wound (encircle one): a. full thickness b. partial thickness
5. Extent of burn (% of total burn surface area) _____
6. Duration of time before getting medical attention after burn injury_____

Annex II: Laboratory data collection format (on admission, 7th day and 14th day of admission)

1. Patient ID---------
2. Sample code number----------
3. Type of specimen collected a. swab b. blood c. both
4. Culture result ----------
5. Gram stain result (morphology of the bacteria, gram reaction)----------
6. Biochemical test result----------
7. Name of the identified bacteria----------
8. Antimicrobial sensitivity test result (Sensitive, Intermediate or Resistant for commonly used antibiotic discs)----------

Any remark or comment----------

Name of the investigator----------

Signature ---------- Date----------

Annex III: Information sheet and consent form

- ✓ **Name of Investigator**: Awoke Deribie (B.Sc, M.Sc candidate)
- ✓ **Introduction**

You are invited to participate in a research study conducted by M.Sc candidate, from Addis Abeba University. Your participation is voluntarily. This research team includes one principal investigator, data/specimen collectors, senior laboratory professionals and two advisors from Addis Abeba University.

Please take as much time as you need to read/listen the information sheet.

- ✓ **Purpose of the Research Project**

We are asking you to take part in the study because we are trying to learn more about longitudinal bacterial colonization of burn patients for better management of infection complications.

- ✓ **Procedure**

In order to assess the longitudinal bacterial colonization of burn patients at Yekatit 12 hospital; we invite you to take part in this project. If you are willing to participate in this project, you need to understand and give your consent. The required clinical sample will be collected by experienced professionals. Then, you are requested to give your consent to the data collectors.

- ✓ **Risks and Discomforts**

There are no anticipated risks to your participation. From your burn wound periodic swab will be taken. To check your status of septicaemia blood will also be collected peredically. During collection of blood you may feel some discomfort but this does not produce serious pain.

- ✓ **Benefits to subjects and/or to the society**

You will be directly benefited from your participation in the study. Based on the diagnosis result you will be treated accordingly and at the same time you are indirectly benefiting through the generation of this data for better management of patients like you attending this hospital and others hospitals in the country associated with burn care.

- ✓ **Payment /compensation for participation**

You will not receive any payment for your participation in this research study.

✓ **Confidentiality**

There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential. The information collected about you will be coded using numbers.

✓ **Participation and withdrawal**

You can choose whether to be a part of this study or not. If you are volunteer in this study, you may withdrawal at any time without consequences of any kind. You may also refuse to give any sample.

✓ **Person to contact:**

If you have any question you can contact any of the following and you may ask at any time you want.

1. Awoke Deribie(BSc, MSc candidate):
   Cell phone: +251- 09 13 05 98 87 E-mail: awe.love2000@yahoo.com
2. Institutional Review Board, Faculty of Medicine , Addis Abeba University

Patient consent form

Patient ID----------

Name of the patient ----------

I have been informed about the study, which plans to determine longitudinal bacteriology and anti microbial sensitivity pattern of bacterial isolates among the burn patients at burn center at Yekatit Hospital Addis Ababa. The objective and application of the study were explained to me. I am also informed that all information contained within the questionnaire is to be kept confidential. More over I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care and hospital access.

It therefore with full understanding of the situations that I agreed to give the written voluntarily to the researcher to use the specimen taken from my wound and blood; for investigation. I also agreed the organisms isolated might be stored and investigated further on similar grounds. In addition I have had the opportunity to ask a question about the project and I have got the clarification to my satisfaction.

More over I was also told that results will be reported timely to physician in charge for appropriate treatment and management of my case. And hence I agreed that I am contributing to the treatment of my fellows and myself by contributing in this project.

I _____ the undersigned hereby give my consent for giving the requested information and specimen for the purpose of determination of longitudinal bacteriology and antimicrobial sensitivity of the bacterial isolates.

Signature (participant/parent/ guardian): ----------

Witness (Illiterate) ----------

Physician/principal investigator/data collector----------

Date ------/-------/----

የጥናቱ ማብራሪያና ስምምነት ቅጽ

1. ጥንቱን የሚያካሂደዉ ሰዉ

አወቀ ደርቤ (የመጀመሪያ ዲግሪ ምሩቅ ና በአዲስ አበባ ዩኒቨርሲቲ የሁለተኛ ዲግሪ ተማሪ)

2. የጥናቱ ዓላማ

ጥናቱ የሚካሄደዉ የቃጠሎ አደጋ በደረሰባቸዉ የካቲት 12 ሆስፒታል ተኝተዉ በሚታከሙ በሽተኞች ላይ ሲሆን ዓላማዉ በሽተኞችን ለባሰ ህመም የ ሚዳርጉ በሽታ አምጭ ባክቴሪያዎችን ተከታትሎ መለየትና ፈዋሽ መድሐኒቶች አነዚህን ባክቴሪያዎች ለመግደል ያላቸዉን አቅም መሞከር ይሆናል።

3. በጥናቱ ስለመሳተፍ

በዚህ ጥናት መሳተፍ በሙሉ ፈቃዳኝነት ላይ የተመሰረተ ነዉ። ስለሆነም በጥናቱ አንዲሳተፉ ፈቃደኝነትዎን እንጠይቃለን። በጥናቱ ሊሳተፉ ከተስማሙ ሐኪሙ ወይም ዋናዉ ተመራማሪ ለሚጠይቅዎት ጥያቄዎች መልስ መስጠት ይጠበቅብዎተል። ለምርመራ የሚሆን ደምና ከቁስለዎ ላይ ቲንሽ ናሙና አስከ ሦስት ጊዜ ድረስ እንዲወሰድ ፈቃደዎን እንጠይቃለን።

4. ሊከሰቱ የሚችሉ ስጋቶች

ለጥቱ በሚወሰድ ናሙና ምክንያት የተለየ ችግር አይከሰትብዎትም። የናሙና አወሳሰዱ በሽተኛ ዉ ለራሱ ብሎ ከሚሰጠዉ ናሙና የተለየ አይደለም። ደም በሲሪንጅ በሚወሰድበት ጊዜ አንደማንኛዉም በሽተኛ የተለመደ ቲንሽ አለመመቸት ለተወሰነ ደቂቃ ሊኖር ይችላል።

5. በጥናቱ በመሳተፍ የሚገኝ ጥቅም

በሽተኞች ከምርመራዉ ዉጤት ቀጥተኛ ተጠቃሚ ይሆናሉ። የምርመራዉን ዉጤት ከሀኪሞች ጋር በመነጋገር በአጠቃላይ ለሕመምተኞች በሆስፒተሉ የተሻለ ፈጣን አንክብካቤ ለማድረግ ያግዛል።

6. ክፍያን በተመለከተ

በዚህ ጥናት በመሳተፍዎ ምንም አይነት ክፍያ አይከፈለዎትም።

7. የጥናቱ መረጃዎች ሚስጢራዊነት

በጥናቱ ዉስጥ የተሰበሰቡ ማናቸዉም ግላዊ መረጃዎች ሚስጢራዊነት የተጠበቀ ይሆናል። ከማንነትዎ ጋር በቀጥ ተያያዥነት ያላቸዉ መረጃዎች በሙሉ በዋና ተመራማሪዉ ሚስጢራዊ በሆነ የመረጃ ጥንቅር ዘዴ ከተቀየሩ በኋላ ብቻ ለምርምር ሂደቱ የሚዉሉ የሆናሉ።

8. ከጥናቱ ስለመዉጣትና ስለማቋረጥ

ይህ ጥናት በፍቃደኝነት ላይ የተመሰረተ አንደመሆኑ መጠን በማንኛዉም ወቅት በፈቃድዎ ከጥናቱ መዉጣት ይችላሉ።ከጥናቱ ቢዎጡም የተለመደዉን የሕክምና እርዳታ በጤና ተቋሙ ዉስጥ በማንኛዉም ጊዜ የማግኜት መብት አልዎት።

9. ከትናቱ ጋር በተያያዘ ማናቸዉም ጥያቄ ቢኖርዎ በሚከተለዉ አድራሻ ጥያቄዎን ማቅረብ ይችላሉ፦

1. አወቀ ደርቤ
   አድራሻ፦ አዲስ አባበ ዩኒቨርሲቲ ሕክምና ፋካልቲ፤ ስልክ 0913-05 98 87
2. አዲስ አባበ ዩኒቨርሲቲ ሕክምና ፋካልቲ ስነምግባር መቆጣጠሪያ ክፍል (institutional review board)

ስለስምምነቱ ማረጋገጫ ፊርማዎች

የበሽተኛዉ ምስጢር ቁጥር----------

የበሽተኛዉ ሙሉ ስም----------

አኔ ስሜ ከዚህ በታች የተገለጸዉ በዚህ ጥናት ተሳታፊ ለመሆን ስወስን የጥናቱ ዓላማዎች አሰራሮችና ቅድመ ሁኔታዎች በግልጽ በመረዳትና እንዲሁም ከጥናቱ ተሳተፊነት ፈቃደኝቴን በማንኛዉ ደረጃ የማስወገድ መብቴን በማረጋገት ነዉ።

እኔ---------- በጥናቱ ተሳተፊ መሆኔን በፊርማየ እያረጋገጥኩ ይህንን ስወስን በትናቱ ሳቢያ ሊከሰቱ የሚችሉ ስጋቶችን በሚገባ የተረዳሁና ከጥናቱ በማንኛዉም ደረጃ ራሴን ለማግለል ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እንደማይነፈጉኝ በማመን ነዉ።። እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጹልኝ መሆኑን በፊርማዬ አረጋግጣለሁ።።

የበሽተኛዉ ሙሉ ስም---------- ፊርማ----------

የተመራማሪዉ ሙሉ ስም---------- ፊርማ----------

የምስክር ሙሉ ስም---------- ፊርማ----------

ቀን------/------/------

ASSURANCE FORM

I, the undersigned, assert that this MSc. thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for the thesis have been accordingly acknowledged.

M. Sc candidate: ***Awoke Deribie, BSc.*** Signature: _____ Date_____

Advisors:

1. ***Tamrat* Abebe *(BSc., MSc., PhD.*** candidate***)***

   Signature: _____ Date _____

2. ***Adane Mihret (DVM, MSc., PhD.*** candidate***)***

   Signature: _____ Date _____

3. **Yohannes Demisie** (MD, Chief Plastic Surgeon)

Signature: _____ Date_____