MICROBIOLOGY OF BURN UNIT AT YEKATIT 12 HOSPITAL, ADDIS ABABA

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OCTOBER, 2005

MICROBIOLOGY OF BURN UNIT AT YEKATIT 12 HOSPITAL, ADDIS ABABA

A THESIS PRESENTED TO GRADUATE STUDIES PROGRAMME, ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL MICROBIOLOGY

By

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October, 2005

Declaration

I, the undersigned, declare that this M.Sc 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 duly acknowledged.

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AKNOWLEDGEMENTS

I would like to express my sincere and heart felt gratitude to:

Dr Yimtubezinash Woldeamanuel, my supervisor in the Department of Microbiology; Faculty of Medicine, Addis Ababa University; for providing unreserved, constructive advice and encouragement throughout the course of this research.

Dr Yohannis Mengiste, my supervisor, for providing constructive advice literature beginning from the research.

W/o Mekdes Gebeyehu and Dr Eshetu Lemma, my co-supervisors, in the Department of Bacteriology at Ethiopian Health and Nutrition Research Institute (EHNRI), for their advice and supporting me in the laboratory with full heart especially in providing me with necessary reagents.

All staff of bacteriology laboratory at EHNRI for their technical assistance throughout the research.

All staff of the Burn unit at Yekatit 12 hospital for their kind cooperation during sample collection.

This study is supported by Graduated studies, Addis Ababa University.

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LIST OF ABBREVIATIONS

BSI Blood stream infection

BWI *Burn wound infection*

CDC *Center for Disease Control*

CFU *Colony forming unit*

HCW *Health care worker*

IgG *Immunoglobin G*

MDR *Multi-drug resistant*

MRSA *Methicillin resistant* Staphylococcus aureus

MSSA Methicillin sensitive *Staphylococcus aureus*

NNIS National Nosocomial Infections Surveillance

RFLP *Restriction fragment length polymorphism*

TBSA *Total body surface area burned*

ABSTRACT

Burn patients are at risk of acquiring infection because of the loss of skin barrier and suppressed immune system, compounded by prolonged hospitalization and invasive therapeutic procedures. Since specialized burn units were non-existent in Ethiopia before the establishment of one at Yekatit 12 hospital, Addis Ababa, no study was done on burn patients in the country. Hence, to study the microbiology of burn patients admitted at Yekatit 12 hospital, a prospective study was undertaken on 52 patients from March to August 2005. Periodic swabs were taken from the burn patients as well as from the patients' attendants, attending staffs, and the burn unit environment in order to associate prevailing burn pathogens with endogenous or exogenous sources. The pattern of colonization in the burn wound changed during the hospital stay from a predominance of Gram-positive bacteria (69.8%) at admission to Gram-negative bacteria (68.6%) after the second week. On comparing infected patients (n=38) with non-infected patients (n=14), there was significant difference in age distributions (p=0.035), in burn types (p=0.017), hospital stay (p<0.0001), and total burned surface area (p=0.005) but no significant difference in the frequency of infections by sex (p=0.535). Among the 33 patients without infection on admission, 20(60.6%) developed at least 1 type of nosocomial infection with the most frequent nosocomial infection being burn wound infection (N=20; 60.6%), followed by urinary tract infection (N= 10; 30.3%), bloodstream infection (N=4; 12.1 %) and pneumonia (N=1; 3.0%). 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). All of the isolates of *S. aureus* were sensitive to methicillin, clindamycin and vancomycin and were moderately sensitive to chloramphenicol, cephalothin, and augmentin but highly resistant to ampicillin and penicillin G. Thirteen isolates of *P. aeruginosa* (86.7%) strains were designated multi drug resistant to the commonly used drugs in the burn unit and the country at large. Burn wound infection was the most common infection in the burn unit and *S. aureus* and *P. aeruginosa* with high degree of resistance to the commonly used antibiotics in the burn unit were the most commonly isolated organisms from the burn wounds. Since bacterial isolates with identical sensitivity pattern to the clinical isolates were identified from both endogenous sources and exogenous ones, it can be deduced that acquisition of the major burn pathogens was likely multifactorial.

CHAPTER I: INTRODUCTION

GENERAL INTRODUCTION

Infection remains a foremost concern in the management of the burn wound because the large raw area with its serous exudates may act as huge culture plate on which organisms can establish and multiply little affected by the body immune response (Mayhall, 1999). For patients who survive the acute phase of a burn injury, infections are the most common cause of death. It has been estimated that 75% of all deaths following thermal injuries are related to infection (Neelam *et al*., 2004; Salah *et al*., 2003; Zorgani *et al*., 2002). The most common sites of nosocomial infections in burn patients are the burn wound and the lungs (Apelgren *et al*., 2002; Revathi *et al*., 1998).

Extensive burns contribute to immunosuppresion and this renders such patients prone to invasive bacterial infections. The burn injury destroys the skin barrier that normally prevents invasion by microorganisms, making the burn wound the most frequent origin of sepsis in such patients (Mayhall, 1999; Atiyeh *et al*., 2001; Agnihotri *et al*., 2004).

Infection risk for burn patients is different from other patients in several important respects. Sources of organisms are found in the patient's own endogenous (normal) flora, from exogenous sources in the environment, and from healthcare personnel (Pruitt et al., 1998). After microorganisms are transmitted from the above sources to the surface of the burn wound, host and microbial factors determine whether the organisms will survive, colonize the surface, and invade the burn wound (Mayhall, 1999; Atoyebi *et al*., 1992). Survival in burn patients has improved tremendously with the control and prevention of the exogenous sources of infections and with various methods of elimination of endogenous sources (Dayoub, *et al*. 1995; Mayhall, 1999).

The methods for managing burn injury have evolved during the past 50 years. This evolution has been accompanied by changes in the etiology, epidemiology, and approach to prevention of burn wound infections (BWIs). From 1950s to mid-1980s, burn wounds were treated by the exposure method, with application of topical antimicrobials to the burn wound and gradual debridement with immersion hydrotherapy.

As early burn wound excision and wound closure became the focal point of burn wound management, accompanied by a change from immersion to showering hydrotherapy, the rate of BWI appeared to decrease. Few epidemiologic studies have been done since this change in the approach to management of burn injury (Mayhall, 2003).

1.1. Literature review

1.1.1. Pathogenesis of burn wound colonization and infection

Burn wound infection is defined as the massive involvement of the burn wound and the adjoining tissue as recognized locally by inflammatory signs and exudation of pus. In addition, it can also qualitatively defined by culturing the bacterial flora from surface swabs and by quantitative bacteriology which reveals an excess of 10^5 organisms in each gram of involved tissue. Burn wound sepsis is predominantly due to a mixed flora of organisms and may be locally or generally invasive but is lethal in either form (Nagoba *et al*., 1999).

Loss of the integument combined with the immune defects that accompany thermal injury place the burn patient at high risk for burn wound infection. Initially, the burnt area is considered free of major microbial contamination. However, Gram-positive bacteria in the depths of sweat glands and hair follicles may survive the heat and unless topical antimicrobial agents are used, these bacteria heavily colonize the wounds within the first 48 hours after injury (Salah *et al*., 2003). Further colonization with other potentially invasive bacteria and fungi derived mainly from the patient's gastrointestinal and upper respiratory tracts as well as from the hospital environment occurs. Reports of the mean times of burn wound colonization slightly differed among different studies. In any case, colonization will occur in fresh burns irrespective of administration of systemic antibiotic prophylaxis or daily cleaning and application of topical antimicrobial agents.

In addition, there was really no remarkable difference in the mean colonization times in one series (3.7 days for *S. aureus*, 5.6 days for *Pseudomonas* species), in which cases were managed in surgical wards (Bowen *et al,* 1990) and another series in which cases were managed in specialized burn units (Lawrence, 1985).

Following colonization, the organisms on the surface start to penetrate the burn eschar to a variable extent, depending on their invasive capacity, local wound factors, and the degree of patient's immunosuppression (Mayhall,1999; Salah *et al*., 2003; Al-Akaylhl, 1999). The number of organisms on the wound is also crucial for infection to follow. If organisms reach a concentration of at least 10^5 CFUs (colony forming units) per gram of tissue, they may spread from the hair follicles along the dermal subcutaneous junction. Perivascular colonization may result in thrombosis, vascular occlusion, and necrosis of the remaining viable elements. The resultant ischemic and bacterial autolysis may convert a partial thickness injury to a full thickness injury (Mayhall, 1999).

Abnormalities of Host Defense Following Burn Injury

Abnormalities can occur in almost every component of host defense following a burn injury, but the relative importance of each varies with age of the patient, extent of burn, and interval following injury (Mayhall, 1999).

Acute burn injury causes a generalized vascular response involving both burned and non-burned areas, which is characterized clinically by the accumulation of water and plasma proteins in the extravascualr spaces. This is associated with an inability of the host to localize inflammatory cells in the burned tissue of other areas subjected to bacterial contamination and causes a transient but profound susceptibility to infection (Mayhall, 1999).

After the first few days, a layer of granulation tissue gradually develops beneath the burn wound eschar which affords the host a progressively increasing resistance to invasion of bacteria in the eschar. This capability diminishes as granulation tissue gradually develops beneath the burn wound scar, partially explaining the late susceptibility of burn patients to local invasive infections. Because of the exudation and loss of plasma proteins into the burn wound and interstitial tissues, complements and IgG fall in concentrations immediately following burn injury.

An accentuated catabolic destruction of IgG can result in failure to recover normal levels and this can be associated with sepsis (Munster, *et al*., 1970). Immunosuppression in the burn patient involves the nonspecific immune system (abnormal neutrophil and macrophage functions), cellular immunity (diminished ratio of helper to suppressor lymphocytes, decreased Natural Killer cell activity), and humoral immunity (activation of complement with drop in complement activity, diminished serum immunoglobin levels) (Heidman *et al*., 1992; Lutterman *et al*., 1986).

1.1.2. Pathogens associated with burn wound colonization and infection

History indicates that the relative importance and cyclic pathogenicity of various organisms have changed and may be expected to continue to change as systemic and topical antibacterial treatment develops and changes (Komolafe *et al*., 2003; Mayhall, 2003).

For example, before the discovery of penicillin and the sulphonamides, streptococcal infections were the most frequent cause of septic death in burns. In the late 1920s and mid-1930s Pack, Aldrich, and Cruickshank separately reported that burn patients were mostly colonized with hemolytic streptococci by days 1-6 post-burn (cited in Komolafe *et al*, 2003). However, by the late 1940s it was noted that streptococcal infections in burns had been essentially eliminated with the advent of penicillin with only few focal outbreaks (McGregor, 1998). As streptococcal infections declined, *S. aureus* became the major burn pathogen and by the mid-1950s to early 1960s it was reported as the primary isolate recovered in 75% of the burn patients dying of septicaemia. Once Staphylococcus infections were controlled, the Gram-negative organisms came into increasing prominence and replaced *S. aureus* in frequency of occurrence. The decade of the 1970s heralded an increase in the predominance of yeast, fungi, and viruses in the burn wound flora profile. Studies that span the period of the late 1970s and early 1980s (Bonny *et al.,*1984 cited in Komoafe *et al*., 2003) indicate that *S. aureus* once again emerged as the predominant burn wound pathogenic isolate as Gram-negative organisms seemed to be on the decline. Furthermore, these researchers demonstrated a significant association between increasing burn size and increasing incidence of Gram-negative pathogenic organisms.

The predominance of any particular bacterial organism in BWIs depends among others, on the extent of burn wound and the time specimen collection for bacteriology was carried out. In addition, like burn wound colonization, the organisms that predominate as causative agents of burn wound infections in any burn unit also change over time. Gram-positive organisms are initially prevalent, and then gradually become superceded by the Gram-negative opportunists that appear to have a greater propensity to invade (Salah *et al*., 2003; Komolafe *et al.*, 2003; Ulku *et al*., 2004).

Organisms are brought to the burn unit in the wounds of new patients, which would then persist as dominant flora in the unit until new organisms are introduced. In one study, for example, a new MDR (multi-drug resistant) strain of *P. aeruginosa* was introduced into the burn unit from newly admitted patient and eradication of the organism was possible only when the infected patients were removed (Mark *et al*., 2001). Furthermore, alterations in the protocols of burn wound management and the introduction of new topical and systemic antimicrobial agents eventually influences the nature of the microbial flora of the burn unit. Therefore, it is often not sufficient to be aware of the organisms that are generally considered a problem for burn patients. Knowledge of the flora that predominantly colonize the burn wounds in a particular burn unit would allow prompt management of imminent clinical sepsis with proper antibiotics, before the results of microbiological cultures become available (Salah *et al*., 2003; Ulku *et al*., 2004).

Bacteria as colonizing and infecting organisms:

Currently, the most common pathogens isolated from burn wound are bacteria particularly *S. aureus, P. aeruginosa*, other gram-negative bacilli, *Enterococcus* spp. and *S. pyogenes* (Ulku *et al.,* 2004; Neelam *et al*., 2004; Pandit *et al.,* 1993; Gastmeier *et al*., 2002). Anaerobes cause up to 2-7% of all burn wound infections (Karyoute, 1989; Mayhall, 1999). The relative frequency and importance of each organism varies among different studies (Valerie *et al.* 2003). In a retrospective study by Komolafe and his colleagues in Balantyre, Malawi, 53.6% of specimens (170/317) yielded a single isolate while 25.6% (81/317) and 20.8% (66/317) of burn wound specimens had double and triple isolates, respectively. Of the total 535 bacterial isolates studied, 44.1% were Gram-negatives and 55.9% Gram-positives.

Among the Gram-negatives*, P. aeruginosa, Proteus mirabilis, E. coli* and *K. pneumoniae* were the most common accounting for 94.1% while in the Gram-positive group, staphylococcal and streptococcal spp. predominated (100%). Taken together, the three most common isolates in this study were *S. aureus* (37.6%), *P. aeruginosa* (22.4%) and beta-hemolytic streptococci (13.6%) (Komolafe *et al,* 2003). Similar predominance of *S. aureus* and *P. aeruginosa* were seen in other studies (Heberal *et al*., 1987; Pezzino *et al*., 1989; Agnihotri *et al*., 2004).

S. aureus continues to be the most important cause of BWIs (Ulku *et al.*, 2004). There is increasing evidence that methicillin-resistant *S. aureus* (MRSA) has become a significant problem in many burn units (Ulku *et al.,* 2004; Embil *et al*., 2001).

P. aeruginosa is a well-recognized cause of nosocomial infections among patients with burns (Agnihotri et al., 2004). It is usually spread from patient to patient by direct contact, via staff involved in direct patient care, or through contact with contaminated surfaces. It survives well in the hospital environment in damp areas such as sinks, taps and rubber hosing (Kolmos et al., 1993; Spyros et al., 1972). Once established in these environmental niches, the organisms can persist for months within a unit, posing an infection risk for patients being treated there. Staff members' hands can become transiently contaminated and transfer infection between patients. Other shared facilities such as chairs, cushions, dressing's trolleys and mattresses can facilitate cross-infection (Fujita et al., 1981). There is a significantly higher mortality and morbidity in terms of length of stay, number of surgical procedures and the amount of blood products used among patients infected with aminoglycoside resistant Pseudomonas than control patients not infected with this organism (Tredget et al., 2004).

Enterococci have become one of the most important causes of BWIs in some units most likely due to the widespread use of third generation cephalosporins over the last decade to which enterococci are resistant (Still *et al*., 2001).

Other important bacterial burn wound pathogens include *E. coli, Proteus* spp*., Klebsiella* spp*., Serratia marcescens, Enterobacter* spp*., Acinetobacter* spp. and *S. pyogenes* (Karyoute, 1989; Rameshwar *et al*., 1999).

Fungi as colonizing and infecting organisms

The great preponderance of BWIs caused by fungi is due to filamentous fungi. The filamentous fungi that most often cause burn wound infection are *Aspergillus* spp., Zygomyctes, and *Fusarium* spp. *Candida* spp are the fungal organisms that most commonly colonize the burn wound but do not invade and cause infection as often as do filamentous fungi (Karyoute, 1989; Becker *et al.,* 1991). Thus the surfaces of burn wounds are frequently colonized by *Candida* spp., but rarely do patients develop BWI due to *Candida* spp. according to histopathologic examination of burn wound tissue (Karyoute, 1989). Candida colonization appear to be primarily from endogenous sources while true fungi are ubiquitous in the environment and can be found in air handling and ventilation systems, plants and soil (Becker *et al.,* 1991). Factors that appear to have markedly reduced bacterial BWI, including patient isolation, topical agents, and wound excision do not appear to have had a similar effect on fungal wound infection. The mechanism of spread and colonization of fungi, and the lack of effective topical antifungal agents may explain this finding (Becker *et al*., 1991).

Viruses infecting burn wound

Symptomatic Herpes simplex infections, mainly reactivations, involving the burn wound tend to occur in healing partial-thickness burn wounds that involve the face. In one study, 25% of children with burn wound had serologic evidence of Herpes simplex infection, but only one had a burn infection due to Herpes simplex (Mayhall, 1999).

1.1.3. Epidemiology of Burn wound colonization and infection

- ***Incidence of colonization and infection***

It has been observed in Center for Disease Control (CDC) surveillance during 1995 to 2002 that the rates of nosocomial infections for burn units are higher than that seen in wards, and other intensive care units (ICUs) (NNIS, 2002). The rate of nosocomial infections varies among different studies but lies between 28% and 86 % (Geyik *et al*., 2003; Oncul *et al*., 2003; Askarian *et al.,* 2003; Rastegar *et al*., 2000).

It has also been demonstrated that patients with burn injuries acquired MRSA (methicillin-resistant *S. aureus*) more often than patients without burns and that the burn unit is the major foci of endemic MRSA infections within a hospital (John *et al*., 1983; Kooistra, *et al.,* 2004).

Incidence of nosocomial infection is affected by the TBSA (total burned surface area). The overall incidence of nosocomial infections (including BWIs) is low for patients with <30% TBSA burn injuries (Oncul *et al*., 2003).

- ***Sources of colonization and infection***

Some of the reservoirs of burn wound colonization and infection that warrant discussions are:

i. Burn wounds of patients: The collective burn wound surfaces of the patients in a burn unit may make up an important reservoir of microorganisms that can cause infection. The burn wound has been shown to be a reservoir for *P. aeruginosa, S. pyogenes,* and *S. aureus* and hence the patient colonized or infected with these pathogens represents an important reservoir, which may be subsequently dispersed to other patients via the hands of HCWs (health care workers) or through the inanimate environment (Wiliam *et al.,* 1983; Mayhall, 1999).

ii. Gastrointestinal tract: There is substantial evidence that microorganisms that colonize the burn patient's bowel may colonize the burn wound and lead to BWI. For example, *P. aeruginosa* may reach the bowel by ingestion of these organisms as a result of cross-contamination from one burn patient's wound surface to the oropharynx of a patient in a nearby bed or by ingestion of food contaminated by *P. aeruginosa.* Such organisms from the gut when passed in faeces may then colonize areas near the anus including buttocks, perineum, lower abdomen, and inside of the upper thighs. The gut flora may also contaminate the burn wound by translocation from the gastrointestinal tract (Mayhall, 1999).

iii. Endogenous flora: Early BWIs caused by Gram-positive cocci are due to organisms from the endogenous skin flora (Mayhall, 1999). Another endogenous source for potential burn wound pathogens is the nose.

In one study which used molecular typing of strains of *S. aureus* recovered from patient's nares on admission and from sites of colonization, it was observed that 78% of the colonizing strain was identical to the strain recovered from the nares at admission (Kooistra *et al*., 2004). Importantly, 64% of the patients who did not carry the organism at admission also developed burn wound colonization. In another study, 20 % of HCWs at a burn unit carried *S. aureus* (all methicillin sensitive) in the nose (Neelam *et al*., 2004) while Preetha and colleagues (1998) found a 76% rate of carriage among HCWs of which 50% were MRSA carriers.

iv. Environment: Microorganisms that cause BWI have been recovered from a number of inanimate sites in the environments of burn units (Wiliam *et al*., 1983). Table 1.1. lists sites from which burn wound pathogens have been recovered in the environment of burn units. Among the most important inanimate reservoirs or sources for microorganisms that cause burn wound infection is hydrotherapy equipment (Embil *et al*., 2001).

Table 1.1. Sites of environmental contamination in burn units

Site	organism	Reference
Hydrotherapy equipment	MRSA	Embil et al., 2001; Wiliam et al., 1983
Sinks	*P. aeruginosa*	Jean et al., 2003
Stretchers at hydrotherapy	*P. aeruginosa*	Jean et al., 2003
Sink basins	*P. aeruginosa*	Spyros et al., 1972
and drains	MRSA	Wiliam et al., 1983
Water supply	*P. aeruginosa*	Torregrossa et al., 2000
Shelves	*Acinetobacter spp*	Bayat et al., 2003,
Bed rails	*P. aeruginosa*	Mayhall, 1999
Air	MRSA	Neelam et al., 2004
		Wiliam et al., 1983
Mattresses	*P. aeruginosa*	Mayhall, 1999
	Acinetobacter spp	Bayat et al., 2003
Floor	MRSA	Wiliam et al., 1983
Sterile dressing	*E. coli*	Neelam et al., 2004
Saline bottles	*Proteus* and *Enterobacter* spp.	Neelam et al., 2004
Door handle and ventilator	*Acinetobacter* spp.	Roberts et al., 2001
Antiseptics	*P. aeruginosa*	Oie et al., 1996
Chlorhexidine	*P. aeruginosa*	Jean et al., 2003

- ***Modes of Transmission***

Hands of Health care workers: There is evidence that microorganisms are transmitted between patients by the hands of medical personnel (patient to hands to patient) or they may be indirectly transmitted by contaminated hands (patient to hands to inanimate environmental surface to hands to patient) (Wiliam *et al*., 1983; Spyros *et al*., 1972). It has been also demonstrated that environmental contamination with MRSA was sufficient to contaminate the gloves of personnel who had contact with the inanimate environment of the patient's room, but had no direct contact with the patients (Lemmen *et al*., 2004; John *et al*., 1983). Contamination of the hands of HCWs together with environmental contamination has been crucial modes of transmission in other MDR bacteria, too (Simor *et al.,* 2002).

Gastrointestinal tract of patients: Organisms that gain entrance to the gut of a patient may be carried to the patient's burn wound surface by feces. The gut may be inoculated with a pathogen by contact of the patient's oropharynx with the contaminated hands of a HCW or by ingestion of contaminated food (Mayhall, 1999).

Inanimate environmental surfaces: It has been documented that transfer of pathogens from hydrotherapy equipment and mattresses subsequent to patient contact results in colonization of the burn wound. In one study, an outbreak of MRSA in a burn unit from hydrotherapy equipment was controlled with cessation of a stretcher shower protocol in the common hydrotherapy room (Embil *et al*., 2001).

- ***Risk factors for colonization and infection***

The major predisposing factors to the high incidence of infection are poor personal hygiene and socio-economic conditions of the patients, compounded by materials (vegetable oils, ink, and cow dung) smeared on their wounds and a delay in the transportation of the patients to the burn unit (Mathangi *et al*., 1985). Other risk factors associated with infection are patient factors such as age, extent of wound, presence of preexisting diseases, impairment of blood flow and acidosis. Microbial factors such as virulence, numbers of the organisms, secreted products (such as enzymes, toxins) and antimicrobial resistance all contribute to the invasiveness of the etiologic agents of burn wound infections (Morsi 1990; Ozumba *et al*., 2001; Askarian *et al*., 2003).

Some studies have demonstrated that patients with infection were older, had larger burns and were submitted to more procedures such a ventilator therapy, central venous catheters and arterial catheters (Apelgren *et al.,* 2002; Gastemier *et al.,* 2002).

The length of hospital stay is an important risk factor for colonization and infection of burn wounds. It has been observed that there is a significant positive association between length of stay and colonization and infections with pathogens (Apelgren *et al*., 2002; Neelam *et al.,* 2004).

Studies also demonstrated a significant association between burn wound size and BWI as well as sepsis. Patients with higher TBSA were more likely to develop BWIs and sepsis (Neelam *et al*., 2004; Gastmeier *et al.,* 2002). Infected patients also had higher percent of full-thickness burns as compared to uninfected ones (Neelam *et al*., 2004).

The type of burn injury was also found to be a risk factor in one study with patients with flame and inhalation injury being at high risk of infectious complications (Rodgers *et al.,* 2000).

When organisms in a burn patient population become resistant to the topical antimicrobial agent used for suppression of growth on the burn wound, for any given patient the risk of uncontrolled growth of microorganisms in the wound increases and invasion of viable tissue becomes more likely. For this reason, outbreaks of colonization or infection due to gram-negative bacilli resistant to topical antimicrobial agent such as gentamicin, silver sulfadiazine or silver nitrate have been reported (Jean *et al*., 2003; Mayhall, 1999).

Resistance to systemically administered antibiotics may also result in a selective advantage for the resistant microorganisms and place patients at greater risk for BWI. In one study which used gentamicin and tobramycin empirically to treat BWIs, the antibiotics well reached the layer of the burn wound and eliminated the microorganisms on the surface. During the therapy, six patients developed superinfection of the burn wound with an opportunist, *Serratia marcescens,* and five of these isolates were highly resistant of the antibiotic being administered. Thus, when a microorganism present on the wounds of patients in a burn unit becomes highly resistant to an antibiotic used frequently to treat BWI, particularly when used empirically, use of this antibiotic may place patients at risk for BWI.

This has also been substantiated in patients colonized with vancomycin-resistant enterococcus who were found to have risk factors such as prior vancomycin use, prior use of third generation cephalosporins, critical illness with severe underlying disease or immunosuppression, and a prolonged hospital stay (Mayhall, 1999).

There are well recognized risk factors for mortality in burn patients such as large burns, late primary excisions, old age and polymicrobial bacteremias (Apelgren *et al.,* 2002).

- ***Outbreaks of infection in burn units***

Infections in patients with burns do increase mortality; morbidity and hospital stay especially during outbreaks of infection with antibiotic resistant organisms (Apelgren *et al.,* 2002; Oncul *et al*., 2003).

It is difficult to pinpoint the definite source of infection by the etiologic agents in any outbreak situation. However, various studies have postulated that carriage of antibiotic resistant organisms particularly MRSA by HCWs contributes to the occurrence of the respective infections in clinical situations. The problem is compounded in burn unit as patients are severely immuno-compromised and receive numerous antibiotics. Care of these patients often requires many hours of hands-on contact (Richard *et al*., 1982; Torregrossa *et al.,* 2000). Besides the hands of HCWs, carriage in other parts of the body is a possibility and hence be responsible as involuntary vehicles for microorganisms for cross-transmission (Roberts *et al.,* 2001).

Studies have also demonstrated that inanimate environment around the patient including furniture are often contaminated and can be important source of outbreak of infection (Bollerao *et al*., 2003; Roberts *et al.,* 2001). An outbreak of MRSA in a burn unit had also resulted from contamination of a hand held shower and stretcher for showering in the hydrotherapy room (Embil *et al.,* 2001).

MDR Gram-negative bacteria particularly *P. aeruginosa* have frequently been reported as the cause of nosocomial outbreaks of infection in burn units or as colonizers of the wounds of burn patients. Such infections caused by the Gram negative pathogens continue to be a common complication in burn patients and to contribute substantially to burn-related morbidity and mortality worldwide despite advances in surgical care and the introduction of potent antimicrobial agents.

One study (Jean *et al.,*2003) has disclosed that more than one clone of MDR *P. aeruginosa* can colonize not only burn wounds but also other sites of the bodies of burn patients and that a single clone of MDR *P. aeruginosa* can persist in different body sites of burn patients for weeks and months and can subsequently cause outbreaks of various severe infections despite antibiotic therapy, reinforcement of isolation precautions, and improved environmental decontamination measures (Mark *et al*., 2001).

Previous results also support that a single MDR strain could persist in the same patient for many months and cause recurrent episodes of clinical infection (Hsueh *et al*., 1998).

Although eradication of MDR organisms may not be achieved during outbreaks of infection and may be very costly, infection control interventions can decrease new cases (Lai *et al*., 1998).

1.1.4. Diagnosis in burn wound colonization and infection

- ***Clinical Diagnosis***

Changes in the wound characterized by dark brown, black, or violaceous discoloration; unexpectedly rapid separation of the eschar; hemorrhagic discoloration of subeschar tissue and edema; and violaceous discoloration of unburned skin at the wound margin suggest burn wound infection. Clinical suspicion of infection is heightened when these local wound manifestations are accompanied by hypothermia, hyperthermia, hypotension, oliguria, ileus with abdominal distension, or altered mental status (Mayhall, 1999). But certain factors confound the clinical diagnosis of infection in burn patients. Such factors are inhalation injury, central nervous system dysfunction and injury, narcotics, and hormonal response to injury (Morsi, 1990; Pruitt, 1984).

- ***Microbiologic Diagnosis***

Surface swab cultures either qualitative or quantitative cultures have been used in the diagnosis of colonization and BWIs (Mayhall, 1999; Karyoute, 1989).

When qualitative results and quantitative bacterial counts of surface swabs and wound biopsies were taken from burn patients, a significant correlation between the total bacterial counts of surface swabs and the total bacterial counts of biopsies were seen.
Qualitatively, when *S. aureus* was present in the burn wound biopsy, it was present on surface culture 95% of the time, and when *P. aeruginosa* was recovered from the burn wound biopsy, it was cultured from the surface swabs 92% of the time. While *S. aureus* and *P. aeruginosa* in the burn wound may be detected by surface swabs, surface cultures are generally not useful for predicting the quantitative microbiology of the burn wound (Mayhall, 1999).

Quantitative culture of wound biopsies has high sensitivity but a low specificity. Burn wounds with $< 10^5$ CFUs/g of tissue are highly unlikely to be infected, whereas only about one-third of burn wounds with at least 10^5 CFUs/ g of tissue are really infected (Mayhall, 1999). Gram's stain or hematoxylin and eosin stain are most useful for the identification of bacteria while periodic acid-Schiff stain or silver methenamine stain are best for fungal identification (Moris, 1990).
The problem with interpretation of biopsies has been the uneven distribution of microorganisms, both qualitatively and quantitatively, throughout the burn wound (Mayhall, 1999).

- ***Histopathologic Diagnosis***

Histologic examination of the biopsy specimen is a very reliable means of differentiating wound colonization from invasive infection. Identification of the histologic changes characteristic of bacterial, fungal, and viral infections facilitates the selection of appropriate therapy (Pruitt *et al.,* 1998). Therefore, in burn wounds with unexcised eschar, the diagnosis of BWI can be made by histopathologic examination of a full-thickness burn wound biopsy. The specimen is divided- one-half to be cultured quantitatively, and one-half to be processed for histopathologic examination (Mayhall, 1999).

- ***Epidemiological investigation***

Environmental sampling. The role of the inanimate environment surrounding the colonized or infected patient in disease transmission has been studied by different authors (Embil *et al.,* 2001; Wiliam *et al*., 1983; Roberts *et al.,* 2001). Volumetric air samplers and Rodac plate cultures were used in the survey of inanimate environment in patient rooms and adjacent rooms for airborne and surface level contamination with MRSA (Wiliam *et al.,* 1983).

HCW screening. Screening cultures for all staffs and attendants can be obtained from nares, hairline, hands, axilla, or throat (Embil *et al.,* 2001; Kooistra *et al*., 2004).

Epidemiological typing: Typing can be used to test hypotheses about the reservoirs, sources and vehicles of transmission, to verify the efficacy of control measures, and to study the epidemiology of outbreak strains, and in confirming their clonality. It can be performed by using a diversity of phenotypic and increasingly today, genotypic methods (Struelens *et al.,* 2002).

Among phenotypic methods currently used for hospital epidemiology, the antibiogram is still extremely useful as a routine technique to detect hospital infection with antibiotic-resistant bacteria and determine the similarity of isolates in hospitals (Mayhall, 1999).A good concordance of discriminatory power of antibiogram and molecular typing has been demonstrated in one study (Hsueh *et al*., 1998) but not for MRSA isolates in another study (Namiko *et al.,* 2001). Serotyping is a moderately discriminating phenotypic method that is available only for a limited range of nosocomial pathogens, including *P. aeruginosa* (Richard *et al.,* 1994. However, its usefulness is limited by the high frequency of both nonserotypeable and polyagglutinable strains (Hsueh *et al.,* 1998).

Molecular (Genotyping) methods are done by comparing the restricted fragment length polymorphism patterns using pulsed field gel electrophoresis (PFGE) or Southern blot hybridization with DNA probes. Currently, PFGE is commonly used for most nosocomial pathogens because of its excellent discriminatory power and its broad applicability to bacteria and yeasts (Embil *et al*., 2001; Roberts *et al.,* 2001; Mark *et al*., 2001).

1.1.5. Antimicrobial resistance in burn units

The incidence of antimicrobial resistance among burn pathogens has markedly increased over the past years resulting in limitation of therapeutic options (Neelam *et al*., 2004; Singh *et al*., 2003; Nagoba *et al*., 1999).

This increasing emergence and spread of MDR 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.

The frequency of cross-transmission of MDR organisms varies between 13 and 34.6% and is especially high in intensive care units and the burn units (Agnihotri *et al.,* 2004; Lemmen *et al*., 2004). Antibiotic resistant pathogens that are nowadays increasingly being isolated include MRSA, MDR *P. aeruginosa*, *Acinetobacter spp*. and vancomycin resistant enterococci (Neelam *et al.,* 2004; Singh *et al.,* 2003; Valerie *et al.,* 2003; Still *et al*., 2001; Paul *et al.,* 2002). For example, 45% of all *S. aureus* isolates in one study were MRSA (Bagdonas *et al.,* 2003).

For *P. aeruginosa*, MDR is considered when an isolate is resistant to ceftazidime and at least three of piperacillin, cefoperazone, aztreonam, imipenem, cefepime, cefpirome, ofloxacin, ciprofloxacin, minocycline, and aminoglycosides (Hsueh *et al.,* 1998).

Among the panels of antibiotics used in susceptibility tests by Komolafe *et al* (2003), between 1994 and 1999 a broad-spectrum resistance to most antibiotics used in the tests was observed. For example, *S. aureus* showed only 33.3% susceptibility to penicillin, while *P.aeruginosa* showed 53.3% susceptibility to gentamicin, *P. mirabilis* 48.7% and coliforms 32.6%. Surprisingly, 90% of *P. aeruginosa* were resistant to the commonly used antipseudomonal antibiotics in another study (Estahbanati *et al.,* 2002).

Such pattern of antibiotic resistance observed in this analysis is seen in other studies involving burn centres as hospitals especially burn units are an important breeding ground for the development and spread of antibiotic resistant bacteria (Ram *et al*., 2000; Neelam *et al.,* 2004; Geyik *et al.,* 2003). This is the consequence of exposing to heavy antibiotic use, a high-density patient population in frequent contact with health care staff, and the attendant risk of cross-infection (Gold et al., 1996).

For example, the use of broad-spectrum antibiotics such as third generation cephalosporins in burn units exerts selective pressure on bacteria, thereby promoting infections with MDR strains (Richard *et al.*, 1994).

Although routine surveillance cultures of environmental samples are notorious for being nonproductive and for yielding results which usually are not clinically relevant, it is suggested that if an MDR *P. aeruginosa* and MRSA are isolated and an infection caused by these organisms is documented, extensive cultures of samples from all body sites of all patients in the same burn unit and an environmental survey for the strain, as well as the use of isolation precautions, may be crucial for the early control of an ongoing outbreak (Salah *et al.,* 2003; Jean *et al.,* 2003; Tredget *et al*., 2004).

1.1.6. Other nosocomial infections in the burn patient

Though the most common nosocomial infections are burn wound infections (70-75%), other infections such as pneumonia (9%), bloodstream infections (BSI) (8%) and urinary tract infections (UTI) (11%) are surging (Neelam *et al*., 2004; Geyik *et al*., 2003; Santucci *et al.,* 2003).

This is particularly true for those with extensive burn wounds and those in whom life saving aids and procedures have been undertaken (e.g., bladder catheterization, intubation, intravenous infusion) (Neelam *et al.,* 2004).

Thus, since thermal injuries suppress specific and cellular nonspecific immune function, and massive invasive therapeutic and diagnostic procedures like tracheal intubation, intravascular access, and urinary catheterization are required to save these patients' lives, burn patients are at high risk for acquiring additional nosocomial infections (Rodgers *et al.*, 2000).

BSI occurs in 8 % of burn patients within burn unit (Bang *et al.,* 2004; Geyik *et al.,* 2003; Sanyal *et al.,* 1998). The rate is increased when intravenous lines are used (Askarian *et al.*, 2003). The source of BSIs in most burn patients has been demonstrated to be the burn wound. In one study, *S. aureus* was the main isolate and the authors indicated that patients were infected with strains from their wounds (Apelgren *et al*., 2002).

Other studies had also *S. aureus* as their commonest organism isolated from blood culture which was closely followed by *P. aeruginosa* (Neelam *et al.,* 2004; Bang *et al.,* 2004). Pneumonia is significantly associated with inhalation injury and prolonged ventilation (Gastmeier *et al.,* 2002) while urinary tract infections in burn patients are most commonly associated with urinary catheterization (Neelam *et al.,* 2004; Askarian *et al*., 2003).The etiologic agents of UTI in burn patients are similar to those of other patients and they do differ from strains causing BWI (Leseva *et al,* 1998).

1.1.7. Prevention and control of infections in burn units

Despite the fact that the use of effective antimicrobial agents has not solved the problem of serious infections in severe burns fully, much can be done to control the incidence and seriousness of infections (Mayhall, 1999). A major infection control issue around the world that is being increasingly recognized is the colonization of burn units with MDR strains of pathogenic bacteria. It is usually not possible to eradicate carriage of MDR organisms with antibiotic therapy. Repeated isolation of the same strain of the same strain of MDR organism from burn patients after antibiotic therapy would support this. Studies have suggested that to break the cycle of ongoing transmission and infection it is necessary to remove infected patients from the burn unit (Mark *et al.,* 2001).

- ***Barrier Techniques***

Important barrier techniques are those used to prevent contact transmission of organisms from patient to patient by the contaminated hands and clothing of personnel who provide direct patient care besides isolation care in single rooms (Thompson *et al*., 2002).
Hand washing with washing agents containing antiseptics and use of gowns, masks, and gloves by HCWs decrease cross contamination of burn patients and has been effective in to prevent transmission of MRSA and other MDR microorganisms (Apelgren *et al.,* 2002; Embil *et al.,* 2001; Rafael *et a*l., 2001).

- ***Prevention of Cross-contamination from Inanimate Surfaces and Food***

Items of equipment that must be shared between patients should be thoroughly cleaned and disinfected between patients. Particular attention should be paid to mattress covers, since outbreaks have been related to damaged mattress covers that led to contamination of the mattress foam (Mayhall, 1999). Attention should also be paid to avoiding contamination of kitchen utensils with raw fruits and vegetables that may later contact uncontaminated foods before they are served to burn patients (Mayhall, 1999).

- ***Application of topical antimicrobial agents to the burn wound***

The use of topical antimicrobial agents such as mafenide acetate, aqueous silver nitrate, sulfadiazine silver and gentamicin sulfate to diminish the colonization and growth of microorganisms on the surface of the burn wound has brought tremendous improvements in the effective control of wound sepsis (Alexander, 1971; Heberal *et al*., 1987). Therefore, following daily cleaning, topical antimicrobial therapy is instituted in all patients hospitalized for burn injuries to aid in control of the growth of bacteria in the wound (Alexander, 1971).

- ***Appropriate Use of Systemic Antimicrobial Agents***

Prophylactic systemic antibiotics are avoided since it can result in a disturbance of wound ecology and the emergence of opportunistic pathogens. Hence systemic treatment is given only upon specific indications and frequent bacteriological monitoring of the burn wound should be performed. With such appropriate and rational use of systemically administered antimicrobial agents, it is possible to reduce the pressure for selection of resistant microorganisms (Apelgren *et al.,* 2002; Alexander, 1971).

- ***Early Excision and Closure of the Burn Wound***

Excision of the burn wound the dead and devascularized tissue (eschar), which provides a favorable milieu for the growth of pathogens, aids early grafting and is being currently practiced worldwide (Alexander, 1971; Chai, 2000). However, it is unclear whether early excision and closure of burn wounds significantly reduces the rate of BWIs.

Early burn wound excision has not been scientifically proven to be an effective modality for the prevention of BWIs. However, it is a widely held belief among burn surgeons that early excision and closure significantly reduce BWI and mortality from burn injury (Pruitt *et al.,* 1998).

- ***Specific Immunization***

The common occurrence and seriousness of *Pseudomonas* spp. infections in the burn patient led to the development of an effective polyvalent Pseudomonas vaccine that has been strikingly effective in reducing mortality from Pseudomonas infections (Fisher, 1971). Vaccination of burn patients with antiserum to the most common serotypes (O:6, O:11, O:5, O:16) will produce immunity in more than half of the burn patients (Estahbanati *et al.,* 2002).

- ***Selective decontamination of the digestive tract (SDD)***

It is postulated that the elimination of potentially pathogenic microorganisms from the intestinal tract of burn patients by the oral administration of non-absorbable antibiotics will diminish colonization and infection of burn wounds. In a nonrandomized study, it was noted that there is a significant decrease in burn wound colonization with Gram-negative organisms in patients given SDD. In addition, significant reductions in respiratory infections, septicemia and mortality rate was noted among those who received SDD (Mackie *et al.,* 1992). However, from the overall available published data, SDD is unproven as an effective modality for prevention of BWI and the side effects of such therapy are not well defined and data are insufficient to determine whether such prophylaxis will lead to selection of resistant microorganisms in burn units (Mayhall, 1999).

1.2. Relevance of the study

The Burn unit at Yekatit hospital is a newly established unit, the only of its kind in the country. Because isolated burn units were non-existent in the country prior to the establishment of the Yekatit Burn unit, practically no study has been done on the general microbiology of Burn unit in Ethiopia. Each individual unit varies in its baseline population of microorganisms over time, and generalizations drawn on the basis of results in other units may have little applicability to that of a unit in Ethiopia (Sanyal, *et al.,* 1998). In addition, since the microorganisms that predominate in a particular burn unit often change in relation to newly admitted patients and altered therapy protocols, knowledge of the microorganisms that are generally a problem in burn practice might result in wrong selection of empirical systemic antibiotics (Salah *et al.,* 2003; Komolafe *et al.,* 2003). It becomes therefore essential for the burn unit at Yekatit 12 Hospital to determine its specific pattern of burn wound microbial colonization, time-related changes in predominant flora, and antimicrobial resistance profiles. This would allow early management of septic episodes with proper systemic antibiotics in a setting like Yekatit 12 Hospital, where no facility for microbiological culture was available at the time of the study.

At Yekatit12 hospital, there is no known baseline study for routine surveillance of environmental colonization and staff carriage in the Burn unit. Hence, a comprehensive microbiologic study of the burn unit is warranted.

There are compelling reasons for the identification and control of MRSA and MDR organisms. The drugs for the treatment of infections with such antibiotic resistant organisms (e.g., vancomycin to treat MRSA infection; aztreonam and amikacin for Pseudomonas infections) are beyond the reach of the majority of infected patents in Ethiopia (Walton *et al.,* 1997).

Thus, the scarcity of treatment options and the morbidity and mortality associated with infections by these organisms provide a strong argument for our intention to identify colonized burn patients, screen staff, attendants and patients and survey environment for these organisms thereby implementing strict rules to control their spread.

This study will be a starting ground work for a continuous surveillance of microorganisms and a regular update of their antibiotic resistance pattern which is essential to maintain good infection control programmes in the burn unit of the hospital.

It is therefore important to find out whether the inanimate environment of colonized patients is a major natural reservoir of Gram-negatives and/or Gram-positives. This further substantiates the necessity of environmental culturing in our study to determine whether the environment is an important reservoir of MDR organisms to cause cross-transmission (Lemmen *et al.,* 2004).

In addition, this study will be the first of its kind on nosocomial infections in burn units since no information exists on nosocomial infections (other than burn wound sepsis) among burn patients in Ethiopia.

In conclusion, since adequate bacteriological surveillance and monitoring from the moment of admission into the burn unit is required in order to diagnose any infection, study the colonization flora and assess the more pathogenic or MDR organisms (Zorgani *et al*., 2002) and since there has been no comprehensive microbiological data with regard to burn unit in Ethiopia, this study had to be undertaken.

1.3. Objectives

1.3.1. General objective

- To assess the microbiology of burn unit at Yekatit 12 Hospital prospectively for 6 months.

1.3.2. Specific objectives

- To analyze the pattern of burn wound microbial colonization and to evaluate the time-related changes in the predominant flora throughout the patient's hospital stay.

- To determine microbial pattern of burn wound infections and their antimicrobial susceptibility pattern.

- To detect reservoir patients, patients' attendants and burn unit staffs and to assess the degree of contamination of the inanimate environment with the organisms isolated from the burn patients.

- To study prospectively the pattern of other nosocomial infections namely, blood stream infections, pneumonia and urinary tract infections among the burn patients.

CHAPTER II. MATERIALS AND METHODS

2.1. Setting.

Yekatit 12 hospital is a tertiary care center that has been on renovation for the last few years. The burn unit consists of 5 rooms all adjacent to one another with the operation theatre at the end of the rooms in front of the common hydrotherapy room. There is a room for pediatric patients with 7 beds; two adult male rooms with 2 beds each, a female room with 8 beds, a common hydrotherapy hall with additional small room attached to it, a nursing station and a protective zone (room) where staff and guests change into burn unit clothes, shoes and caps.

Hand washing using detergent skin cleansing soaps and solutions is required before patient contacts; caps, masks, shoe covers, and gloves are required at all times in the hydrotherapy room. There is a separate sterilization room for the burn unit where saline for hydrotherapy; rubber sheets for patients and the different surgical instruments are sterilized.

Patients are bathed everyday after removing all dressings and the burn wounds are cleansed thoroughly using warm shower and sterile saline (with the exception of under-five children with small TBSA who are only cleansed with sterile saline). Silver sulfadiazine (1%) is then applied to the burn wounds and dressed. Early excision and skin grafting was performed as soon as the patients' condition permits.

2.2. Study Design.

A prospective study was undertaken from March to August 2005 to study the pattern of nosocomial infections and colonization of burn wounds at Burn Unit of Yekatit 12 Hospital, Addis Ababa.

2.3. Study Population

Patients with open burn wounds admitted to Yekatit 12 burn unit for skin grafting were included in the study. Patients with chronic burn wounds and those admitted with burn wound contracture were excluded from the study. The patients were divided into two groups.

The first group consisted of patients who had been admitted to the unit without any signs of infection at the time of admission and the second group consisted of those admitted with signs of infection at admission to prospectively follow for any change in the pattern of infection and microorganisms in this subgroup (Karyoute, 1989).

Since there is no previous study of this kind in Ethiopia to refer as baseline, colonization rate from other countries was used to calculate sample size for studying the rate of colonization (Karyoute, 1989). Taking a proportion of 94%, a 95% confidence interval and tolerable error of 0.04, the sample size was taken to be 51.

The formula used to calculate the sample size was:

$$n_f = \frac{z\acute{\alpha}^2 p(1-p)}{d^2}$$

where n= sample size; z= confidence interval; α= level of significance; d= tolerable error and p= proportion.

Accordingly, a total of 52 burn patients were included during the study period. Out of these, 33 patients were presented with no signs and symptoms of infection on admission whereas 19 patients had clinical signs and symptoms of BWI and had longer duration of burn injury having being cared for at local clinics or at home. Those presenting without signs and symptoms of infection on admission (n=33) were included for the prospective study on pattern of all types of nosocomial infections whereas those admitted with clinical signs of BWI were included to study for the microbial pattern of the BWI and any changes in the pattern of infections and their microbial etiology. In addition, 218 environmental samples, 84 samples from burn unit staff and 150 samples from attendants were collected as described below.

2.4. Collection, handling, and transport of specimens

2.4.1. Data collection:

For every subject presenting with no signs and symptoms of infection within the first 48 hours of admission, a daily surveillance for nosocomial infections (BWIs, pneumonia, BSIs, and UTIs) were conducted (Mehrdad, *et al*., 2004).

Surveillance was conducted by direct patient examination for signs and symptoms of infection and follow up of microbiology and hematology results.

To define nosocomial infections in the study group, CDC criteria with slight modifications (appendix-iii) were used (Apelgren *et al*., 2002).

An individual was considered colonized if a pathogen was isolated without any discernable signs of inflammation and disease on the site.

A Gram-negative isolate was considered multidrug-resistant (MDR) when it was resistant to at least three of the agents: ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, kanamicin, polymyxin B, carbenicillin, and amoxicillin-clavulanic acid (Hsueh *et al*., 1998).

2.4.2. Sites of specimen collection:

I Burn wound colonization and infection:

Swabs were taken for each 10% of open burns preferably from upper and lower extremities avoiding oral, genital, scalp, and anal regions. After a routine patient sample gave a positive microbiological result, a detailed survey of the environment, caring staffs, and the patient were performed by the principal investigator as follows:

II Environmental sampling: involved different locations (Jean et al. 2003, Lemmen *et al*., 2004; Bollerao *et al*., 2003):

Gowns of patients and burn unit personnel, toilet seats, bed pans, hydrotherapy equipments (showers, shower tables and swabs of sink surfaces), water from sink and shower, door handles (of the ward and the common treatment room), floors, walls, window ledges, mattresses and sheets, side rails of bed and chairs, trolleys where burns

patients are washed and have their dressings changed, antiseptic solutions and cleaning solutions, topical agent and the air.

For large surfaces, sterile gauzes (about 8cmX8cm) moistened with sterile saline were used. An area of about 30cm by 30cm was wiped by making vertical S-strokes to cover the entire sample area and then the exposed side of the pad was folded to make horizontal S-strokes over the same area.

For small surfaces (bed rails, door handles, sink tap handles, shower heads etc.), sterile cotton tipped applicator was used. The swabs were first moistened with sterile saline and rolled several times making vertical S-strokes to cover the entire sample area of around 5cm X 5cm (Lemmen *et al.,* 2004).

For air sampling, three 10-cm petri-dishes containing Blood agar (Oxoid Ltd., Basingstoke, Hampshire, England), MacConkey (Oxoid, England), and Sabouraud's agar (Oxoid, England) were exposed in the 5 patients' rooms and the hydrotherapy rooms for 1 hour at a distance of 1 meter from every obstacle (Fischer's 1-1-1 scheme) (Cucchiara *et al*., 1994).

III ***Patient screening****:* involved swabs from the hands of patients, neighboring patients, swabs from throats (two patients), nostrils, axilla, perineal region, of index patient.

IV ***Staff screening****:* involved swabs taken from the commonly recommended sites of all staff members working in the burn unit (n=21) for screening of colonization, including hairline, nostrils and hands (gloved and ungloved) (Lemmen *et al*., 2004; John *et al*., 1983;). A staff was classified as a carrier if at least one of the swabs taken from the different sites tested positive for a burn pathogen isolated from patient(s) cared for by the staff (Preetha *et al*., 1998).

V ***Screening attendants****:* involved the same sites as staffs.

2.4.3. Frequency and time of specimen collection:

For every patient meeting the inclusion criteria:

- Burn wound swabs were taken initially on admission (for those with injury longer than 24 hours) and once per week till wound closure. From patients with injury shorter than 24 hours, samples were taken on admission, on 5th day and then twice per week till wound closure (Mehrdad *et al*., 2004). They were taken before dressing changes and before administration of antibiotics whenever possible. Wound swabs were also taken whenever there were clinical signs of grafted skin infections.
- Urine cultures were performed once per week for those with indwelling urinary catheters and on request for those with signs and symptoms of UTI (Mehrdad *et al.*, 2004; Karyoute, 1989)
- 2 consecutive blood cultures were drawn during fever or clinical features of sepsis (Mehrdad *et al*., 2004).
- All environmental, patient and staff samples were taken before any isolation precautions and disinfections were implemented.

2.4.4. Transport of specimens

All swabs were dipped in Amies transport medium and taken to the microbiology laboratory at EHNRI for plating on Blood Agar (Oxoid, England), Mc-Conkey (Oxoid, England), and Sabouraud's Dextrose Agar media (Oxoid, England).

2.5. Processing of specimens

I. ***Wound colonization***

The wound swab specimens were inoculated on blood agar plate, Mac Conkey, and Mannitol salt agar (Oxoid, England) and were incubated at 35^{0}C for 24-48 hours in addition to inoculating on saboraud dextrose agar, which was incubated at room temperature and at 35^{0}C for 7 days. Identification of bacterial isolates was done using colony morphology, Gram-stain, and conventional biochemical tests. For the identification of *Aspergillus spp*. isolated, colony morphology and microscopy were used.

II. ***Survey of environment*:**

The swabs from the environment were inoculated into 5 ml tryptic soy broth. After 24 hours of incubation at 35-37^{0}C, the broth were subcultured on blood agar plate and Mac Conkey agar (Oxoid, England). Identification of the isolates was then done as described above. Samples of antiseptic solutions were poured directly onto nutrient agars and inoculated into Triptic soy broth as described elsewhere (Mark *et al*., 2001).

Samples of normal saline and water (tap water and hydrotherapy baths) were collected in sterile bottle and 10 and 50ml were filtered through sterile membrane filters. The filters were placed on blood agar and MacConkey agar and incubated at 35^{0}C (Calvario *et al*., 1994).

III. ***Staff, patient and attendant screening***:

The swabs taken from the aforementioned sites of the caring staffs, the attendants and the patient were inoculated on Amies transport media (Oxoid, England) and then taken to the laboratory for inoculation on blood agar, MacConkey and Mannitol salt agar (Oxoid, England). The plates were incubated at 35^{o}C for 48 hours. Identification of colonies was verified using the conventional biochemical tests.

IV. ***Specimens from other sites of infection****:*

Urine samples collected were plated on Cysteine lactose electrolyte deficient (CLED) agar (Oxoid, England), incubated as above and isolates identified by standard procedures. Blood cultures collected were incubated for 7 days or till diagnosed positive. The positive bottles were Gram-stained and sub-cultured on Blood agar and MacConkey agar and identification proceeded as mentioned above (Zorgani *et al.*, 2002). Sputum samples were inoculated on Blood agar and MacConkey agar and identification of isolates was done as above.

2.6 Antimicrobial susceptibility testing:

Inoculums were prepared by transferring 5 colonies from pure culture into 5 ml Tryptic Soya Broth (Oxoid, England) and incubated at 35^0C for 2 hours. Turbidity equivalent to 0.5 Mc Farland was obtained by diluting the broth and the entire surface of Muller Hinton agar (Oxoid, England) was streaked with the inoculums using a cotton swab according to National Committee for Clinical Laboratory Standards (NCCLS, 2003).

Different panels of antimicrobial agents for Gram-positive and Gram- negative bacteria were used. The antibiotic disks used for Gram positive isolates were: Penicillin G 10 IU (BBL), Ampicillin 10µg (Oxoid), Amoxicillin 10µg (Oxoid), Methicillin 5µg (Oxoid), Erythromycin 15µg (Span, India), Clindamycin 2µg (Span), Chloramphenicol 30µg (Oxoid), Vancomycin 30µg (BBL), Cephalothin 30µg (Oxoid), Amoxicillin-clavulanic acid 30µg (Oxoid) and tetracycline. For Gram negative isolates, Gentamicin 10µg(Span, India), Kanamycin 30µg (Oxoid, England), Ampicillin 10µg (Oxoid, England), Amoxicillin 10µg (Oxoid), Cotrimoxazole 25µg (Oxoid), Ciprofloxacin 5µg (Span, India), Ceftriaxone 30ug (Span, India), Chloramphenicol 30µg (Oxoid, England), Amoxicillin-clavulanic acid 30µg (Oxoid), Ceftazidime 30ug (Oxoid), Carbenicillin, Polymyxin-B 300units (Span) and tetracycline were used.

Antibiotypes were considered identical if the zone of inhibition of all antibiotic disks tested was identical as used in previous studies (Hsueh *et al*., 1998). Strains of isolates with identical antibiotic susceptibility pattern (identical antibiotypes) were sought for in order to associate the possible source with the infection.

2.7 Quality control

All plates and disks were stored at 4^0C and the plates were incubated at 35^0C for 48 hours before use to assure sterility .The standard reference strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were tested weekly as controls on the biochemical tests and agar plates including Mueller Hinton with antibiotic discs.

2.8 Statistical analysis:

Using the socio-demographic data, clinical data, microbiologic data, which were collectively documented for each patient on a questionnaire (appendix-ii) - after retrieving the data from the patient's chart, the patient, and the microbiology report, risk factors associated with infection of the burn patient were identified and analyzed statistically.

The incidence of nosocomial infections was calculated by dividing the total number of infections that developed during the study period over the total number of patient-days (hospital stay) which was then multiplied by 1000 to obtain a figure that was expressed per 1000 patient-days and compared with results from other studies (Askarian *et al.,*2003).

Results were analyzed using Statistical package for Social Sciences (SPSS) version 11.5, and all p-values <0.05 were considered significant.

2.9 Ethical considerations

The MSc research project was approved by the Department of Microbiology, Immunology and Parasitology, the Faculty Research Publications Committee, was endorsed by the Faculty Academic commission. Ethical clearance was given by the Faculty Research and Publication Committee (FRPC) Addis Ababa University Medical faculty, from Yekatit 12 hospital management committee and Ethiopian Health and Nutrition Research Institute. Written informed consent was obtained from the burn patients, attendants, and the HCWs prior to sampling (Appendix-IV). Plastic surgeons were involved in the clinical diagnosis and patients were not subjected for unnecessary sample collection unless it was warranted for the benefit of the patient.

CHAPTER III: RESULTS

The data collected in this study consisted of 52 burn patients admitted to Yekatit 12 burn unit and stayed for more than 48 hours during the 6-month study period. The mean age of the patients was 18.7 years (range: 1-65 years). There were 21(40.4%) male patients and 31(59.6%) female patients. The most common cause of burn was exposure to flame (78.8%); the remaining patients had scalds by boiling water and hot liquids (kerosene) (13.5%) and electrical injuries (7.7%). The TBSA ranged from 9.0 % to 65.0 % with a mean of 22.3% $\pm$ 12.1%. Mean length of stay in the burn unit was 53.6 days (range 9-89 days). The majority of patients (28/52) were admitted later than 24 hours after injury while 24 patients (46%) were admitted within the first 24 hours of injury.

3.1 Pattern of burn wound microbial colonization

One group of the study population consisted of 33 patients who had no clinical signs of infection on admission and did not develop infection within 72 hours after arrival to the hospital. Two hundred twenty five burn wound swabs were taken altogether from these patients till grafting was completed. The distribution of different species of bacteria which were recovered at different periods of hospital stay is presented in table 3.1.

On admission, 3 samples out of the 33 yielded no growth (9.1%). There was a predominance of Gram-positive isolates as they constituted 69.8% of all organisms grown, while Gram-negative isolates accounted for 18.2%. No mixed growth was observed at this time.

From the second week onwards, a total of 74 swabs were taken yielding 89 microbial isolates. A predominance of Gram-negative isolates was noted throughout the 2-6 weeks (depending on the time for skin grafting to be completed) of follow-up of each patient as Gram-negative isolates accounted for 68.6% (while Gram positive isolates accounted for 29.2% this time) of all organisms grown. Mixed growth occurred during this time in 15 samples (20.3%).

Table 3.1. Pattern of colonizing organisms isolated from burn wound swabs from 33 patients at Yekatit 12 hospital, Addis Ababa, Ethiopia, 2005.

Organism	At admission n (%)	5th day n (%)	7th day on n(%)
S. aureus	12 (36.4 %)	10 (27.8)	15(16.9%)
CN Staphylococci*	10 (30.4%)	8(22.2%)	10(11.2%)
P. aeruginosa	1 (3.0%)	5(13.9%)	21(23.6%)
E.coli	2 (6.1%)	5(13.9%)	15(16.9%)
Klebsiella pneumoniae	2(6.1%)	4(11.2%)	12(13.5%)
Enterococcus spp.	1(3.0%)	1(2.7%)	1(1.1%)
Proteus mirabilis	1(3.0%)	1(2.7%)	8(9.0%)
Enterobacter spp.	0	0	2(2.2%)
Acinetobacter spp.	0	0	3(3.4%)
Candida albicans	1(3.0%)	2(5.6%)	2(2.2%)
No growth	3 (9.0%)	0	0
Mixed growth	0	3(8.3%)	15(20.3%)
Total (Isolates)	30	36	89
Sampling occasions	33	33	74

* CN= coagulase negative

3.2 Burn wound infections

There was significant difference in age distribution on comparing infected patients (n=38) with non-infected ones (n=14) (p=0.035). However, there was no significant difference in the frequency of infections by sex (p=0.535) (Table 3.2.).When comparing infected patients who had different types of burn injuries, 80.5% of those with flame injury, 75% with electrical injury and 28.6% with scald injury developed BWI and the difference was statistically significant (p=0.017). Infected patients stayed longer in the hospital than non-infected patients (p<0.0001). Patients with higher TBSA were more likely to develop wound infection; the difference was statistically significant (p=0.005).

Infection attack rates among the burn patients increased with increasing burn size (TBSA) as shown in table 3.4.

Table 3.2 Comparison of all infected and non-infected burn patients at Yekatit 12 Hospital, 2005.

	Infected patients	Non-infected patients	Statistical test value
Patients(n)	38	14	
Male	15	6	X^2= 0.049, p =0.535
Female	23	8	
Age(years)			
Range	1-65	1-38	
Median	22	5	p=0.035[a]
Mean	21.4	11.3	
Burn Type			
Flame	33	8	
Scald	2	5	X^2=8.199, p=0.017
Electrical	3	1	
TBSA(%)			
Range	9-65	9-16	
Median	19	13	X^2=18.599, p=0.005
Mean	22.3	12.3	
Burn Duration (days)			
Range	1-45	1-13	
Median	4	1	p=0.009[a]
Mean	11.4	0.8	
Hospitalization (days)			
Range	9-60	16-30	
Median	37	19	p< 0.0001[a]
Mean	41.2	20.6	

a- paired t-test and one-way ANOVA used

When only nosocomial infections were taken for those patients without infection at admission (n=33), age distribution was still significantly associated with infections (p=0.005) (table 3.3). Sex was not significantly associated with the development of nosocomial infections (p=0.503). Higher TBSA was significantly associated with the development of nosocomial infections (p=0.009) and patients who developed BWIs had more prolonged hospitalization than those without BWIs (p=0.002). Four patients died during their hospital stay and all the deaths were associated with sepsis and high TBSA. Besides, 2 patients had graft failures following mixed burn wound infections with *P. aeruginosa* and β-hemolytic Streptococci and two patients following *P. aeruginosa* infection alone.

Table 3.3 Comparison of infected and non-infected burn patients with no infection at admission at Yekatit 12 hospital, 2005.

	Infected patients	Non-infected patients	Statistical test value
Patients(n)	20	13	
Male	8	6	X^2= 0.122, p =0.503
Female	12	7	
Age(years)			
Range	1-65	1-34	
Median	21	4	X^2= 10.608, p=0.005
Mean	20.2	10.1	
Burn Type			
Flame	16	8	
Scald	2	4	X^2=2.285, p=0.319
Electrical	2	1	
TBSA(%)			
Range	9-65	9-16	
Median	18	13	X^2=6.864, p=0.009
Mean	20	11	
Burn Duration (days)			
Range	1-7	1-3	
Median	1	1	p=0.415
Mean	1.2	0.8	
Hospitalization (days)			
Range	9-56	16-30	
Median	35	19	X^2 =9.8, p=0.002
Mean	37.6	21.6	

Table 3. 4 Infection attack rate by burn size (TBSA) in the burn patients at Yekatit 12 burn unit, Addis Ababa.

TBSA(%)	0-10	11-20	21-30	31-40	>41	Total
Total no. of patients	17	12	13	3	5	52
No. of infected patients	8	10	13	2	4	38
Attack rate (%)	47.1	83.3	100	66.7	80	100

3.3 Other nosocomial infections

Among the 33 patients with very recent burn injury (within 7 days) and without infection on admission, 20(60.6%) developed at least 1 type of nosocomial infection during the course of hospitalization. Whereas, 18/19(94.7%) of those patients with injury longer than 7 days developed infection and the duration of burn injury before admission was significantly associated with development of infection (p=0.009) (table 3.2). The most frequent nosocomial infection was burn wound infection (N=20; 60.6%), followed by UTI (N= 10; 30.3%), bloodstream infection (N=4; 12.1 %) and pneumonia (N=1; 3.0%).
The overall nosocomial infection rate in the burn unit was found to be 33.3/1000patient-days. Bladder catheterization and UTI were significantly associated (p<0.0001), with only one case of UTI not associated with catheterization. Like BWIs, UTIs and BSIs were also significantly associated with higher TBSA (p=0.018 and p<0.001 respectively). All patients who developed BSI had flame injury.

3.4 Pattern of microbial isolates:

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) (table 3.5). *E.coli*, *Proteus sp*. and *Citrobacter sp*. accounted for 5.5% each (3/55) while there were 2 isolates (3.6%) of *Klebsiella sp., Enterococcus sp*. and group A β-hemolytic Streptococci each. The rest 3 isolates (5.4%) were *Acinetobacter sp., Enterobacter* sp. and *Aspergillus sp*.
On the other hand, 32 isolates were recovered from the 33 patients who presented with no infection at admission of which 12 (37.5%) isolates were *S. aureus*, 12 (37.5%) *P. aeruginosa*, 2 (6.3%) *Citrobacter spp*., and 1(3.1%) isolate was *E.coli spp*., *Klebsiella sp., Enterococcus sp*., group A β-hemolytic Streptococci, *Acinetobacter sp.,* and *Aspergillus spp*. each. Table 3.5 shows the distribution of the isolates in BWIs and UTI. Fourteen patients (26.9%) developed UTI of which 11 had their urinary bladder catheterized.

The most common organism isolated from their urine was *E. coli* (n= 10), followed by *P. aeruginosa* (n=3) and *Klebsiella sp* (n=1). Five patients developed BSI during their hospital stay. *S. aureus* was isolated in 3 of these patients while *P. aeruginosa* was isolated in 2 patients. *S. aureus* was the organism isolated from the 2 patients that developed pneumonia.

Table 3.5. Frequency of isolates recovered in BWIs and UTIs at Yekatit 12 hospital, Addis Ababa, Ethiopia.

Microorganisms	BWI[a] n(%)	UTI [a] n(%)	BWI [b] n(%)	UTI[b] n(%)
S. aureus	22(40.0)	0	12(37.5)	0
P.aeruginosa	15(27.3)	3(21.4)	12(37.5)	3(30)
E.coli	3(5.5)	7(50)	1(3.1)	3(30)
Proteus sp.	3(5.5)	1(7.1)	0(0)	1(10)
Citrobacter sp.	3(5.5)	1(7.1)	2(6.4)	1(10)
Klebsiella sp.	2(3.6)	2(14.4)	1(3.1)	2(20)
Enterococcus sp.	2(3.6)	0	1(3.1)	0
β-hemolytic Streptococci	2(3.6)	0	1(3.1)	0
Acinetobacter sp.	1(1.8)	0	1(3.1)	0
Enterobacter sp.	1(1.8)	0	0(0)	0
Aspergillus sp.	1(1.8)	0	1(3.1)	0
Total	55(100)	14	32(100)	10(100)

[a] infections in all patients studied;
[b] infections in patients without infection at admission).

3.5 Antibiotic susceptibility pattern of isolated bacteria

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, 8(30.8%) to tetracycline and to erythromycin each. On the other hand, 24(92.3%) and 25(96.2%) were resistant to ampicillin and penicillin G respectively, as shown in table 3.6.

A high level of drug resistance was seen among the Gram negative isolates, particularly *P. aeruginosa*. Thirteen isolates of *P. aeruginosa* (86.7%) strains were resistant to at least three of: amoxicillin-clavulanic acid, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, or kanamycin, hence designated MDR to the commonly used drugs in the burn unit and the country at large. Ninety percent of the isolates (18/20) were resistant to ciprofloxacin and to amoxicillin-clavulanic acid each, 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 and polymyxin B. Tables 3.7 and 3.8 present the resistance patterns of *P. aeruginosa* and *S. aureus* respectively to 2 or more antibiotics.

Table3.6. Susceptibility pattern of major pathogens isolated from burn patients with nosocomial infections at Yekatit 12 Hospital.

Bacteria Isolated	Total No.	Antimicrobial agents tested: No.(%)														
		M	AMP	AMC	PEN	CRO	CLO	CIP	CN	KN	SXT	CAZ	T	C	ERY	CAR
S. aureus	26	26	2(7.7)	24(92)	1(3.8)	-	22(84)	7(27)	10(39)	-	5(19)	-	8(31)	12(46)	8(31)	-
P. aeruginosa	20	-	0	2(10)	-	10(50)	-	2(10)	8(40)	6(30)	0	19(95)	2(10)	0	-	14(70)
E. coli	10	-	0	5(50)	-	9(90)	-	6(60)	6(60)	5(50)	3(30)	10	1(10)	3(30)	-	8(80)
Klebsiella spp.	4	-	0	2(50)	-	4(100)	-	3(75)	3(75)	2(50)	3(75)	3(75)	0	2(50)	-	3(75)
Proteus spp.	4	-	1(25)	3(75)	-	4(100)	-	4(100)	3(75)	2(50)	2(50)	4	1(25)	1(25)	-	3(75)
Total	64	26	3(8)	36(56)	1	27	22	22(34)	30(47)	15	13(20)	36	12(19)	18(28)		

M= Methicillin AMP= Ampicillin CN= Gentamicin AMC-Amoxicillin-clavulanic acid PEN= Penicillin G
CRO= Ceftriaxone CLO= Cephalothin CIP= Ciprofloxacin CN= Gentamicin KN= Kanamycin CAZ-Ceftazidime
T-Tetracycline SXT-trimethoprim sulfamethoxazole C= Chloramphenicol ERY= Erythromycin CAR-Carbenicillin

Table 3.7. Antibiograms of *P. aeruginosa* isolates from the infected burn patients

No. of resisted Antibiotics	Types of antibiotics resisted	No. of isolates tested (%)
R-2	CN, AMC	11(55)
	CN, KN	9(45)
	CN, CIP	11(55)
	CN, CRO	7(35)
R-3	CN, AMC, KN	8(40)
	CN, KN, CIP	9(45)
	CN, KN, CRO	5(25)
	CN, AMC, CRO	5(25)
	KN, AMC, CRO	5(25)
R-4	CN, AMC, KN, CIP	8(40)
	CN, AMC, KN, CRO	4(20)
	CN, KN, CIP, CRO	5(25)
	KN, AMC, CIP, CRO	6(30)
R-5	CN, AMC, KN, CIP, CRO	4(20)
	CN, AMC, KN, CIP, CAR	4(20)
	CN, AMC, KN, CRO, CAR	4(20)
	CN, AMC, CIP, CRO, CAR	4(20)
	CN, KN, CIP, CRO, CAZ	2(10)
R-6	CN, AMC, KN, CIP, CRO, CAR, TET	4(20)
R-7	CN, AMC, KN, CIP, CRO, CAR, TET, SXT	4(20)
R-8	CN, AMC, KN, CIP, CRO, CAR, TET, SXT, CAF	4(20)
R-9	CN, AMC, KN, CIP, CRO, CAR, TET, SXT, CAF, AMX	4(20)
R-10	CN, AMC, KN, CIP, CRO, CAR, TET, SXT, CAF, AMX, CAZ	2(10)
Total		20(100)

R= Resistant to

CN- Gentamicin, AMC-Amoxicillin-clavulanic acid, KN-Kanamycin, CIP-Ciprofloxacin, CRO-Ceftriaxone, CAZ-Ceftazidime, TET-Tetracycline, SXT-trimethoprim sulfamethoxazole, AMX-Amoxicillin, CAR-Carbenicillin.

Table 3.8.Antibiograms of *S. aureus* isolates from the infected burn patients

No. of resisted Antibiotics	Types of antibiotics resisted	No. of resistant isolates (%)
R-2	AMP, PEN	23(88.5)
	AMP, T	16(61.5)
	AMP, ERY	17(65.4)
	ERY, TET	12(46.2)
	TET, CAF	10(38.5)
R-3	AMP, PEN, TET	16(61.5)
	AMP, PEN, ERY	17(65.4)
	AMP, ERY, AMC	2(7.6)
	ERY, TET, CAF	10(38.5)
	ERY, CN, CIP	13(50)
R-4	AMP, PEN, ERY, CN	15(57.7)
	AMP, AMC, ERY, CLO	2(7.6)
	AMP, PEN, ERY, CIP	14(53.8)
	AMC, TET, CAF, ERY	2(7.6)
	ERY, TET, CAF, CLO	2(7.6)
R-5	AMP, PEN, AMC, ERY, CLO	2(7.6)
	AMP, AMC, ERY, CLO, TET	2(7.6)
	AMP, PEN, ERY, CLO, CAF	3(11.5)
	AMC, TET, CAF, ERY, AMP	2(7.6)
	AMC, TET, CAF, ERY, PEN	2(7.6)
R-6	AMP, PEN, AMC, ERY, CLO, TET	2(7.6)
	AMP, AMC, ERY, CLO, TET, CAF	2(7.6)
	AMP, PEN, ERY, CLO, TET, CIP	3(11.5)
	AMC, TET, CAF, ERY, AMP, SXT	2(7.6)
R-7	AMP, PEN, AMC, ERY, CLO, TET, CN	2(7.6)
	AMP, AMC, ERY, CLO, TET, CAF, CLI	1(3.8)
R-8	AMP, PEN, AMC, ERY, CLO, TET, CN, CLI	1(3.8)
Total		26(100)

R= Resistant to

CN- Gentamicin, AMC-Amoxicillin-clavulanic acid, CIP- Ciprofloxacin, CAZ-Ceftazidime, TET-Tetracycline, SXT-trimethoprim sulfamethoxazole,CLI-Clindamycin, CLO- Cephalothin, ERY- Erythromycin, PEN- Penicllin G, AMP- Ampicillin, CAF- Chloramphenicol.

3.6 Environmental sampling:

During the study, no outbreaks or clusters were observed for MDR organisms.

The clinical samples included burn wound swabs (n=274), swabs from hands, nostrils, axilla, hairline, and perineum of patients (n=190), swabs from hands, nostrils, and hairline of patients' attendants (n=150), and staffs (n=84), throat swabs (=4), sputum (=2), urine (=34), blood (=14), and environmental samples which included tap water (n=8 samples), sinks (n=8), cleaned bathtub (n=1), cleaned stretchers from hydrotherapy room (=2), cleaned floor (n=6), clean trolley (n=3), savlon solution (n=4), topical antibiotics (n=8), gowns (n=59), bed pans (n=33), window ledges and walls (n=6 each), mattresses (n=33), bed rails (n=33), door handles (n=8) were studied.

Five different swabs were taken individually for an infected patient yielding a total of 165 swabs for the 33 patients with no infection on admission. Examination of the water (from the hydrotherapy room and the wards), savlon, normal saline, and the topical antibiotics at three different times during the study, revealed no burn pathogen. Similarly, the shower head and shower faucet, stretchers with their sterilized rubber sheets, gowns of HCWs did not harbor burn pathogens with identical antibiogram to the infecting isolates during the study. Table 3.9 summarizes the frequency of different bacteriological isolates from the environment which were found to have similar antibiograms with clinical isolates.

Table 3.9. Environmental bacteriological screens at Yekatit 12 hospital.

Sites	No. of sites[a]	*S. aureus*	*P. aeruginosa*	*E. Coli*	*Proteus sp.*	*Klebsiella sp.*	Total
Gown (pt)	33	2	1	0	1	0	4(9.3%)
Floor	6	3	1	0	0	0	4(9.3%)
Bed pan	33	1	1	1	1	0	4(9.3%)
Window	6	1	0	0	0	0	1(2.3%)
Mattress	33	3	1	0	1	1	6(14.0%)
Bed rail	33	2	0	1	0	0	3(7.0%)
Sink surface	8	0	2	1	0	0	3(7.0%)
Door handle	8	3	1	0	0	1	5(11.6%)
Hydrotherapy	1	1	1	2	1	1	6(14%)
Air	6	5	2	0	0	0	7(16.3%)
Total (%)		21(48%)	10(23.3%)	5(11.6%)	4(9.3%)	3(7.0%)	43(100%)

[a]- Number of samples on a site could be more than one at a time and was taken more than once.

Strains of *S. aureus* with identical sensitivity pattern to isolates from the burn wounds of 12 patients (10 of which were infected) were isolated from the air of hydrotherapy rooms, the adult female room, one of the male rooms and the corridor in front of the wards(figure 3.1). None of the isolates from the other rooms had identical sensitivity pattern with patients' isolates.

P. aeruginosa, Acinetobacter spp., Proteus spp., and *E. coli* were detected in the hydrotherapy rooms, the corridors, and all the wards but at much lower microbial charge than *S. aureus*. Figure 3.1 indicates the mean values of measurements of *S. aureus* made in the air of various rooms in the Burn unit.

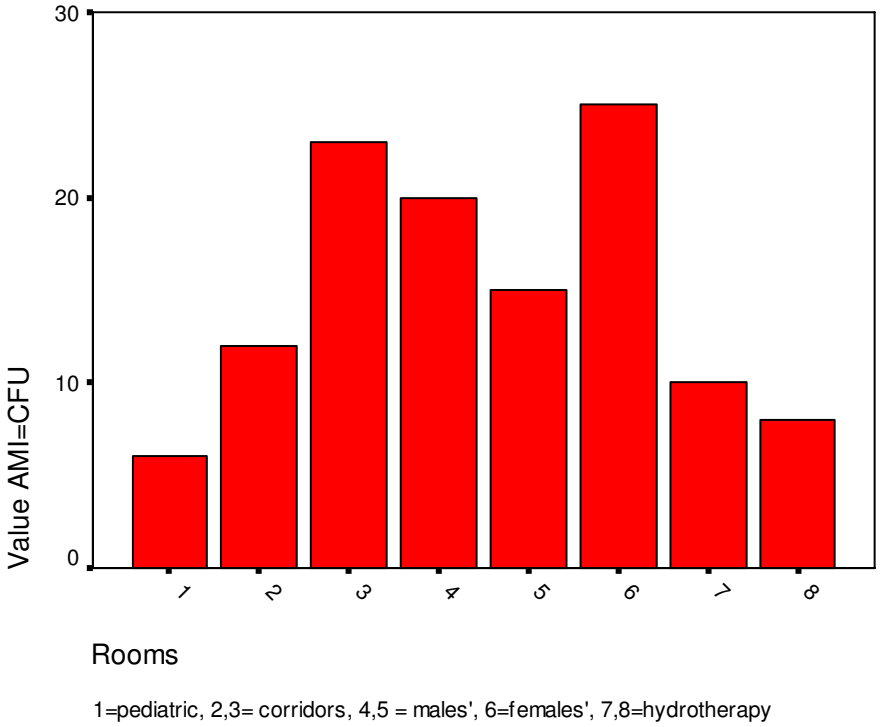

Figure 3.1: Air microbe index measurements (fall-out) in high risk rooms at Yekatit12 Burn unit (mean values of CFU)

3.7 Staff, patients, and patients' attendants screening:

Swabs from staff hands yielded coagulase negative Staphylococci in all and *S. aureus* was isolated in 3/21 (14.3%) staffs of which 2 (9.5%) had identical pattern of antibiogram with 2 infected patients and their own nares. Only one of the samples from the HCWs was positive for *P. aeruginosa* and none for other Gram-negative bacteria identical to the colonizing or infecting organisms prevailing during the study (Table 3.10).

Table 3.10 Frequency of isolation of the major burn pathogens from different sites of patients, attendants and staffs at Yekatit 12 hospital, Addis Ababa, Ethiopia.

Sites	*S. aureus*	*P. aeruginosa*	*E. coli*	*Proteus sp.*	*B-H -Strept.*	Total(%)
Patients (n=52)						
Nostrils	9	0	-	-	2	11(16.9%)
Hands	10	3	-	-	-	13(20.0%)
Axilla	5	0	-	-	-	5(7.7%)
Hairline	4	0	-	-	-	4(6.2%)
Perineum	0	9	8	4	-	2(32.3%)
Attendants(n=50)						
Nostrils	6	-	-	-	1	7 (10.8%)
Hands	4	2	1	1	-	8(12.3%)
Hairline	1	-	-	-	-	1(1.5%)
Staffs(n=21)						
Nostrils	2	-	-	-	-	2(3.1%)
Hands	2	1	-	-	-	3(4.6%)
Hairline	-	-	-	-	-	-
Total(%)	43(66.2%)	15(23.1%)	9(13.8%)	5 (7.7%)	3(4.6%)	65 (100%)

Nine of the patients' attendants (out of 50) carried *S. aureus* with similar antibiograms to infecting organisms in one or more of their body parts. Two attendants also carried *P. aeruginosa* likely to be responsible for infection of the two patients.

Fourteen patients (53.8%) harbored *S. aureus* in their nose, hands, axilla, and/or hairline with identical antibiogram to the organism causing their infection(s). On the other hand, 9 patients (45%) infected with *P. aeruginosa* had the same organism isolated from their perineal region while *S. aureus* with identical antibiogram was not isolated from this site. Similarly, the perineum proved to be positive in 18 (75%) infected patients with the other Gram-negative organisms. β- hemolytic streptococci were isolated from the nose and

throat of two patients who developed infection by the same organism and with the same antibiogram.

Perineal swabs from the index patients (n=2) with enterococcal infection yielded isolates with the same antibiogram as the isolates from their burn wound swabs (Table 3.11).

None of the hands of the neighboring patients carried the same bacteria as the index patient.

Table 3.11 Frequency of isolation of various microbial strains from patients and different environments in the Burn unit.

Isolate	Patients (n)	No. of strains with identical antibiogram isolated in:			
		Patient (endogenous)	Environment	Attendant	HCW
S. aureus	26*	14	10	9	2
P. aeruginosa	20	9	10	2	1
E. coli	10	8	2	1	0
Klebsiella spp.	4	3	2	0	0
Proteus spp.	4	4	2	1	0
Enterococcus spp.	2	2	0	0	0
Enterobacter spp	1	1	1	0	0
Acinetobacter spp.	1	0	1	0	0
Citrobacter spp.	4	2	1	0	0
β-hemolytic Streptococci	2	2	0	1	0

* the sum of isolates from the different sources exceeds that of the isolates from sites of infection because isolates with identical antibiograms were found in more than one source.

Altogether, it was possible to locate the likely sources of *S. aureus* for 23 isolates (88.5%) which were isolated from patients themselves, their attendants, caring staffs and the environment. For *P. aeruginosa*, all isolates from the patients could also be located in the aforementioned possible sources (they had identical antibiogram). This was also true for the rest of Gram negative as well as Gram positive bacteria.

CHAPTER IV DISCUSSION

In this study, out of the 33 burn patients admitted without signs of infections only 3(9.1%) had sterile wound swabs. This might be because of the presentation time being longer than 6 hours in the rest of the patients. Ugburo and his colleagues (2004) had reported that burn wounds were usually sterile up to a maximum period of 6 h after injury and contamination was almost invariably present in cases admitted after 6h.

The usually reported predominance of Gram-positive organisms in the first week after burn injury, and predominance of Gram-negative organisms from the second week on, was also demonstrated in this study (Table 3.1). The most frequent colonizing organism after the first week of admission was *P. aeruginosa* which was cultured in 23.6% of the cases. Nevertheless, *S. aureus* closely followed by coagulase negative Staphylococci are the predominating isolates on admission. These findings are not unique to the burn unit but were reflected in many studies (Salah *et al*, 2003; McGregor, 1998).

In this study, a higher burn wound infection attack rate (100 %) occurred in the age range of 21-30 years, while a lower percentage (47 %) was seen early in life (<10 years of age) (table 3.4). This is in agreement with the findings of Mathangi *et al* (1985), Karyoute (1989) and that of Sherertz *et al.* (1983).

It is clear from this study that the rate of BWI (60.6%) is higher than that found in other units in developed countries (33.9%) (Geyik *et al.,* 2003), (38%) in the work of Oncul *et al.* (2002) and (27%) in USA (Rodgers *et al*., 2000) but comparable to those of developing countries (85%) in Iran's study (Askarian *et al*., 2003). Although early replacement of the damaged burn skin with skin grafts can control and reduce the incidence of infection, this method requires prompt admission to hospital which is not the case in the majority of the burn patients admitted to this Burn unit. For patients who were admitted earlier than 7 days, 20/33 (60.6%) developed burn wound infection whereas 18/19(94.7%) of those admitted with injuries later than 7 days developed BWI. Therefore, it was clear that delay in the admission of burn patients to burn unit was the major reason for higher wound infection rates as the burn patients might acquire pathogens at home or at emergency outpatient department of local clinics or hospitals.

In our study, it can be seen that BWIs are very high among patients with delay in referring patients to the Burn unit. A previous study had concluded likewise that

relatively poor socioeconomic conditions of patients in developing nations and the delay in referring patients to specialized burn units are the most important predisposing factors in BWIs (Karyoute, 1989).

Besides a delay in the time of admission, other factors have been associated significantly with the development of infections in this study. TBSA was significantly associated with all types of nosocomial infections (p=0.009) in general and specifically; TBSA was significantly associated with BWI (p=0.009), UTI (p=0.018), and BSI (p<0.001). There was the same observation in other studies where higher TBSA was significantly associated with the development of nosocomial infections (Table 4.1).

Patients with infection stayed in the hospital longer than non-infected burn patients (p=0.002). The same finding was observed in a study by Neelam *et al*. (2004). Burn type (whether flame, scald or electrical injury) was not found to be a risk factor for developing infections in this study (p=0.319) (Table3.3) although some have found a significant association (Rodgers *et al.,* 2000). Similarly, there was no significant difference in sex distribution on comparing infected and non-infected patients. Neelam *et al*. (2004) could not also demonstrate any significant difference in sex and age distribution between the two groups. As all injured patients had full-thickness burn, we could not demonstrate the risk of burn depth in developing infections but some studies have indicated that full-thickness burn is one of the risk factors for infection (Rodgers *et al.,* 2000).

Table 4.1 Influence of the percentage of TBSA on the development of nosocomial infections in different studies.

Studies	TBSA compared	BWI	BSI	UTI	All infections
Schlager *et al*., 1994	<20 vs ≥ 20%	p<0.00001	not tested	not tested	p<0.00001
Weber *et al*. 1997	<30%, 30-60%, >60%	p<0.0001	p=0.19	p=0.08	p<0.0001
Gastmeier *et al*., 2002	<30 vs. ≥ 30%	p=0.007	p=1.0	p=0.71	p=0.22
Apelgren et al., 2002	<20, 20-40, 4-60%	p<0.001	p<0.001	p<0.001	p<0.001
Present study	<30 vs. ≥30%	p=0.009	p<0.001	p=0.018	p=0.009

When comparing infection attack rate in patients with lower TBSA (TBSA<20) with that of higher TBSA (>20), the present study as well as other studies (Karyoute, 1989; Sheretz, 1983) demonstrate that attack rate increased with higher TBSA .

The overall incidence of nosocomial infection in the burn unit, 33.3 per 1000 patient-days was comparable to other studies which had such rates as 32.3/1000 patient days (Wurtz *et al.,* 1995), 44.7/1000 patient days (Askarian *et al.,* 2003) and 36.2/1000 patient days (Neelam *et al.,* 2004). The lower incidence of nosocomial infection at Yekatit 12 burn unit might be the result of the prolonged total hospital stay in the unit compared to the other units (Mehrdad *et al*., 2004, Oncul *et al.,* 2002). Wound and urinary tract infections were more common in this study in contrast to some studies (Neelam *et al.,* 2004) in which BWIs and BSIs were more common but in agreement with the work of Geyik and his colleagues (2003). BSI was less common because this unit practiced intravenous line usage, a less invasive procedure, instead of central venous line insertion used in burn units in developed world. That all of the patients who developed BSI had flame injury goes with the fact that flame injury caused considerable immunosuppression and massive tissue damage making colonization and growth of potent pathogens easier as other studies have also indicated that patients with extensive flame burns were more prone to developing septicemia (Askarian *et al.,* 2003; Bang *et al.,* 2004). Pneumonia was also infrequent as inhalation injury and intubation were uncommon whereas in centers where endotracheal intubation and respiratory support were done, the incidence of pneumonia far excels the incidence of the other nosocomial infections (Richard *et al.,* 1982). All the deaths (n=4) were related to sepsis and high TBSA in this study as noted in other studies which demonstrated a major (>50%) cause of death to be sepsis (Neelam *et al.,* 2004; Geyik *et al*., 2003). None of those patients with TBSA exceeding 50% (n=2) survived in this study as Neelam *et al* (2004) have demonstrated the significant risk of larger TBSA for mortality in a larger sample size.

The lower mortality in this study compared to other studies (Geyik *et al*., 2003; Wong *et al.,* 1995) might be because there were fewer patients who developed sepsis and patients with very high TBSA (>50%) were also fewer than the other studies as such patients die at the site of injury or on their way to health institutions which usually takes many hours at which time patients might succumb due to hypovolemic shock.

The predominance of *S. aureus* although supported by several workers (Komolafe *et al*., 2003; Leseva et al., 1996; Santucci *et al*., 2003), is in contrast to the observation of numerous others (Salah *et al*, 2003; Revathi *et al*, 1998; Oncul *et al*., 2002; Agnihotri *et al*., 2004 Atoyebi *et al*., 1992; Pandit *et al.*, 1993) where *P. aeruginosa* was most prevalent.

Results of previous studies which are also confirmed in this study have shown that *S. aureus* and *P. aeruginosa* are the two most common isolates in burn injuries, although Gram-negative bacilli are generally thought to prevail in burn infections (Komolafe *et al*., 2003; Revathi *et al*, 1998; Atoyebi *et al*., 1992; Pandit *et al*., 1993).Nonetheless, this study and others have also demonstrated a significant frequency of mixed infections by these two organisms (Calvario *et al.,* 1994; Komolafe *et al*., 2003). The finding of a low isolation rate of β-hemolytic Streptococci is in agreement with some of the previous studies (Agnihotri *et al.,* 2004; Atoyebi *et al*., 1992) but in contrast to the findings of others (Komolafe *et al.,* 2003) where an isolation rate of 13.6% was seen. As the organisms are known to have a deleterious role of inducing skin graft failures (Revathi *et al*., 1998), a low isolation rate in the unit is a fortunate coincidence. Among the Gram negative bacilli (other than *P. aeruginosa*), *E.coli, Proteus spp., Citrobacter spp.* are the next important pathogens in this study albeit the big difference in their isolation rate from the former organism. This is in agreement with the work of Komolafe and his colleagues (2003) but in contrast to the study of Agnihotri *et al* (2004) where *Acinetobacter spp*., *Klebsiella spp.* and *Enterobacter spp*. came next to *P. aeruginosa* which could be due to empirical use of broad-spectrum antibiotics (as it was practiced in the unit) and non-adherence to hospital antibiotic policy resulting in the emergence of MDR strains of *Acinetobacter spp* over other susceptible organisms.

There was persistence of *P. aeruginosa* on the burn wound samples taken at different times, partly due to the ineffectiveness of the usual topical and antibiotic therapies used in the burn unit, and was responsible for the partial failure of skin grafts take-in four patients, as also reported elsewhere in the literature (Herzog *et al*., 1988). The major isolates that caused bacteriuria in this study were *E. coli* followed by *P. aeruginosa* (table 3.5) as was also observed in Leseva and Zozikov's study (1998).

Fungal infection could be detected in only one patient in this study as was also noted by the study of Neelam and colleagues (2004) while Mathangi *et al* (1985) detected a 6 % rate in 600 burn patients. Hence, with very large sample size, it is likely to demonstrate fungal isolates in infected burn patients.

Out of the multiple swabs taken throughout the burn unit, looking for possible reservoir of infecting organisms, none demonstrated an ongoing environmental reservoir for MRSA, an organism significantly more common in burn units than in any other unit in hospitals (John *et al.,* 1983). On the other hand, the hydrotherapy room especially the tank that passes contaminated water already used by patients during washing is in close proximity to the patient and the caring staffs during showering. We have demonstrated that after every patient is treated, the surface of the tank harbors burn pathogens that presumably arose from patients' burn wounds. This can readily contaminate the staffs' gowns and most importantly other patients' wounds by water splashed from the surface of the tank during washing procedures. The results of this study showed some environmental strains presenting a profile of antibiograms identical to that of clinical strains, suggesting a link between the environment and the patients (Table 3.10).

The data indicated that the inanimate environment of patients infected with either Gram positive or Gram negative bacteria were frequently contaminated with the organisms, and therefore surfaces and objects may likely serve as not only a primary source but also as a secondary reservoir for cross-transmission.

Unlike a study by Lemmen *et al.* (2004) who demonstrated an isolation of Gram-positive bacteria at significantly higher frequency than Gram-negative isolates from the inanimate environment, this study could not demonstrate such association.

The observation of fall-out (sediment) organisms in the petri-dishes located in the hydrotherapy room and the adult rooms with similar antibiograms to isolates from some of the infected and colonized patients at different periods of the study suggests that the air can be a potential reservoir for burn pathogens. The levels of *S. aureus* obtained in this study (figure 3.1) exceed the recommended limit for very high risk areas (all of the areas studied were considered very high risk areas) but many of the levels were good high or fair according to the reference standards for assessment of degree of environmental contamination according to the Air Microbe Index (Cucchiara *et al.,* 1994).

In contrast, *P. aeuruginosa* was detected at lower frequency and at much lower microbial charge in the air of the different rooms than *S. aureus* (data not shown). This result agrees with an environmental study by Calvario *et al* (1994). Interestingly, the isolates of *P. aeruginosa* from the air had identical sensitivity pattern with most of the burn wound isolates but this may be because this hospital pathogen is already resistant to most of the antibiotics in the panel we used and may not tell whether the isolates were in reality of the same clonality. Air contamination regardless of the magnitude of the microbial charge (air microbe index) becomes particularly significant when considering the fact that airborne organisms do fall on the exposed burn wound easily. Therefore, exogenous infections from organisms circulating in the air of the burn unit should be included in the management and preventive measures to interrupt the air-patient system and reduce the incidence of infections from this source.

Sampling of staff members' exposed body sites within the burn unit failed to demonstrate ongoing carriage of MDR Gram-negative bacteria or MRSA. This is in accordance with the work of Crossely *et al*., (1979) who found only 0.8% of HCWs being colonized by a burn pathogen responsible for an outbreak in their unit.

However, others observed a very high figure of staff carriage (24%-50%) of burn pathogens particularly MRSA (John *et al.,* 1983; Preetha *et al*., 1998).

We have also demonstrated that staffs do not appear to be major reservoirs at least in their exposed body parts. However, there is a high likelihood that staffs temporarily carry burn pathogens and serve as routes of transmission to uninfected patients.

It can be observed from this study that carriage in patients could play a crucial role in auto-infection as well as cross-colonization as patients harbored significant number of the major infecting organisms. This had also been demonstrated in another study where the majority of patients (95%) carrying a pathogen on admission were subsequently colonized and infected by the same strains of bacteria (Kooistra *et al.,* 2004).

Patients' attendants, on the other hand, were found to carry some of the burn pathogens colonizing and infecting the burn patients, particularly *S. aureus* (table 3.10).

In addition, there was sufficient overlap in the time spent in the burn unit by the patients to allow cross-infection among patients since there was no single-bed room in the unit.

As some studies have demonstrated that isolates with the same antibiotype also had the same molecular patterns (Hsueh *et al*., 1998; Richard *et al.,* 1982), we have used antibiograms to identify the possible source and route of transmission of the burn pathogens in this unit. Antibiograms was the only method that we could afford to locate the possible sources of colonizing or infecting organisms on the burn patients. In fact it was possible from the findings that some of the environmental surveys and patients and attendants screenings are suggestive of being reservoirs for the burn pathogens or colonizers. When multiple isolates from individual patients were compared for their similarity or difference in antibiogram pattern, it was shown that for each patient the particular isolate was consistent over follow-up time.

For those where no apparent source could be identified, the possibility is that endogenous organisms or environmental ones that were overwhelmed by other bacteria were not isolated in our attempt. Some studies have also failed to identify any source of transmission (Mark *et al*., 2001; McGregor, 1998).

The two isolates of *Enterococcus sp*. from the perineum of the index patients infected with the organisms had identical antibiogram with the burn wound isolates. Perineum and rectum as sources of Enterococci are well documented in other studies (Lai *et al.,* 1998). Whether these isolates were mere contaminants in the stool from nearby infected wound or were pathogens from the intestinal tract colonizing and infecting the burn wound is unclear.

Concerning antimicrobial susceptibility pattern of all isolated bacteria, although subject to frequent modifications, its assessment is important for clinical and epidemiological purposes. This is because the resistance of various organisms to antimicrobials poses a challenge to burn care since it decreases the effectiveness of treatment and increases morbidity, mortality and cost of care (Tredget *et al*., 2004).

Most of the isolates of *P. aeruginosa* were found to be resistant to the commonly used antibiotics but not to the antipseudomonal cephalosporin ceftazidime and polymyxin B. This is probably because the burn patients have never been treated with the two antibiotics as the prior treatment with such antibiotics has been demonstrated to be an independent risk factor for such MDR *P. aeruginosa* infections (Richard *et al*., 1994).

There was no isolate that was found to be pan-resistant (resistant to all antibiotics tested) in contrast to one study which had demonstrated the frequency of *P. aeruginosa* resistance to gentamicin, ceftazidime, carbenicillin, cephalothin to be over 90% (Estahbanati *et al*., 2002). In that study, the organism was most sensitive to amikacin and tetracycline while in our study; only 2 isolates (20%) were sensitive to tetracycline. However, Appelgren and his colleagues (2002) found low rates of antibiotic resistance and multi-resistance was a rare phenomenon. In the present study, there was also a moderate degree of resistance to ceftriaxone and gentamicin and high resistance to kanamycin.

Likewise, all isolates of *S. aureus* were sensitive to methicillin (it means they will also be sensitive to the commonly available antibiotic cloxacillin) and vancomycin in contrast to studies in developed countries (Richard *et al.,* 1982; Santucci *et al.*, 2003) and developing countries (Kehinde *et al*., 2004; Neelam *et al.,* 2004; Singh *et al.*, 2003) where the use of multiple antibiotics including potent anti-staphylococcal penicillins have increased the risk of colonization and infection by MRSA. The Indian study has also reported the endemicity of MRSA in their unit (Neelam *et al.,* 2004). However, a Malawi study identified only one isolate of MRSA out of 201 isolates of *S. aureus* (Komolafe *et al*., 2003).

In addition to the aforementioned antibiotics, isolates of *S. aureus* in the study were highly susceptible to clindamycin, augmentin (amoxicillin-clavulanic acid) and cephalothin (Table 3.6), and moderately susceptible to chloramphenicol and gentamicin which is in agreement with the work of Komolafe *et al* (2003). But the isolates were highly resistant to pencillin G (in contrast to the study of Komolafe *et al*., 2003) and ampicillin. Given the difficulty to control outbreaks due to MRSA, the ineffectiveness of antibiotic therapies used in this burn unit against MRSA infection, and the absence of antibiotics for eradication of carrier state in the unit, it is a fortunate state for the burn unit to have no isolates of MRSA and *P. aeruginosa* isolates resistant to ceftazidime.

Hence, many cases of burn patients in the unit with MSSA can be treated solely by hydrotherapy and applying dressing with antiseptic solutions, early removal of necrotic tissues and covering the injury with a skin graft without any systemic antibiotic therapy (Bagdonas *et al.,* 2003).

Limitations of the study

In the present study, the phenotypic method (antibiogram) of epidemiologic typing used in an attempt to identify the possible reservoirs or sources and vehicles of transmission of colonizing and infecting burn wound pathogens has a drawback (Mayhall, 1999). Since unrelated clones of a single species can undergo evolutionary convergence to the same resistance phenotype under antibiotic selective pressure, through mutations and genetic exchanges (Namiko *et al.,* 2001), unless confirmed by genomic typing, no definite conclusions can be drawn with regard to the role of the possible sources and vehicles reported in this study.

The number of isolates from the different possible sources with identical antibiogram to those from infection sites was greater than the actual number of isolates from the sites of infection in 71 isolates of the total 74 isolates. This resulted in difficulty of identifying exactly one source of the organisms causing the infection as the same strain was located in more than one source.

Another possible limitation of this study is that only burn wound swabs were used for isolating pathogens from infected wounds. The fact that there was only one possible fungal infection of the burn wounds in this study might have been due to our failure to use burn wound biopsies and histologic examination which is considered to be the most suitable technique for the diagnosis of fungal infection of burn wounds (Pruitt *et al*., 1998; Mayhall, 1999) despite the low frequency of fungal isolates in burn wounds (less than 5%) in most studies (Mathangi *et al*., 1985; Mayhall, 1999; Calvario *et al.,* 1994). But it should be noted that biopsies taken at different points, though very close together, in the same burn area can yield extremely variable microbes both qualitatively as well as quantitatively (Calvario *et al.,* 1994). Besides, burn wound swabbing is a simple, inexpensive, non-invasive, and convenient procedure that is still being widely practiced in different centers (Agnihotri *et al.,* 2004; Komolafe *et al*., 2003).

It can also be argued that any microorganism present in deeper tissue are also likely to be presenting the superficial layer and consequently, it is most likely that superficial wound exudates display a full spectrum of the wound microflora which is involved in

pathogenesis of infection. Hence, wound swabs can be justified as simple procedures for assessing the microflora of burn wounds that are clinically infected (Mayhall, 1999).

The sample size used in this study is smaller than many of the studies involving burn patients, as the duration of the study was also shorter than many. This might have an impact in the interpretation of some of the results reported. Yet, there are studies including the present study which have used smaller number of patients than this study and demonstrated similar findings with those studies with large sample size (Gastemeir *et al.,* 2002, Neelam *et al.,* 2004; Oncul *et al*., 2002).

None of the specimens were cultured anaerobically and hence no comment can be made as to the rate of anaerobic infection in the burn patients in this study. However, as anaerobes account for a very small rate of infections in burn patients for the very nature of open wounds like burn wounds not allowing good growth of anaerobes (Karyoute, 1989; Mathangi *et al,* 1985), the absence of anaerobic culturing in our study may not have a detrimental effect on the overall pattern of bacterial isolates in BWIs.

Conclusions and Recommendations

Gram-positive organisms predominated as colonizing agents of the burn wounds but later dominated by Gram-negative bacteria in the burn patients. The most common nosocomial infection developed in the burn patients was burn wound infection followed by urinary tract infection. The most common infectious organisms in BWIs in the burn unit were *S. aureus* and *P.aeruginosa.* The isolates showed a moderate degree of resistance to the commonly used antibiotics. The design and discipline in the unit can be considered satisfactory in managing the burn population served as environmental contamination and staff carriage of major burn pathogens was not high. However, as patients with longer duration of injury are admitted, there is always a possibility of introduction of infected wounds (including infections with MDR organisms) into the burn unit.

From this study, it can be deduced that acquisition of the major burn pathogens was likely multifactorial, related to environmental contamination and contact with transiently colonized HCWs, patients' attendants, and endogenous sources.

Another important finding worth mentioning is that infected patients did shed the pathogens to the environment and this could serve as secondary reservoir for new patients.

Focusing attention on the present study results, it is recommended that to reduce the rate of nosocomial infection;

- personnel must continue to follow the satisfactory infection prevention and control rules (since treatment of infection with available antibiotics according to the results obtained proves to be difficult for economical reasons);
- assess nosocomial control methods;
- diagnose epidemics and identify endemic burn pathogens through a programme of surveillance with microbiological monitoring both of the patients and of the more significant spaces in the burn unit to be conducted continuously.
- though prophylactic antibiotics are not given, a stricter antibiotic policy is required instead of giving antibiotics to uninfected patients with clinical suspicion only.
- The Burn unit should be a model for future units to be constructed in other hospitals in the country as gross contamination of surfaces and air with MRSA or MDR organisms are hardly identified in this study.
- The community at large must be enlightened through proper health education programmes about the danger of keeping burn patients at home and smearing the wounds with herbs and other materials and that burn patients are better treated in hospitals with isolated burn units. Unfortunately, patient isolation in single rooms may not be possible in our setting due to limited resources.
- In this study, serotyping and genotyping of bacterial isolates especially multi-drug resistant organisms was not possible. Therefore, a retrospective analysis of the collected isolates in addition to a prolonged prospective study that analyzes the molecular epidemiology of all isolates using serotyping and genotyping by PFGE or other suitable techniques is essential in the future.

- Although this study has demonstrated the distribution of burn pathogens in the burn unit environment, the routes of infection and transmission could not be ascertained. Future studies should be conducted to determine the most important routes of infection and transmission in burn patients from the environmental reservoirs as this information is still controversial even in other units worldwide (Torregrossa *et al*., 2000).
- At last it is recommended for the burn unit to participate in the worldwide survey being conducted by the International Society for Burn Injuries (ISBI) in an attempt to better understand the problem of major infectious complications from multi-drug resistant organisms from the global perspective.

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Appendix I

Investigation form for follow-up of admitted burn patients in Yekatit 12 Hospital

I. Socio-demographic data

1. Serial number_____ 2. Card number_____

3. Age_____ 4. Sex_____ 5. Address_____

6. Occupation_____8. Monthly income(in birr)_____

II. Clinical data

1. Etiology of burn trauma (encircle one);

a. Scald c. electricity

b. Flame d. chemical agents e. others(specify)

3. Place of injury (encircle one): a. home b. factory c. others(specify)

2. Anatomic location of the burn (encircle as many): a. extremities b. trunk

c. head & neck d. perineum

3. Depth of the burn wound (encircle one): a. full thickness b. partial thickness

4. Extent of burn (% of total burn surface area) _____

5. Duration of the burn injury (in hours) _____

6. Other nosocomial infections developed (encircle as many):

a. Pneumonia c. bloodstream infections

b. Urinary tract infections d. others (specify)

7. Duration of stay in hospital (in days) _____

8. Minor procedures done on the patient (encircle yes or no):

a. intubation- yes / no c. urethral catheterization – yes / no

b. intravenous infusions- yes / no d. surgical drainage - yes / no

8. Preexisting disease (specify) _____

9. Prior administration of antimicrobial agents (type, route, duration)_____

10 Antibiotic prophylaxis given (encircle one)- yes / no

11. Treatment (encircle as many):

a. Topical chemotherapy c. hydrotherapy

b. Systemic therapy d. surgical debridement

Appendix II

Assessment of standard of hygiene within the burn unit

Surfaces	Window ledges	Door frames	Rough wall	Floors	Cupboards
Visible dust collected(yes/no					
Remarks					

I. Disinfection practices

a. Frequency (____X/day) c. Surfaces disinfected (list or specify)

b. Type of disinfectant used_____

II. Staff & patient traffic

a. Staff movement : -extent from patient to patient

- to other wards

b. Patient movement: - to hydrotherapy (frequency, interval b/n patients)

- - to operation theatre

III. Hand washing practices(by the staff)-------yes/no answers

a. before entering patient room

b. after entering patient room

c. before entering hydrotherapy room

d. after entering hydrotherapy room

IV. Personal protective (yes or no answers):

a. gowns b. cap c. mask d. gloves

Appendix-III

Criteria for nosocomial infection

A.1. ***Blood stream infection***

1. Verified blood steam infection

   One or several positive blood cultures and at least one sign from the list below

2. Suspected blood stream infection

   No positive blood culture but sign (f) and at least another two signs and antimicrobial therapy instituted

A.1.1. *Clinical and laboratory signs of BSI*

a. temperature> 38.5 or <36^{0}C during at least 12 h or ongoing antipyretic therapy
b. pulse rate>100/min
c. oliguria (< 20ml/hr)
d. respiratory rate > 28/min
e. septic emboli
f. disturbance of mental orientation or level of consciousness
g. hypotension<90 mmHg or inotropic drugs
h. C-recative protein >100mg/l
i. Leukocyte count<4 or >12X10^9/l

A.2. ***Pneumonia***

Patients diagnosed with pneumonia ‘‘had rales or dullness to percussion on physical examination of the chest’’ or ‘‘a chest radiographic examination that showed new or progressive infiltrate or consolidation, cavitation, or pleural effusion’’ and ‘‘new onset of purulent sputum or change in character of sputum.’’

A.3. Burn wound infection

1. Pus and/or foul smelling discharge from skin, wound, blister, abscess, drain, fistula or vascular insertion site above fascia.
2. Change in burn wound appearance or character, such as dark discoloration of the eschar, increased bleeding tendency, signs of inflammation in or around the wound or vascular insertion site and positive swab culture
3. Skin graft detached more than 2 days after grafting and positive swab culture

4. Positive swab culture from burn wound and at least two signs from the list below
5. Pus and/or foul smelling discharge from wound, abscess, drain or fistula beneath fascia.

A.3.1.Clinical and laboratory signs of burn wound infection

a. temperature>38.5 or <36^0C during at least 12 h or ongoing antipyretic therapy
b. leukocyte count,4 or >$12X10^9$/l in blood

A.4. Urinary tract infection

In cases of urinary tract infection, patients showed at least one of the following signs or symptoms with no other recognizable cause: ‘‘fever (T[388C), urgency, frequency, dysuria, or suprapubic tenderness and at least one of the following’’: ‘‘a. positive dipstick for leukocyte esterase and/or nitrate’’ or ‘‘b. physician diagnosis of a urinary tract infection.’’

Appendix- IV

Patient consent form

I, the undersigned, have fully agreed to participate in the study 'microbiology of burn unit at Yekatit Hospital' after it was explained to me by the investigator/s. I hereby give my consent for taking specimens from me for the study.

Name_____

Signature_____

Date_____