CHAPTER I
INTRODUCTION

1.1 Background

An intact human skin surface is vital to the preservation of body fluid homeostasis, thermoregulation and the host's protection against infection. The skin also has immunological, neurosensory and metabolic functions such as vitamin D metabolism**.** The skin is an essential component of the nonspecific immune system, protecting the host from potential pathogens in the environment (Chalise *et al.*, 2008).

Thermal burns are burns to the skin caused by any external heat source. This may be in the form of a naked flame from an open fire place or house fire, a scald from steam, hot or molten liquid, or via direct contact with a hot object such as a hot oven rack or hot cooking pan. Other types of burns include radiation burns, chemical burns and electrical burns (Chalise *et al.,* 2008; Lawrence and Florencia, 2008). Burns remain a significant public health problem in term of morbidity, long-term disability and mortality throughout the world; especially in economically developing countries (Ekrami and Kalantar, 2007). Thermal injury destroys the physical skin barrier that normally prevents invasion of microorganisms. During the first weeks following thermal trauma, the affected sites are colonized with bacteria (Song *et al.,* 2001). Following colonization, these organisms of the surface start to penetrate the burn eschar to available extent and viable sub eschar tissues become invaded (Agnihotri *et al.,* 2004; Nasser *et al.*, 2003).

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

wound invasion still represents a major cause of infection in burn intensive care units (Santucci *et al.,* 2003). The pattern of infection differs from hospital to hospital; the varied bacterial flora of infected wound may change considerably during the healing period (Rajput *et al.,* 2008). When an abrasion is created on the skin, microorganisms, usually the opportunistic organisms invade the abrasion and multiply leading to a delay in the healing process and finally infectious condition. The spectrum of infection ranges from asymptomatic colonization to bacteraemia and death (Abubakar 2009).

Burn patients are at a high risk for infection as a result of the nature of the burn injury itself, the immunocompromising effects of burns, prolonged hospital stays and intensive diagnostic and therapeutic procedures (Lari and Alaghehbandan, 2000). The pathogenesis of colonization, infection and invasion of microorganisms is related to the fact that there is a disruption of the normal skin barrier at the site, as well as a large amount of necrotic tissue and protein-rich wound exudates at the burn surface, providing a rich growth medium for colonization and growth of microorganisms (Erol *et al.*, 2004). In addition, the control and prevention of infectious diseases among burned patients present a greater and more specialized problem, because the skin barriers are disrupted, the environment in burn units can become contaminated with resistant organisms and these organisms can be transmitted easily from one patient to another. Thus, burn care units can be the sites of explosive and prolonged outbreaks caused by resistant organisms (Falks *et al*., 2000).

The burn site remains relatively sterile during the first 24 hour; thereafter, colonization of the wound by Gram negative bacteria is common (Pruitt *et al.*, 1998). *Pseudomonas aeruginosa* has emerged as a predominant member of the burn wound flora and in the absence of topical therapy is cultured from the burn injuries of 70% patients by the third week (Church *et al.*, 2006).

A variety of organisms have been isolated from burn wound colonisation and

infections. Aerobic bacterial isolates from burn wounds have ranged from Gram positive organisms like *Staphylococcus aureus*, coagulase negative staphylococci and *Enterococcus* spp., to Gram negative organisms like *P. aeruginosa, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Enterobacter* spp., *Proteus* spp. and *Acinetobacter* spp. (Church *et al.*, 2006). The surface of every burn wound is contaminated to some degree by bacteria (Lawrence and Lilly, 1972). Because of this, surface bacterial growth is routinely monitored in most centers to facilitate management and treatment. It has been found by many investigators that the distribution of various species of bacteria from burn wound surfaces is similar to that from blood specimens (Li 1989).

The increase rate of burn wound infection and sepsis is due to overcrowding, inadequate sterilization and disinfection practices, gross contamination of environment, lack of isolation facilities, inadequate hand washing and absence of barrier nursing (Taneja *et al.*, 2004). Patients have to stay for long period in the hospital and many intravascular and other devices are put in them. Hence they are at greater risk of acquiring hospital-acquired infection. The organisms that predominate as causative agents of burn wound infection in any burn treatment facility change over time. Gram positive organisms are initially prevalent during hospital stay of patients; then gradually become superseded by gram negative opportunists that appear to have a greater propensity to invade (Pruitt 1984).

An effective infection control policy is very much required to reduce or eliminate endemic pathogenic and/or antibiotic resistant organisms, prevent the establishment of antibiotic resistant organisms as the predominant nosocomial flora of the burn unit and prevent cross-contamination. For every burn institution there should have separate burn management protocol, as the prevalence and type of organisms may vary from center to center (Salah *et al.*, 2003). Infection in burns is not only important in being responsible for death but it is also an important factor in the prolongation of hospitalization time and delay in skin grafting. It is therefore essential for every burn institution to determine the time

related changes in predominant flora and antimicrobial sensitivity profiles. This would allow early management of imminent septic episodes with empirical systemic antibiotic before the results of microbiologic culture becomes available thus improving overall infection related morbidity and mortality (Ulku *et al.*, 2004).

Burn injuries remain a major cause of morbidity and mortality in low and middle-income countries. Health statistics are generally lacking; published data about burn injuries are scarce in Nepal. Also, the data on the changes in microbial profile in burn wound with respect to time are limited. Rapidly emerging nosocomial and community acquired pathogens and the problem of multidrug resistance necessitates periodic review of isolation patterns and antibiogram in the burn ward. Although eradication of infection in burn patients is impossible, a well conducted surveillance; infection control and prevention programme can help reduce the incidence, mortality rates, length of hospitalization and associated costs. The present study is undertaken to study the time related change in microflora in burn wounds of the burn patients from a tertiary care medical hospital. This study will help to assess the burden of infections at the center and antimicrobial susceptibility testing will help to formulate antibiotic policy for better management of these patients and the incidence of infections.

1.2 Objectives

1.2.1 General objective

a. To determine the time related changes in microbiological profile and their antibiogram in burn patients.

1.2.2 Specific objectives

a. To determine the bacterial isolates of burn wound.
b. To evaluate the time related changes of bacterial isolates in burn wound.
c. To determine the change of antimicrobial resistance pattern of bacterial isolates of burn with time.
d. To describe the risk factors (age, gender, TBSA, burn sites, burn cause and burn degree) of burn with burn wound infections.

CHAPTER II
LITERATURE REVIEW

2.1 Human skin

The skin is one of the largest organs in the human body, in terms of both its overall size and weight. The two skin layers together are upto several millimeters thick, but both epidermal and dermal thickness varies depending on the body site. The epidermis is the thinnest (0.05 mm) over the eyelid but thicker (up to 1 mm) over the soles of the feet (Dyer and Roberts, 1990). The dermis is thickest on the back. Males generally have thicker skin than females. General skin thickness peaks in midlife and gradually thins as part of the aging process. Infants, young children, and elderly adults have a much thinner dermal layer to their skin, resulting in an increased propensity for deeper burn injury. Epidermal cells are constantly being shed and replaced every month through a process that continually pushes new cells to the surface. The epidermis therefore heals itself after superficial injury (Hunt and Purdue, 1992).

2.2 Burn injury

Burns are one of the most common and devastating forms of trauma. Burn wounds are especially prone to infection. Thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humeral immune responses are pivotal factors contributing to infectious complications in

patients with severe burns (Heideman and Bengtsson, 1992). The burn wound surface provides a protein-rich environment consisting of a vascular necrotic tissue (eschar) that provides a favourable niche for microbial colonization and proliferation (Erol *et al.*, 2004). The avascularity of the eschar results in impaired migration of host immune cells and restricts delivery of systemically administered antimicrobial agents to the area, while toxic substances released by eschar tissue impair local host immune responses.

Burn injury is a major problem in many parts of the world. Burn injury destroys the skin barrier that normally prevents invasion by microorganisms. This makes the burn wound the most frequent origin of sepsis in these patients. Multi-organ failure and infection complications are the major causes of morbidity and death in serious burn injury (Lawrence and Florencia, 2008; Wolf and Herndon, 1999). The highest fatality rates occur among children 4 years of age or younger and adults over the age of 55 years. Burn related deaths in these two age groups account for more than two-thirds of all fire deaths (Lionelli *et al.*, 2005).

2.3 Pathophysiology

Loss of the cutaneous barrier facilitates entry of the patient's own flora and of organisms from the hospital environment into the burn wound. Initially, the wound is colonized with Gram positive bacteria from the surrounding tissue. The avascularity of the eschar, along with the impairment of local immune responses, favors further bacterial colonization and proliferation. Burns provide a highly nutritious medium for bacteria. Colonization may be limited to the eschar, invade deeper tissues, or spread systemically through lymph and blood (Church *et al.*, 2006; Lawrence and Florencia, 2008).

The cascade of events that follow a severe burn injury and that lead to multi-organ system failure and death are thought to represent a two step process. The burn injury itself, with ensuing hypovolemia and tissue hypoxia, is followed by invasive infection arising from large amounts of devitalized tissue. The frequency

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

2.4 Clinical manifestations

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

Early surgical excision of devitalized tissue is now widely used and burn-wound infections can be classified in relation to the excision site as burn-wound

impetigo; burn-related surgical wound infection; burn wound cellulitis infection that is secondary to a partial or full-thickness burn wound and is manifested by separation of the eschar or by violaceous, dark brown, or black discoloration of the eschar (Chalise *et al.,* 2008; Khajuria *et al.,* 2009).

In addition to infection of the burn wound itself, a number of other infections due to the immunosuppression caused by extensive burns and the manipulations necessary for clinical care put burn patients at risk. Pneumonia, now the most common infectious complication among hospitalized burn patients, is most often nosocomially acquired via the respiratory route; among eighth risk factors associated with secondary pneumonia are inhalation injury, intubation, full-thickness chest wall burns, immobility and uncontrolled wound sepsis with hematogenous spread. Septic pulmonary emboli may also occur. Suppurative thrombophlebitis may complicate the vascular catheterization necessary for fluid and nutritional support in burns. Endocarditis, urinary tract infection, bacterial chondritis (particularly in patients with burned ears) and intra abdominal infection also complicate serious burn injury (Lawrence and Florencia, 2008).

2.5 Microbial etiology

Bacteria rapidly colonize open skin wounds after burn injury. Microorganisms colonizing the burn wound originate from the patient's endogenous skin and gastrointestinal and respiratory flora (Erol *et al.*, 2004). Microorganisms may also be transferred to a patient's skin surface via contact with contaminated external environmental surfaces, water, fomites, air and the soiled hands of health care workers (Weber *et al.*, 1997).

The distribution of organisms changes over time in the individual patient. Immediately following injury, Gram positive bacteria from the patient's endogenous skin flora or the external environment predominantly colonize the burn wound (Barret and Herndon, 2003). Endogenous Gram negative bacteria from the patient's gastrointestinal flora also rapidly colonize the burn wound

surface in the first few days after injury (Manson *et al.*, 1992). Microorganisms transmitted from the hospital environment tend to be more resistant to antimicrobial agents than those originating from the patient's normal flora (Clark *et al.*, 2003). For example: the dominant flora of burn wounds during hospitalization changes from Gram positive bacteria such as *S. aureus* to Gram negative bacteria like *P. aeruginosa.* The majority of *P. aeruginosa*, an opportunistic human pathogen, isolates from burn patients were multidrug resistant (Lari and Alaghehbandan, 2000).

S. aureus became the principal etiological agent of burn wound infections (Phillips *et al.*, 1989) shortly after the introduction of penicillin G in the early 1950s, which resulted in the virtual elimination of *Streptococcus pyogenes* as a cause of infection in thermally injured patients. Although *S. aureus* remains a common cause of early burn wound infection, *P. aeruginosa* from the patient's endogenous gastrointestinal flora and/or an environmental source is the most common cause of burn wound infections in many centers (Altoparlak *et al.,* 2004). The incidence of infections due to less commonly encountered microbes, including other Gram positive and Gram negative bacteria, fungi and viruses, has also increased steadily in subsequent decades (Agnihotri *et al.,* 2004). While less common, infections due to anaerobic bacteria typically occur secondary to electrical burns or when open wound dressings are used in place of occlusive dressings (Murray and Finegold, 1984).

2.6 Antibiotics resistance in burn centers

Microbes have genetic plasticity, which means that they have the capacity to evolve in response to their environment. The major impetus for developing resistance is selective pressure resulting from antibiotic use. The bacteria that survive are those that develop mechanisms to avoid being killed by antibiotics. The treatment of several pathogens is becoming problematic (Melnick 2007).

The emergence worldwide of antimicrobial resistance among a wide variety of

bacterial burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections (Altoparlak *et al.,* 2004). Antibiotic resistant bacteria can be associated with infections with higher mortality than those caused by antibiotic susceptible strains (Laura *et al.,* 2009). MRSA, methicillin resistant coagulase negative staphylococci, vancomycin-resistant enterococci, and multiply resistant Gram negative bacteria that possess several types of -lactamases, including extended spectrum -lactamases (ESBL), ampC -lactamases, and metallo- -lactamases (MBL), have been emerging as serious pathogens in hospitalized patients (Mathur *et al.,* 2005).

The therapy of both nosocomial and community-acquired infections is affected by the continuing evolution of and challenges presented by antimicrobial resistance. This increasing emergence and spread of multi drug resistant bacteria in hospitals in general and burn centers in particular is of great concern and continues to challenge infection control and hospital epidemiology practice worldwide (Lorian 2005). Most of the antimicrobial resistance, which is now making it difficult to treat some infectious diseases, is due to the extensive use and misuse of antimicrobial drugs, which have favored the emergence and survival of resistant strains of microorganisms (Cheesbrough 2006).

2.7 Prevention and control of burn wound infections

Overcrowding, inadequate sterilization and disinfection practices, gross contamination of the environment, lack of isolation facilities, inadequate hand washing and barrier nursing are some of the reasons for high infection and sepsis rates in burn centers of resource poor settings in many developing countries (Taneja *et al.,* 2004).

Burn wound infections should be rigorously monitored according to the standard definitions in order to generate accurate epidemiological data about infection rates. Routine surveillance should also be carried out for other types of

nosocomial infections commonly diagnosed in burn patients, including catheter-related infections, pneumonia, and urinary tract infections. Laboratory surveillance cultures as well as routine microbial surveillance cultures of the burn wound and other sources (*i.e.*, blood, respiratory, and urine samples) should be monitored to rapidly identify epidemic pathogens and/ or antibiotic resistant strains so that control measures can be immediately implemented (Altoparlak *et al.,* 2005).

Antibiotic utilization should be rotated or changed based on monitoring of antibiograms within individual burn centers. Finally, adverse outcomes, including morbidity and mortality due to burn wound infection, sepsis, or another nosocomial infection complication, should be monitored in burn patients according to the extent of burn injury in order to assess the effectiveness of existing infection control practices within the center's modern burn therapy programme. Specialized burn care centers, using a multidisciplinary approach, not only successfully treat large burns and their complications, but provide the necessary rehabilitation and psychological support required for readjustment back into society (Thuan *et al.,* 1996).

2.7.1 Topical antimicrobial therapy

The efficacy of various topical antimicrobials in common use in modern burn centers is dynamic due to the ability of microorganisms to develop resistance rapidly (Church *et al.,* 2006). The four widely used topical antimicrobial agents are silver sulfadiazine cream, mafenide acetate cream, silver nitrate cream, and nanocrystalline silver dressings dramatically decrease the bacterial burden of burn wounds and reduce the incidence of burn wound infection (Lawrence and Florencia, 2008). Selection of topical antimicrobial therapy should be based on the agent's ability to inhibit the microorganisms recovered from burn wound surveillance cultures and monitoring of the nosocomial infections acquired in the burn unit (Church *et al.,* 2006).

2.7.2 Prophylactic systemic antibiotics

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

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

2.8 Epidemiology

Burn injury is a major public health problem in many countries of the world. Infection is the most common cause of death and serious problems following thermal injury. Developing countries have a high incidence of burn injuries, creating alarming public health problem. Moreover, high population density, illiteracy and poverty are the main demographic factors associated with a high risk of burn. The high incidence makes burns an endemic health hazard. Social, economic, and cultural factors interact to complicate the management, reporting and prevention of burns (Khajuria *et al.,* 2009).

2.8.1 Common bacterial isolates and their antibiogram

Shaikh *et al.* (2004) conducted the study of 100 bacterial isolates from burn wound sepsis, *P. aeruginosa* 30 (30%) and *S. aureus* 28 (28%), were the commonest organisms isolated from burn wound infections followed by *Klebsiella* spp. 16 (16%), *Proteus* spp. 14 (14%), *E. coli* 6 (6%) and *S. epidermidis* 6 (6%). *P. aeruginosa*, the most common burn wound isolates exhibited high resistance to ampicillin (80%), amoxycillin (80%), cefazolin (93.3%), erythromycin (80%) and doxycycline (100%). *S. aureus* isolates from burn wounds exhibited highest susceptibility against amikacin and tobramycin 100% and 100% respectively. Burn wounds isolates of *E. coli* exhibited 100% resistance to ampicillin, amoxycillin, ceftizoxime and doxycline. However cefaclor, aztreonam, aminoglycosides and fluoroquinolones were found to be highly effective agents. (Shaikh *et al*, 2004).

Similarly, fifty burn patients were investigated for bacterial profile of blood and burn wound infections. Gram negative organisms were found to be more prevalent. *P. aeruginosa* was found to be the most common isolate followed by *S. aureus, S. epidermidis, E. coli, Klebsiella* and *Salmonella*. In most of the cases, same organisms were found in blood and pus sample. Amikacin, norfloxacin, erythromycin and cefotaxime were more effective antimicrobials while co-trimaxazole, amoxyclav and ceforperazone were found to be the least effective. *Psudomonas* was found to be resistant to most of the therapeutic agents (Kaur *et al.*, 2006).

In the retrospective study of the 182 patients by Alireza *et al*. (2007) in Iran, 140 (76.9%) acquired at least one type of infection of the 140, 116 patients (82.8%) were culture positive on day 7 while 24 (17.2%) on 14 days after admission. Primary wound infection was most common (72.5%), followed by blood stream (18.6%) and urinary tract infections (8.9%). The microorganisms causing infections were *P. aeruginosa* (37.5%), *S. aureus* (20.2%) and *A. baumanni* (10.4%). Among these isolates *P. aeruginosa* was found to be 100 percent resistant to amikacin, gentamicin, carbenicillin, ciprofloxacin, tobramycin and

ceftazidime; 58 percent of *S. aureus* and 60 percent of coagulase negative *Staphylococcus* were methicillin resistant (Ekrami and Kalantar, 2007).

In the study of Mehta *et al*. (2007) in India, bacterial isolates were found in 260 (97.01%) samples and only eight wound swabs were sterile (2.99%). *Pseudomonas* spp. was the commonest pathogen isolated (51.5%) followed by *Acinetobacter* spp. (14.28%), *S. aureus* (11.15%), *Klebsiella* spp. (9.23%) and *Proteus* spp. (2.3%). *Pseudomonas* spp. showed marked resistance with amikacin (85.18%), gentamicin (89.22%), ciprofloxacin (78.81%), carbenicillin (88.26%), tobramycin (87.52%) and ceftazidmine (79.09%). On the other hand, *Pseudomonas* spp. was found to be more sensitive to newer antimicrobials, only 4.54% resistance to imipenem, 21.8% resistance to ceftazidime/clavulanic-acid, 25.67% resistance to cefoperazone/sulbactum. Among Gram negative bacilli, resistance percentage varied from 64.50% to amikacin to 86.64% to gentamicin. *S. aureus* were highly resistant to amoxycillin (69.04%), erythromycin (75.27%), and netilmicin (77.75%). However, no strain of *S. aureus* was found to be resistant to vancomycin (Mehta *et al*., 2007).

Also, fifty burn patients admitted in department of surgery in Nepal Medical College Teaching Hospital in Nepal were reviewed retrospectively. *S. aureus* (28%) was the commonest organism isolated from wound swab culture. Others were *Klebsiella* (16.0%), *Pseudomonas* (13%), *Proteus* (13%) and *E. coli* (13%). No growth was noted in 17% of patients. During the treatment, 14% of patients died and 4% left against medical advice. Remaining patients were discharged after complete recovery (Chalise *et al.,* 2008).

Rajput *et al*. (2008) found that the most common isolates from burn was *P. aeruginosa* (55.0%), followed by *S. aureus* (19.29%), *Klebsiella* spp. (11.43%), *Acinetobacter* spp. (7.14%), *Proteus* spp. (4.29%), *E. coli* (2.85%). Resistance of *S. aureus* was 40% observed with oxacillin and 84% to erythromycin whereas all strains were susceptible to vancomycin. *Pesudomonas,* which was the commonest

isolate, was most resistant to ceftazidime (70%) followed by cefotaxime. ciprofloxacin (55.5%) and amikacin (54%) were found to be most effective antimicrobial agent. Other Gram negative organisms were highly resistant to cefotaxime (66%) followed by gentamycin (60%).). Imipenem was found to be less resistant (26%) against *Pseudomonas* (Rajput *et al.,* 2008).

S. aureus was isolated most frequently (20.8%), followed by *E. coli* (13.9%), *P. aeruginosa* (11.8%), CONS (10.9%), *Enterococcus* spp (9.7%), *E. cloacae* (5.6%), *K. pneumoniae* (5%), *Acinetobacter* spp. (3.2%), *P. mirabilis* (2%) and *Stenotrophomonas maltophilia* (1.4%). Susceptibility of *S. aureus* to broad spectrum substances such as ciprofloxacin or penicillinase stable penicillins has waned, others such as cotrimoxazole or netilmicin remained effective. Not a single resistance against vancomycin was recorded. *P. aeruginosa* has shown increasing susceptibility against netilmicin (1986-1989: 84%, 2002-2005: 95%). Susceptibility of *P. aeruginosa* to ceftazidime has decreased markedly. *Acinetobacter* spp. have shown little susceptibility to most antibiotics. Imipenem or meropenem have been very reliable reserve antibiotics throughout the study period for the fermenting enterobacteriaceae (*E. coli, K. pneumoniae, E. cloacae* and *P. mirabilis*), with susceptibilities of or near 100% (Guggenheim *et al.,* 2009).

Gram negative organisms continue to cause the most severe infections in burn patients. Colistin has re-emerged as a highly effective antibiotic against multi-drug resistant *Pseudomonas* and *Acinetobacter* infections of burns (Ludwik *et al.,* 2009).

The study in South Africa reported that the total number of isolates was 629, out of which 269 were Gram positive cocci and 360 were Gram negative bacilli. The commonest organism was *S. aureus* (27.7%), followed by *K. pneumoniae* (13.4%), *P. mirabilis* (12.4%), Group D streptococcus (9.4%), *P. aeruginosa* (8.9%) and *E. coli* (6.2%). Resistance among the Gram negative bacilli was, in

general, least to imipenem, amikacin and ciprofloxacin (Bhat and Vasaikar, 2010).

In another study by Sewnet (2010) among a total of 50 burn patients in Yekati 12 burn center 21(42%) were found bacteremic. The wound swab showed *S. aureus* (34.04%) and *P. aeruginosa* (31.8%) predominantly. High resistance was observed for ampicillin (77.4%), doxycycline (74.0), naldixic acid (70.5%), penicillin G (68.2%), tetracycline (67.5%), augumentin (37.5%), methicillin (29.5%), gentamycin (19.1%) and ceftriaxone (18.5%) (Sewnet 2010).

In the study conducted at Teiba Center for Burns Surgery in Yemen, out of 167 positive cultures, single Gram positive bacteria were the most dominant (44.3%), followed by Gram negative bacteria (28.7%) and mixed Gram positive and Gram negative bacteria (20.4%). *S. aureus* was the most common organism, isolated 100 (47.8%), followed by *P. aeruginosa* (23%), *E. coli* (5.3%), *Serratia plymuthica* (3.8%), *P. mirabilis* (2.9%), *Salmonella* spp. (2.4%), *S. epidermidis* (2.4%), *Acinetobacter* spp. (1.9%), *Streptococcus faecalis* (1.4%), *Bacillus* spp. (0.96%), *Citrobacter freundii* (0.96%), *Klebsiella* spp. (0.96%) and *S. pyogenes* (0.96%) (Alghalibi *et al.,* 2011).

Of 100 samples of burn wounds and multiple swab samples of different hospital environments. One hundred and twelve isolates were analyzed, from which there was a single agent in the majority of cases (73.3%). *P. aeruginosa* was the most common isolate (32.2%), followed by *Enterobacter* spp. (16.9%), CONS (12.5%), *Acinetobacter* spp. (11.7%), *Klebsiella* spp. (8.9%), *S. aureus* (7.2%), -hemolytic streptococci (4.4%) and others (6.2%). *P. aeruginosa* was the most resistant to third and fourth-generation cephalosporins (100%) whereas other Gram negative bacteria were resistant to ciprofloxacin and cephalosporin (70 to 100%) (Beheshti and Zia, 2011).

Lastly, *P. aeruginosa* was identified in 3012 (70.5%), *S. aureus* in 581 (13.6%), *Acinetobacter* 426 (9.9%), the rest organisms were 279 (6%). Frequencies resistant of applied antibiotics were tobramycin (82%), ceftazidim (78%), ceftizoxime (82%), ciprofloxacin (72%), amikacin (73%), gentamycin (80%), tetracyclin (60%), teazabactam (81%) and cotrimocxazol (98%), respectively for *P. aeruginosa* (Bojary Nasrabadi and Hajia, 2012).

2.8.2 Time related changes in bacterial isolates

In a study conducted to assess changes of microbial flora and wound colonization in Turkey; from Periodic swabs taken on admission and 7th, 14th, and 21st days of hospitalization. The most prevalent isolates were CONS (63%) and *S. aureus* (19.7%). There was a gradual decrease in the number of isolates of CONS and a marked increase in the numbers of *S. aureus* and *P. aeruginosa* from admission to 21st day. At the 21st day, the most frequent organisms were *S. aureus* (37.6%), CONS (34.7%) and *P. aeruginosa* (16.2%). While 35.3% of burn wounds were sterile on admission, microbial colonization reached 86.3% within the first week (Erol *et al.*, 2004).

The study revealed that bacterial colonization reached 86.6% within the first week. Although the Gram negative organisms were more predominant, *S. aureus* (28.4%) was the most prevalent organism in the first week. It was however surpassed by *P. aeruginosa* form third week onwards. For *S. aureus* and *P. aeruginosa,* vancomycin and polymyxin were found to be the most effective drugs respectively. Most of the isolates showed high level resistance to antimicrobial agents. The incidence of oxacillin resistance among coagulase negative staphylococci was high (44.6%) and among *S. aureus* were low (4.7%). However all staphylococci were susceptible to vancomycin. *S. aureus* showed high susceptibility to a wide range of antibiotics. CONS showed low to moderate susceptibility to amoxicillin/clavulanic acid, cephalothin, oxacillin, gentamicin, clindamycin, ciprofloxacin, ampicillin/sulbactan, and co-trimoxazole. More than 80% of *A. baumannii* isolated were susceptible only to ticarcillin/clavulanic acid

and imipenem. More than 90% of *E. cloacae* and *K. pneumoniae* were sensitive to imipenem, amikacin, and ciprofloxacin. All strains of *P. aeruginosa* showed low susceptibility to a wide range of antibiotics, except only to polymyxin and amikacin (Macedo and Santos, 2005).

Ekrami and Kalantar (2007) reported that of 182 patients, 140 (76.9%) acquired at least one type of infection. A total of 116 patients (82.8%) were culture positive on day 7th while 24 (17.2%) were positive on day 14th. Primary wound infection was the most common infection (72.5%), followed by blood stream (18.6%) and urinary tract infections (8.9%). The most frequent microorganisms were *P. aeruginosa* (37.5%), *S. aureus* (20.2%), and *A. baumanni* (10.4 %). Among these isolates, *P. aeruginosa* was found to be 100% resistant to amikacin, gentamicin, carbenicillin and ciprofloxacin (Ekrami and Kalantar, 2007).

A prospective study of bacterial wound colonization in 51 burn patients with chronic wounds of more than 3 weeks duration was carried. Of 178 surface swabs taken, out of which 110 microbial isolates were cultured this included 104 solitary and 3 twin isolates. The most frequent isolate was coagulase negative *S. epidermidis* (56.36%) followed by *Pseudomonas* (18.18%) and *S. aureus* (13.63%). There was a high degree of resistance by these organisms to commonly available antibiotics. The most sensitive antibiotics were gatifloxacin and amikacin irrespective of the organism's cultured (Dhar *et al.*, 2007).

During the six-month study period in Motahari Hospital in Iran, 164 burn patients were admitted to the hospital, of which 812 bacterial isolates were obtained from pus culture. The bacterial isolate was 325 (40%) *Pseudomonas*, 140 (17%) *Acinetobacter*, 132 (16%) *S. aureus*, and 215 (27%) other bacteria. More than one kind of bacteria was identified in 95 samples from 717. Forty percent of cultures were positive without *Pseudomonas* and *Acinetobacter* in first 48 hours after admission. Late in the first week 67% of patient had at least one of *Pseudomonas* and/or *Acinetobacter*. This percentage in second, third, and fourth week was 81%,

84%, and 98%, respectively. Mortality is 12% among patients and all of them had *Acinetobacter* (3 samples) and *P. aeruginosa* and *Acinetobacter* (7 samples) in their positive culture (Azimi *et al.,* 2011).

Another prospective study was carried out in 50 burn patients admitted in Burn unit of Dhaka Medical College Hospital. Among the 200 samples, single organism was isolated in 71% samples and mixed organism in 13.5% and no growth in 14.5%. Among single isolates *P. aeruginosa* was leading (28%) followed by *E. coli* (17.5%), *S. aureus* (16%), CONS (4.5%) and *Klebsiella* (2%). Among mixed growth *P. aeruginosa* was still leading (11%) follow by *E. coli* (9.5%) *Staphylococcus* (5.5%), *Proteus* (1%) and *Klebsiella* (0.5%). On admission 42% of the isolated organisms were *S. aureus* and only 6% each *P. aerunginosa* and *E. coli* were isolated. No growth was found in 28% samples. These findings were gradually changing with time and on day 21 *S. aureus* were only 4% whereas *P. aeruginosa* were 40% and *E. coli* 28%. Antimicrobial sensitivity test showed that *P. aeruginosa* was highly resistant to antimicrobial agents. It was most sensitive to imipenem (98.72%) followed by aztreonam (33.44%), ceftazidime (38.32%) and gentamicin (19.23%). *E. coli* was also found most sensitive to imipenem (98.15%) followed by gentamicin (38.95%), chloramphenicol (37.1%), ciprofloxacin (35.25%) and ceftraixone (29.70%). *S. aureus* was 100% sensitive to vancomycin followed by amoxiclav and oxacillin (53.43% each), gentamicin (44.70%) and cloxacillin (39.52%) (Saha *et al.*, 2011).

Similarly the study conducted by Shahzad *et al*. (2012) showed that single isolates were present in 57.85 % of cases and multiple isolates were noted in 34.65 % cases. The frequency of Gram negative organisms was high. The most common isolate was *P. aeruginosa* (54.4%), followed by *S. aureus* (22%), *Klebsiella* spp. (8.88%), *Acinetobacter* spp. (4.63%), *S. epidermidis* (5.79 %), *Proteus* spp. (2.7%) and *E. coli* (1.54%). Multiple isolates were found in 97 samples (34.64% cases), 7.5 % samples showed absence of bacterial pathogens. At the end of first week, 16 results showed sterile culture, 52 showed single isolates, and 29 patients

showed multiple isolates. At the end of 2nd week, sterile cultures were obtained in 14 patients, single isolate in 110 and 68 multiple isolates in patients. In first week *P. aeruginosa* predominated followed by *S. aureus* how ever in second week *P. aeruginosa* was highly significant. *S. epidermidis* was most sensitive to amikacin (82.25%), whereas most of the Gram negative isolates obtained were found to be multidrug resistant. Resistance of *S. aureus* was 40% observed with oxacillin and 84% to erythromycin whereas all strains were susceptible to vancomycin (Shahzad *et al.,* 2012).

CHAPTER III
MATERIALS AND METHODS

3.1 Study design

This study is a prospective study of all burn patients admitted to burn unit in Bir Hospital from September 2011 to Feburary 2012. All burn admitted patients during this period were included in the study. Patient charts were stored in a computerized database for statistical evaluation.

3.2 Study setting

There is a single burn unit in Bir Hospital, Nepal. It has 3 beds in ICU, 6 beds in BCU, and 1 dressing bed. It also has an operation room and physiotherapy room. The laboratory investigations were conducted in the Department of Pathology, Microbiology Section, Bir Hospital, Kathmandu in collaboration with Central Department of Microbiology.

3.3 Study population and sampling

Samples were collected from September 2011 to February 2012. The number of burn wound swabs depended on the number of admitted patients in the burn unit during this period. The microbial colonization of wounds was studied weekly from the date of admission to the 4th week of hospitalization. During the period 42 burn cases were admitted so, the total number of samples were 168.

3.4 Inclusion and exclusion criteria

All burn patients attending medical care at burn unit of Bir Hopital burn center during the study period were included in the study. Those seriously ill burn patients, who had been in the burn center before the study started, were excluded.

3.5 Questionnaire

To achieve the objectives of this investigative work, a questionnaire was used to collect data from the HCWs and patients. The following data were obtained from all burn cases admitted to the burn units; registration data: age, sex, occupation, and past history. Clinical assessment of the wound: cause of burn, site affected, TBSA, degree and complications. Chronological data: dates of admission and discharge.

A personal interview was held for filling in the questionnaire. All interviews were conducted face to face by the investigator himself. The questionnaire was based on the review of literature related with some modifications.

3.6 Materials and equipments

A list of glassware and equipments used for the study was presented in Appendix I.

3.7 Specimen collection

Periodic wound swabs were collected at 1st, 2nd, 3rd, and 4th weeks of hospital stay at Bir Hospital at burn ward**.** The sampling procedure included collection of swab from clinically deep area of burn wound site prior to any cleansing. In each sampling procedure, the bandages were removed, the remnants of topical antimicrobial agents were scraped away and the wounds were swabbed before washing and applying new topical antimicrobial agents. Swabs were collected by using sterile cotton tipped swabs. Specimens were immediately transferred to sterile test tube. In case of collection of sample from dry surface, swabs were moistened with sterile normal saline. After collection, tubes were plugged properly, labeled and carried promptly to the microbiology laboratory of Bir

Hospital. Wound swabs obtained from the burn patients were subjected to microbiological analysis. The isolates were identified by standard microbiological techniques and their antibiotic susceptibility was determined by using Kirby-Bauer disk diffusion techniques.

3.8 Sample processing

The sample was processed as soon as it reached the laboratory following standard laboratory procedures. Of two samples taken from each patient, one was used for Gram stain and other for culture (Collee *et al.*, 1999).

3.8.1 Macroscopic examination

The colour, odour and whether it contained granules were noted.

3.8.2 Microscopic examination

An even smear of the specimen was made on clean slide. The smear was heat fixed and stained by the Gram stain method. The smear was examined for bacteria among pus cells using 10x and 100x objectives.

3.8.3 Culture of specimen

All wound swab specimens were inoculated on Blood Agar (BA) plate, Mac Conkey agar (MA) and Nutrient agar (NA) and incubated at 37°C for 18-24 hours (Benson, 2001; Cheesbrough, 2006).

3.8.4 Isolation and identification of bacteria

Preliminary identification of bacterial isolates were done using colony morphology and characteristics (like pigmentation, haemolysis pattern on blood agar) and also by Gram staining whenever necessary. Conventional biochemical tests from peptone suspensions of the isolates were performed from primary cultures for final identification of the isolates. In brief, Gram negative rods were identified by performing of a series of biochemical tests, namely: catalase test, oxidase test, oxidative-fermentative (OF) test, methyl-red (MR) test, voges-

proskauer (VP) test, indole test, motolity test, hydrogen sulphide (H_2S) production test, triple sugar iron (TSI) reactions, citrate utilization test, and urease test. Gram-positive cocci were identified based on their preference of growth on BA and NA followed by catalase test, oxidase test, OF test and coagulase test. When the colonies were difficult to recognize on BA plate, gram staining was done (Benson 2001; Cheesbrough 2006). The composition of media and reagents used for different biochemical tests and their procedures are given in appendix-II.

3.9 Antibiotic susceptibility testing for isolated organisms

Mueller Hinton Agar (MHA) was used for determining the sensitivity of bacteria by single disk diffusion method of Kirby Bauer against different antimicrobial agents (Bauer *et al.*, 1966; Muller and Hinton 1941). Each isolate was grown in 5 ml nutrient broth at 37°C for 3-4 hours till the turbidity reached or exceeded that of a 0.5 McFarland standard. If the standard was exceeded, the suspension was diluted with broth till it was visually comparable to the standard. A sterile swab dipped into the suspension of the isolate in broth, squeezed free from excess fluid against the side of tube and then spreaded over the MHA agar plate so as to get a matt growth. Sterile antibiotic discs (Hi-media) were equidistantly placed on these plates and gently pressed onto the medium with the help of sterile forceps to ensure complete contact with the agar surface. A zone of inhibition was measured in millimeters and the organisms classified as sensitive or resistant according to the zone size interpretation chart (Benson 2001; Cheesbrough 2006). The different antibiotics used and the test procedures are given in the appendix- III.

3.10 Quality control for tests

Quality control is absolutely essential for good operating procedure. An important criterion of quality for a microbiological test is how much it contributes to the prevention or cure of infectious diseases (Vandepitte *et al.*, 2003).

To maintain quality control, all tests were performed in an aseptic condition. Aseptic method was followed during sample collection using sterile swab in order

to avoid contamination. The sample was also processed in aseptic condition. The sterility of each batch of test medium was confirmed by incubating one uninoculated tube and plate with the inoculated tests as quality control. During the test, one tube of each batch of medium was inoculated with known organism for positive reaction and another tube with stock culture known to give negative reaction. These positive and negative controls were incubated along with test and compared the results.

3.11 Statistical analysis

Frequencies and percentages were calculated for the study variables. The data were analyzed by using statistical packages for social sciences (SPSS) 16.0 statistical softwares. Chi-square (χ2) test was used to calculate probabilities and determined significance. A p-value of less than or equal to 0.5 was considered to be statistically significant (p 0.5), while p-value more than 0.5 was considered to be statistically not significant (SN).

3.12 Ethical considerations

- This M.Sc. thesis proposal was approved by Central Department of Microlobiology, Tribhuvan University, Kiritipur, Nepal.
- Official letter from the department was written to Bir Hospital and other concerning bodies.
- Patients were directly benefited for better management by communicating the lab result with physicians.
- The procedure of specimen collection was also explained for all participants. Patients were not subjected for unnecessary sample collection unless to benefit them.
- All information from participants was kept confidential.

CHAPTER IV
RESULTS

4.1 Description of study sample

During the six months of prospective study, the totals of 42 patients with a new burn incident were investigated at the burns center. Information about patients such as age, sex and date and cause of injury were noted for analysis. Four pus samples were collected from each burn patient at the interval of a week from the burn unit at Bir hospital. So, the total number of pus samples for the analysis was 168.

4.1.1 Age and Sex

Age was not normally distributed and ranged from 16 to 79 years (mean 38.9, median 33.5, SD 18.9). While 50% of the patients were aged below 30 years, adult aged 25-34 were the largest group accounting for 28.6% of the total. More burns occurred in female (60%) compared to male (40%) (Table 1).

Table 1: Age and gender wise distribution of total patients

Age	Sex				Total	
	Male		Female			
	No.	%	No.	%	No.	%
15-24	6	14.3	5	11.9	11	**26.2**
25-34	4	9.5	8	19.0	12	**28.6**
35-44	0	0	3	7.1	3	**7.1**
45-54	4	9.5	4	9.5	8	**19.0**
55-64	1	2.4	2	4.8	3	**7.1**
65-74	0	0	2	4.8	2	**4.8**
75-84	2	4.8	1	2.4	3	**7.1**
Total	**17**	**40.5**	**25**	**59.5**	**42**	**100**

4.1.2 Sites of burn

Studying the site of burn accident on the body of burn patients, the highest percentage of burn affected was extremities and genitalia 13 (31%), followed by genitalia 9 (21.4%); head and neck 7 (16.7%) and abdomen 7 (16.7%) while the trunk accounted for the lowest percentage 1 (2.4%) (Table 2).

Table 2: Patient distribution according to sites of burn

Burn sites	Number	Percent (%)
Head and neck	7	**16.7**
Extremities	5	**11.9**
Trunk	1	**2.4**
Genitalia	9	**21.4**
Abdomen	7	**16.7**
Extremities and genitalia	13	**31.0**
Total	**42**	**100**

4.1.3 Extent of burns

The total body surface area (TBSA) burn range with 15% to 90%; (20-39%) category included the highest percentage of patients (50.0%) and 80% category showed the lowest percentage of patients (2.4%) (Table 3).

Table 3: Patient distribution according to burn extent

TBSA	**Number**	**Percent (%)**
<20%	6	**14.3**
20-39%	21	**50.0**
40-59%	12	**28.6**
60-79%	2	**4.8**
80%	1	**2.4**
Total	**42**	**100**

4.1.4 Causes of burn

Flame burns resulted in 33 (78.6%) cases that admitted to burn unit during the study period, of which fire burn accounted 26 (61.9%) cases; whereas scald and electrical burns were responsible for 3 (7.1%) cases each and the lowest case included acid burn 1 (2.4%) (Table 4).

Table 4: Patient distribution according to cause of burn

S.N.	**Burn cause**	**Number**	**Percent (%)**
1	Scald	3	**7.1**
2	Flame		
i	Fire	26	**61.9**
ii	Kerosene	6	**14.3**
iii	Petrol	1	**2.4**
3	Electrical	3	**7.1**
4	Lightening	2	**4.8**
5	Acid	1	**2.4**
Total		**42**	**100**

4.1.5 Degree of burn

Majority of burn patients were third degree (full-thickness) burn 28 (67%) followed by second degree (partial-thickness) burn 12 (28%). First degree burn 2 (5%) accounted for the least number of burn among total patients (Figure 2).

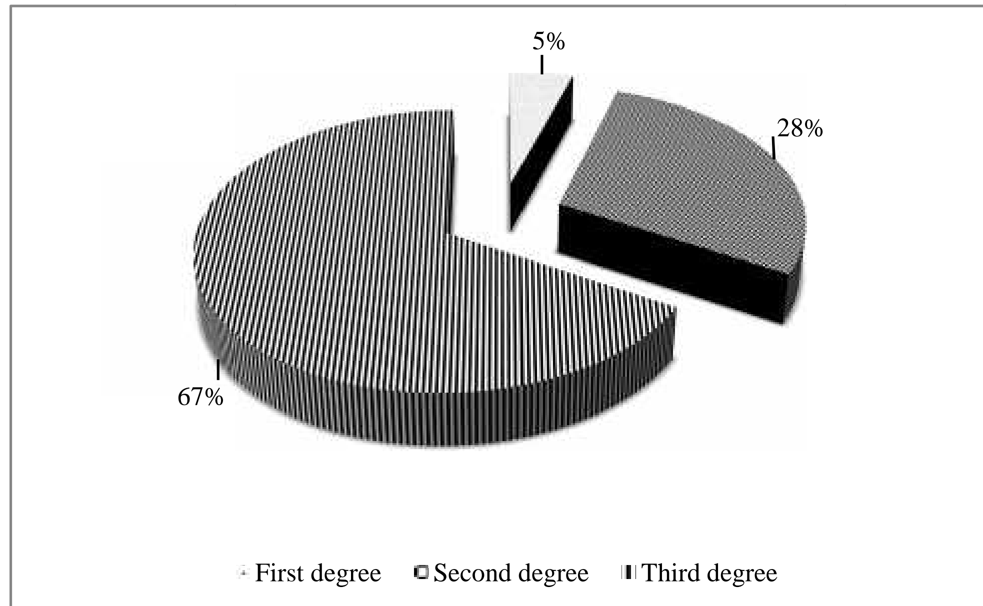

Figure 2: Patients distribution according to the degree of burn

4.2 Risk factors and culture result

Different risk factors were studied for their role in wound infections in burn unit.

4.2.1 Culture results of patient's samples

The overall percentage of positive cultures and no growth were 87.5% and 12.5% respectively. Among the positive growth, single growth accounted for 80 (47.6%) while mixed growth was found to be 67 (39.9%). More than two-thirds of the culture was growth positive.

Table 5: Distribution of pus culture with growth

Growth	Number	Percent (%)
Single	80	**47.6**
Mixed	67	**39.9**
No growth	21	**12.5**
Total	**168**	**100**

4.2.2 Culture results and burn sites

According to the site of burn in the body, extremities and genitalia were the most commonly infected area in comparison to other burn sites which accounted for 28.6% growth. All the pus cultures from genitalia showed positive growth with equal proportion of single and mixed growth 18 (10.7%). This higher contamination reached statistical significance with the sites of burn.

Table 6: Relationship between culture results and burn sites

Burn sites	Culture result – No. (%)			p-value
	Single growth	Mixed growth	No growth	
Head and neck	14 (8.3)	8 (4.8)	5 (3.0)	
Extremities	7 (4.2)	6 (3.6)	7 (4.2)	
Trunk	1 (0.6)	1 (0.6)	2 (1.2)	
Genitalia	18 (10.7)	18 (10.7)	0 (0)	**0.000****
Abdomen	20 (11.9)	6 (3.6)	2 (1.2)	
Extremities and genitalia	20 (11.9)	28 (16.7)	5 (3.0)	

**Significant at 1% level of significance

4.2.3 Culture results and total burn surface area

Studying the risk factor TBSA, the highest growth 74 (44.1%) was obtained in the TBSA (20-39%) group with a higher single growth 45 (26.8%). All cultures 8 (4.8%) were positive for the (60-79%) TBSA group. Growth result from burn was found to be significantly associated with TBSA.

Table 7: Relationship between culture results and TBSA

TBSA	Culture result – No. (%)			p-value
	Single growth	Mixed growth	No growth	

<20%	11 (6.5)	6 (3.6)	5 (3.0)	
20-39%	45 (26.8)	29 (17.3)	9 (5.4)	
40-59%	22 (13.1)	24 (14.3)	2 (1.2)	**0.000****
60-79%	2 (1.2)	6 (3.6)	0 (0)	
80%	0 (0)	2 (1.2)	5 (3.0)	

** Significant at 1% level of significance

4.2.4 Culture results and burn degree

In analyzing the results concerning burn degree, it was clear that patients with third degree burns had more positive cultures 107 (63.7%) compared to the positive cultures 35 (20.8%) isolated from patients with second degree burns. First degree accounted for no mixed growth while there were the highest 55 (32.7%) mixed growth in third degree. Also higher the degree of burn, higher was the growth of organism. There was a highly statistical significant difference between burn degrees and culture results (Table 8).

Table 8: Relationship between culture results and burn degree

Burn degree	Culture result – No. (%)			p-value
	Single growth	Mixed growth	No growth	
First degree	5 (3.0)	0 (0)	3 (1.8)	
Second degree	23 (13.7)	12 (7.1)	13 (7.7)	**0.000****
Third degree	52 (31.0)	55 (32.7)	5 (3.0)	

** Significant at 1% level of significance

4.2.5 Culture results and cause of burn

In regard to the cause of burn, flame burn accounted for the highest 119 (72.6%) growth with the majority of monomicrobial infection 61 (36.3%). All the scald burn resulted in positive growth in contrast to this, no growth was observed in majority of the culture specimens in lightening. There was a significant difference in the culture results between the causes of burn (Table 9).

Table 9: Relationship between culture results and cause of burn

Burn cause	Culture result – No. (%)			p-value
	Single growth	Mixed growth	No growth	
Scald	10 (5.9)	2 (1.2)	0 (0)	
Flame	61 (36.3)	58 (34.5)	13 (7.7)	
Electrical	4 (2.4)	6 (3.6)	2 (1.2)	**0.000****
Lightening	2 (1.2)	1 (0.6)	5 (3.0)	
Acid	3 (1.8)	0 (0)	1 (0.6)	

** Significant at 1% level of significance

4.3.1 Microbiological investigation

Among the forty-two swab samples in the first week, six swabs (14.3%) were sterile and microbial colonization reached 95.9% in the third week, which decreased to 76.2% in fourth weeks of the culture. About twenty-four percent (n=10) of pus swabs collected in fourth week were without growth. Polymicrobial infections (45.2%) predominant in first week which decreased steadily and reached 28.6% at the end of fourth week (Table 10).

Table 10: Pattern of growth of pus culture in different period of time

Growth	Time of sampling (week)							
	First		Second		Third		Fourth	
	No.	%	No.	%	No.	%	No.	%
Single	17	40.5	19	45.3	24	57.1	20	47.6
Mixed	19	45.2	20	47.6	16	38.1	12	28.6
No growth	6	14.3	3	7.1	2	4.8	10	23.8
Total	**42**		**42**		**42**		**42**	

4.3.2 Distribution of total bacterial isolates with types of infections

A total of 215 bacterial species were isolated from 168 pus swabs: *P. aeruginosa* accounts for the highest percentage 98 (45.6%) from the burn patients followed by *S. aureus* 41 (19.1%) and *Acinetobacter* spp. 38 (17.7%). Meanwhile, CONS, *Klebsiella* spp., *E. coli, Proteus* spp., *Citrobacter* spp. and *Enterobacter* spp.

represent the lowest isolated microorganisms and account for 38 (17.7%) isolates. *P. aeruginosa* was found to occurred with most of the organisms particularly *Acinetobacter* spp. followed by *S. aureus* in polymicrobial infection (Table 11).

Table 11: Distribution of organism with types of infections

Types of infection					
Organisms	**Mixed**		**Organisms**	**Single**	
	No.	**%**		**No.**	**%**
P. aeruginosa + *Acinetobacter* spp.	19	12.9	*P. aeruginosa*	47	32.0
P. aeruginosa + *S. aureus*	10	6.8	*S. aureus*	20	13.6
P. aeruginosa + CONS	7	4.8	CONS	3	2.0
P. aeruginosa + *E. coli*	3	2.0	*Acinetobacter* spp.	10	6.8
P. aeruginosa + *Klebsiella* spp.	4	2.7	*Klebsiella* spp.	0	0
P. aeruginosa + *Citrobacter* spp.	2	1.4	*E. coli*	0	0
P. aeruginosa + *Proteus* spp.	4	2.7	*Citrobater* spp.	0	0
P. aeruginosa + *Enterobacter* spp.	1	0.7	*Enterobacter* spp.	0	0
P. aeruginosa + *S. aureus* + *Klebsiella* spp.	1	0.7	*Proteus* spp.	0	0
S. aureus + *Acinetobacter* spp.	4	2.7			
S. aureus + CONS	1	0.7			
S. aureus + *E. coli*	1	0.7			
S. aureus + *Proteus* spp.	1	0.7			
S. aureus + *Klebsiella* spp.	3	2.0			
Acinetobacter spp. + CONS	1	0.7			
Acinetobacter spp. + *Enterobacter* spp.	1	0.7			
*Acinetobacter*spp. + *Citrobacter*spp.	1	0.7			
*Acinetobacter*spp. + *E. coli*	2	1.4			
Proteus spp. + *E. coli*	1	0.7			
Total	**67**	**45.6**		**80**	**54.4**

4.3.3 Pattern of bacterial colonization of burn wounds

P. aeruginosa 22 (38.6%), *Acinetobacter* spp. 12 (21%) and *S. aureus* 11 (19.2%) were the most prevalent isolates on 1st week cultures (pus 1). There was a gradual increase in the number of *P. aeruginosa* 28 (50%) while the number of

Acinetobacter spp. 10 (17.5%) and *S. aureus* 11 (19.6%) remain almost similar from day 1st to 3rd week (pus 3). *Acinetobacter* spp. 6 (13.3%) and *S. aureus* 6 (13.3%) decreased significantly but *P. aeruginosa* 26 (57.8%) remained predominating bacteria from 3rd to 4th week (pus 4). Among the total isolates from burn wound swab, *Klebsiella* spp., *E. coli* and *Proteus* spp. noted with similar frequency (8, 7 and 6 respectively). Similarly *Citrobacter* spp. and *Enterobacter* spp. were isolated with the least number, three and two respectively (Table 12). There is no significant difference in the isolation of organisms with respect to change in time.

Table 12: Isolation pattern of bacteria in different period of time

Organism	**Time of sampling (week)**								**p-value**
	First		**Second**		**Third**		**Fourth**		
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	
P. aeruginosa	22	38.6	22	38.6	28	50.0	26	57.8	
S. aureus	11	19.2	13	22.8	11	19.6	6	13.3	
CONS	3	5.3	2	3.5	2	3.6	5	11.1	
Acinetobacter spp.	12	21.0	10	17.5	10	17.8	6	13.3	
Klebsiella spp.	3	5.3	3	5.3	1	1.8	1	2.2	
E. coli	2	3.5	1	1.8	3	5.4	1	2.2	**0.749**
Citrobater spp.	1	1.8	2	3.5	-	-	-	-	
Enterobacter spp.	1	1.8	1	1.8	-	-	-	-	
Proteus spp.	2	3.5	3	5.3	1	1.8	-	-	
Total	**57**	**100**	**57**	**100**	**56**	**100**	**45**	**100**	

4.4.1 Antibiotic resistant pattern of Gram negative bacteria

Each isolate was tested for their susceptibility pattern against commonly used antibiotics in Bir hospital. A high level of drug resistance was seen among gram negative isolates especially *P. aeruginosa*. It was moderately resistant to Amikacin (64.3%) whereas resistance was more marked with other antimicrobials. Most (70-90%) of the isolates of *P. aeruginosa* were resistant for cotrimoxazol, chloramphenicol, cefotaxime, cefixime, ciprofloxacin, levofloxacin

and gentamycin. On the other hand, almost all *P. aeruginosa* was found to be sensitive to polymyxin B as it is evident by only 1% resistance. Thus it is considered as multidrug resistant to the commonly used drugs in the burn unit. Similarly, almost all (90-97%) *Acinetobacter* spp was resistant to cotrimoxazol, cefixime and cefotaxime whereas it had lower resistant to amikacin (28.9%) and chloramphenicol (36.8%). In addition, almost all isolates of the family enterobacteriaceae that were tested, irrespective of their isolation source, were sensitive to amikacin, whereas most of them were resistant to cefixime (Table 13).

Table 13: Antibiotics resistance pattern of Gram negative bacteria

Organisms	**Antibiotics - No. (%)**								
	AK	**GEN**	**CFM**	**CTX**	**C**	**COT**	**CIP**	**LE**	**PB**
P. aeruginosa n= 98	63 (64.3)	84 (85.7)	89 (90.8)	74 (75.5)	76 (77.6)	71 (72.4)	78 (79.6)	78 (79.6)	**1 (1.0)**
Acinetobacter spp. n= 38	11 (28.9)	26 (68.4)	37 (97.4)	35 (92.1)	14 (36.8)	36 (94.7)	31 (81.6)	23 (60.5)	**-**
Klebsiella spp. n= 8	2 (25.0)	5 (62.5)	7 (87.5)	6 (75.0)	7 (87.5)	5 (62.5)	5 (62.5)	5 (62.5)	**-**
E. coli n= 6	1 (14.3)	5 (71.4)	6 (85.7)	6 (85.7)	6 (85.7)	4 (57.1)	3 (42.9)	2 (28.6)	**-**
Ctrobacter spp. n= 3	0 (0)	0 (0)	3 (100)	1 (33.3)	1 (33.3)	2 (66.6)	1 (33.3)	1 (33.3)	**-**
Enterobacter spp. n= 2	0 (0)	1 (50)	2 (100)	1 (50)	0 (0)	1 (50)	1 (50)	1 (50)	**-**
Proteus spp. n= 6	0 (0)	0 (0)	4 (66.6)	1 (16.6)	1 (16.6)	2 (33.3)	0 (0)	0 (0)	**-**

4.4.2 Antibiotic resistance pattern of Gram positive bacteria

The antibiotic resistance pattern of *S. aureus* showed that most isolates were least resistant to chloramphenicol (19.5%) and levofloxacin (19.5%). However, majority of the isolates were recognized as susceptible for vancomycin as only 1% resistance was seen (Table 14). On the other hand, CONS were highly resistant to cotrimoxazole (91.7%), gentamycin (83.3%) whereas no isolate of CONS was resistant to vancomycin. In addition, they offered moderately resistant to levofloxacin (33.3%) and ciprofloxacin (41.7%) (Table 14).

Table 14: Antibiotic resistance pattern of Gram positive bacteria

Antibiotics	*S. aureus* (n= 41)		CONS (n= 12)	
	No.	%	No.	%
AK	27	65.5	7	58.3
GEN	24	58.5	10	83.3
CFM	32	78.0	7	58.3
CTX	20	48.8	7	58.3
C	8	19.5	8	66.7
COT	26	63.4	11	91.7
CIP	16	39.0	5	41.7
LE	8	19.5	4	33.3
E	19	46.3	7	58.3
VA	1	2.4	0	0
OX	22	53.7	9	75

4.4.3 Antibiotic resistance pattern of total bacterial isolates

Gram negative bacteria were more resistant to most of the antibiotic used than Gram positive bacteria ranging from 47.5% to 91.4% resistant. Among the Gram negative bacteria, amikacin and cefixime offered the lowest and highest resistant antibiotics respectively. Most isolates of Gram positive bacteria were resistant to cefixime 39 (73.6%) and cotrimoxazol 37 (69.8%). Approximately half of the Gram positive bacteria were resistant to erythromycin 26 (49.1%). However, single isolate of Gram positive bacteria was resistant to Vancomycin 1 (1.9%) (Table 15).

Table 15: Antibiotics resistance pattern of total bacterial isolates

Antibiotics	**Gram positive n= 53**		**Gram negative n= 162**		**Total N= 215**	
	No.	**%**	**No.**	**%**	**No.**	%
AK	34	64.2	77	47.5	111	**51.62**
GEN	34	64.2	121	74.7	155	**72.1**
CFM	39	73.6	148	91.4	187	**86.9**
CTX	27	50.9	124	76.5	151	**70.2**
C	16	30.2	105	64.8	121	**56.3**
COT	37	69.8	121	74.7	158	**73.5**
CIP	21	39.6	119	73.5	140	**65.1**
LE	12	22.6	114	70.4	126	**58.6**
PB	-	-	1	0.6	-	**-**
E	26	49.1	-	-	-	**-**
VA	1	1.9	-	-	-	**-**
OX	31	58.5	-	-	-	**-**

4.4.4 Antibiotic resistance pattern of *P. aeruginosa*

Antimicrobial resistance of *P. aeruginosa* recovered from patient's samples was higher than other isolates. *P. aeruginosa* of patients samples were found to be

resistant to most of antimicrobials used. It is interesting to find that the resistance pattern of most of the antibiotics used increased from first to fourth weeks and at the end of fourth week, most of the isolates of *P. aeruginosa* were resistant to all antibiotics except polymyxin B. All isolate of *P. aeruginosa* were resistant to cefixime and cotrimoxazole in contrast, no isolates of *P. aeruginosa* was resistant to polymyxin B at fourth week (Table 16).

Table 16: Antibiotic resistance pattern of *P. aeruginosa*

Antibiotics	Time of sampling (week)				Total
	First n=22 No. (%)	**Second n=22 No. (%)**	**Third n=28 No. (%)**	**Fourth n=26 No. (%)**	**N=98 No. (%)**
AK	12 (54.5)	12 (54.5)	19 (67.9)	20 (76.9)	**63 (64.3)**
GEN	18 (81.8)	17 (77.3)	25 (89.3)	24 (92.3)	**84 (85.7)**
CFM	18 (81.8)	18 (81.8)	27 (96.4)	26 (100)	**89 (90.8)**
CTX	15 (68.2)	16 (72.7)	22 (78.6)	21 (80.8)	**74 (75.5)**
C	13 (59.1)	15 (68.2)	22 (78.6)	22 (84.6)	**72 (73.5)**
COT	14 (63.6)	3 (13.6)	28 (100)	26 (100)	**71 (72.4)**
CIP	16 (72.7)	16 (72.7)	25 (89.3)	21 (80.8)	**78 (79.6)**
LE	18 (81.8)	13 (59.1)	24 (85.7)	21 (80.8)	**76 (77.6)**
PB	0 (0)	0 (0)	1 (3.6)	0 (0)	**1 (1.0)**

4.4.5 Antibiotic resistance pattern of *S. aureus*

Cefixime was the least effective drug against most of *S. aureus* isolated but vancomycin, chloramphenicol and levofloxacin were the best effective drugs against most of *S. aureus* all over the period. The resistant patterns of most antibiotics in the four weeks were not so much pronounced. *S. aureus* was moderately resistant to some antibiotics such as cefotaxime, chloramphenicol, levofloxacin, ciprofloxacin and oxacillin throughout the four weeks. (Table 17).

Table 17: Antibiotic resistance pattern of *S. aureus*

Antibiotics	Time of sampling (week)				Total
	First, n=11	Second, n=13	Third, n=11	Fourth, n=6	N=41
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
AK	8 (72.7)	8 (61.5)	8 (72.7)	3 (50.0)	**27 (65.9)**
GEN	4 (36.4)	10 (76.9)	6 (54.5)	4 (66.7)	**24 (58.5)**
CFM	9 (81.8)	10 (76.9)	8 (72.7)	5 (83.3)	**32 (78.0)**
CTX	5 (45.5)	7 (53.8)	6 (54.5)	2 (33.3)	**20 (48.8)**
C	2 (18.2)	3 (23.1)	2 (18.2)	1 (16.7)	**8 (19.5)**
COT	7 (63.6)	9 (69.2)	7 (63.6)	3 (50.0)	**26 (63.4)**
CIP	4 (36.4)	6 (46.2)	3 (36.4)	3 (50.0)	**16 (39.0)**
LE	3 (27.3)	2 (15.4)	3 (27.3)	1 (16.7)	**8 (19.5)**
VA	1 (9.0)	0 (0)	0 (0)	0 (0)	**1 (2.4)**
OX	4 (36.4)	8 (61.5)	7 (63.6)	3 (50)	**22 (53.7)**

4.4.6 Antibiotic resistance pattern of coagulase negative staphylococci

All isolates of CONS were completely resistant to most of the antibiotics which include amikacin, gentamycin, cefixime, cefotaxime, chloramphenicol and cotrimoxazol in the second week of the culture whereas the resistant pattern decreased on onward weeks. No isolates of CONS was resistant to vancomycin however, cotrimoxazole 11 (91.7%), gentamycin 10 (83.3%) and oxacillin (75%) showed highest degree of resistant (Table 18).

Table 18: Antibiotic resistance pattern of coagulase negative staphylococci

Antibiotics	Time of sampling (week)				Total
	First, n=3	Second, n=2	Third, n=2	Fourth, n=5	N= 12
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
AK	1 (33.3)	2 (100)	0 (0)	4 (80)	**7 (53.3)**
GEN	2 (66.7)	2 (100)	2 (100)	4 (80)	**10 (83.3)**
CFM	0 (0)	2 (100)	1 (50)	4 (80)	**7 (53.3)**
CTX	1 (33.3)	2 (100)	1 (50)	3 (60)	**7 (53.3)**
C	2 (66.7)	2 (100)	1 (50)	3 (60)	**8 (66.7)**
COT	3 (100)	2 (100)	2 (100)	4 (80)	**11 (91.7)**
CIP	1 (33.3)	1 (50)	1 (50)	2 (40)	**5 (41.7)**
LE	0 (0)	0 (0)	2 (100)	2 (40)	**4 (33.3)**
VA	0 (0)	0 (0)	0 (0)	0 (0)	**0 (0)**
OX	2 (66.7)	1 (50)	2 (100)	4 (80)	**9 (75)**

4.4.7 Antibiotic resistance pattern of *Acinetobacter* spp. isolated

Acinetobacter spp. were highly resistant (>80%) to half of the antibiotics during the first week of the culture however they were completely resistant to three-fourth of the antibiotic which include gentamycin, cefotaxime, cefixime, cotrimoxazol, ciprofloxacin and levofloxacin at the end of fourth weeks. Amikacin was the most effective antibiotic for *Acinetobacter* spp. followed by chloramphenicol (Table 19).

Table 19: Antibiotic resistance pattern of *Acinetobacter* spp.

Antibiotics	Time of sampling (week)				Total
	First, n=12	Second, n=10	Third, n=10	Fourth, n=6	N= 38
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
AK	5 (41.7)	2 (20)	2 (20)	2 (33.3)	**11 (28.9)**
GEN	8 (66.7)	6 (60)	6 (60)	6 (100)	**26 (68.4)**
CFM	12 (100)	10 (100)	9 (90)	6 (100)	**36 (94.7)**
CTX	11 (91.7)	9 (90)	9 (90)	6 (100)	**35 (92.1)**
C	3 (25)	4 (40)	3 (30)	4 (66.7)	**14 (36.8)**
COT	11 (91.7)	9 (90)	10 (100)	6 (100)	**36 (94.7)**
CIP	10 (83.3)	7 (70)	8 (80)	6 (100)	**31 (81.6)**
LE	6 (50)	4 (40)	7 (70)	6 (100)	**23 (60.5)**

CHAPTER-V

DISCUSSION

Thermal injury destroys the barrier function of skin, allowing microbial colonization of wounds and even with the use of topical antimicrobials, contamination of wounds is unavoidable (Shankar *et al.*, 2009). It has been estimated that up to 75% of all deaths following burns are related to infection (Sanyal 1998; Shankar *et al.,* 2009). Infection is an important cause of morbidity and mortality in burns. Severe burn patients are very susceptible to infection because of wide exposed raw areas, the presence of necrotic tissue, protein rich exudates, inability of blood to reach the colonized areas of wounds and other host defense mechanisms. The colonization and later invasion of tissues is from patient's normal flora of skin or from gastrointestinal tract or more usually by cross infection (Cheesbrough 2000; Forbes *et al.*, 1998).

In this study, all the patients admitted were above the 15 years old. The findings of this study were that more than half of the patients (54.8%) burnt were below 35 years of age. Ansari and Askarian (2003) observed maximum number of patients between 25 and 34 years age. This finding was consistent with the results of this study in which age group mostly affected by burn injury was between 25 and 34 years.

In this study an increase burn number among female (58.5%) compared to male (41.5%) is observed. This may be attributed to the facts that female in Nepal mostly spend their time in kitchen which increases risks of burn accidents. This is in agreement with similar studies in Iran (Panjeshahin *et al.,* 2001). The result was in contradiction with the studies from Iran (Alaghehbandan *et al.,* 2001) and Palestine (Silfen *et al.,* 2000) in which males were the victims of burns more frequently than females. Other studies also reported that females were the victims of burns more frequently than males (Cutillas *et al.,* 1998, Liu *et al.,* 1998; Mzezewa *et al.,* 1999). This may be due to socioeconomic reasons in our society.

Studying of the site of burn accident on the body of burned patients, it was found that the highest percentage of burns affected in the extremities and genitalia 13 (31%), followed by genitalia 9 (21.4%), head and neck 7 (16.7%) and abdomen 7 (16.7%) while the trunk accounted for the lowest percentage 1%. This results were in contradiction to another study (Silfen *et al.,* 2000) in which the highest percentage of burn happened in head and neck, followed by trunk and extremities. Extremities and genitalia were the most common sites of burn in Nepal which may be due to the cultural habit of wearing more cloths especially females and the cloths made of easily flammable cotton.

Regarding the burn etiology, flame incidence (78.6%), followed by scald (7.1%) and electrical (7.1%) was the main reasons for burn accidents. This may be explained based on the facts that many families of Nepal use poor quality kerosene lamps for lightening; kerosene or open wood fires for cooking and warming as they cannot afford safer heating and lighting devices and stoves. This finding was correlated with other study in Iran (Panjeshahin *et al.,* 2001). Another studies reported that scald burn was the most common cause of burns followed by fire (Haik *et al.,* 2007; Nasser *et al.,* 2009).

The present study found that nearly 64% of patients had less than 40% TBSA burn which was similar to the result that found in Iran where the extent of the burn was less than 40% of the TBSA in 55% of the patients (Panjeshahin *et al.,* 2001) and higher than that observed in occupied Palestine (Silfen *et al.,* 2000) in which 78% of patients had 5% TBSA burn. Higher the TBSA of the patients resulted in higher motility rate. Most patients (90%) with greater than 40% TBSA died in this study. Higher TBSA in this study may be due to lack of fire extinguishing tools in home in Nepal. On Chalise *et al.* (2008) study there was 14% death in Nepal among a total of 50 patients whose mean TBSA was 33.9%.

Despite significant improvement in the survival of burn patients, infectious complications continue to be the major cause of morbidity and mortality (David *et al.*, 1998). Though control of invasive bacterial burn wound infection, strict isolation techniques and infection control policies have significantly minimized the occurrence of burn wound infection (Amin and Kalantar, 2004). This study showed high prevalence of bacterial infections among burn patients as compared to another study from Iran (Askarian and Hosseini, 2004).

In Nepal the studies in time-related changes in burn wound infection were limited. Infection with one or more organisms was present in 87.5% cases in this study. Single organism was isolated in 47.6% and mixed organism in 39.9% and no growth in 12.5% swabs. This result was similar to the study conducted in Bangladesh (Saha *et al.*, 2011). Incidence of infection varies from place to place and country to country due to different therapeutic and preventive policy (Appelgren *et al.*, 2002). The high infections may be due to the cross contamination of the bacteria within or between the patients through contact, air or lack of filtration of air in the burn ward.

The results of this study showed statistically significant relationship between burn sites and infection incidence. All the cultures from the genitalia showed the significant growth of bacteria. Extremities and genitalia exhibited the highest

growth with single (11.9%) and multiple infections (16.7%). Higher growth as well as higher multiple infections around the extremities and genitalia may be due to the contamination of wounds with the microorganisms found in gastrointestinal tract.

In this study, the statistically significant relationship was found between degree of burn and TBSA with culture results. The group with TBSA 20-39% has the highest positive cultures, which is followed by TBSA 40-59%. The high burn percent size and degree increase the chance of pathogenic organism's colonization. In recent studies, it was demonstrated that a significant association between increasing burn size and increasing incidence of pathogenic organisms (Komolafe *et al.,* 2003; Oncul *et al.,* 2009). They also reported that the incidence of invasive-cultures increased as burn size increased. The place was crowded with patients, which may cause direct contact among them. Moreover, there was no special bathroom for patients in the unit so they have to use common the bathrooms. Contact with other patients, cross-infection, contaminated environment, and contaminated air in burn units were the main reasons of increasing infection in such cases.

Third degree burns had more positive cultures (63.7%) comparing to the positive cultures of second degree burns (20.8%) and first degree burn (3%). Polymicrobial infection was highest in third degree while there was no mixed infection in first degree. This could be due to the larger dead tissues or deficient of immunological responses in third degree burn. Also, the antimicrobial drugs given topically and systemically were unable to reach the deeper parts of the tissues due to full-thickness of burn.

Colonisation of burn wounds with microorganisms is almost certain to occur in patients with major burns. Colonisation may occur initially from normal resident flora in skin and throat, like staphylococci; later organisms from the gastrointestinal tract like *E. coli, Klebsiella, Proteus*, etc, may also become

involved (Santucci *et al.*, 2003). In addition, infection may also be transmitted by fomites or the hands of personnel (Bagdonas *et al.*, 2004). Such initial colonisation always carries the potential to cause overt burn wound infection and subsequent invasion of the bloodstream. Therefore, identifying the burn wound isolates and testing their susceptibility to antimicrobial agents are important in the management of burn wound sepsis. Various studies have been conducted to document the spectrum of burn wound isolates and their antibiograms.

In this study, the predominant isolates from burn wound culture were *P. aeruginosa* (45.6%) and *S. aureus* (19.1%). *Acinentobacter* spp (17.7%) was the third most common isolate, followed by CONS (5.6%) This finding that *P. aeruginos*a was the most common isolate coincides with previous reports (Agnihotri *et al.,* 2004; Nasser *et al.*, 2003; Singh *et al.*, 2003) but is in contrast to other studies which report *S. aureus* as predominant organism (Komolafe *et al.*, 2003; Lesseva and Hadjiiski 1996). The difference may be because of the disparity in sampling procedure i.e. in this study there was periodic sampling but that was a cross sectional. Also half of the burn patients were referred from other hospitals after few days stayed.

Analyzing the results of four wound swabs taken from burn wound of each patient it was observed that very high culture positivity 87.5% was found in the samples. It is similar to other study (Agnihotri *et al.*, 2004). By 3rd week most of the samples yielded growth with least number of no growth, number of single growth was highest and Gram negative organisms were predominant. All these changes were gradual from the starting to the end of sample collection. *P. aeruginosa* isolation was maximum in this study in both single (21.9%) and mixed (23.7%) infection (Table 11). These findings were consistent with those of other centers of different countries (Salah *et al.*, 2003; Singh *et al.*, 2003). In this study *S. aureus* was the second most common organism isolated single (9.3%) and mixed (9.8%) followed by *Acinetobacter, Klebsiella* and others. -haemolytic *Streptococcus* was not found in the wounds of any patient. Similarly the complete absence of -haemolytic *Streptococcus* was also reported by some workers (Rahman *et al.*,

1997; Singh *et al.*, 2003). But some other workers found the organism in post burn infection (Gupta *et al.,* 2005; Salah *et al.*, 2003).

There is no doubt that efforts at combating infection in burns must remain a continuing preoccupation, *P. aeruginosa* was the most prevalent single organism (38.6%) colonizing the burn wounds in the first week following burn injuries which became dominant in all subsequent weeks. Gram negative bacteria continued to become the dominant isolates in all four weeks. The isolation of *P. aeruginosa* increased gradually from 38.6% to 57.8% throughout patients' hospital stay of 4th week. However, other Gram negative bacteria decreased with the least isolation at the 4th week. The study results of various worker revealed that the bacteriology of burn infection has been changing from time to time and also the antimicrobial sensitivity pattern.

P. aeruginosa 22 (38.6%), *Acinetobacter* spp 12 (21%) and *S. aureus* 11 (19.2%) were the most prevalent isolates in first week cultures. There was a gradual increase in the number of isolates of *P. aeruginosa* from first week to third week. However, the number of isolates of *S. aureus* and *Acinetobacter* spp remain almost consistence. At the third week, the most frequent organism isolated was *P. aeruginosa* (50%), followed by *S. aureus* (19.6%) and *Acinetobacter* spp (17.8%). This finding is in contrast with the studies done in Turkey and Nepal (Chalise *et al.*, 2008; Erol *et al.,* 2004). Prevalence of *P. aeruginosa* in the burn wards may be due to the fact that organism thrives in a moist environment (Atoyebi *et al.*, 1992).

S. aureus (19.2%) was the third most predominant organism after *Acinetobacter* spp (21%) in the first week which decreased gradually to 13.3% in fourth week of the hospitalization while CONS increased from 5.3% to 11.1% during these periods. Compared to several earlier reports on burn wound colonization and invasive infection, one of the most striking differences is the high frequency of CONS throughout the hospital stay in this study. Even though the pathogenicity

of these microorganisms in burn patients has been questioned, it should be noted that these patients are immunocompromised. Several studies have consistently suggested that CONS should be considered a significant pathogen in both burn patients and critically ill surgical patients (Vindenes and Bjerknes, 1995). In addtion, *Acinetobacter* spp was demonstrated in 17.7% of all isolates in this study. *Acinetobacter* spp can cause infections in patients with burns and these bacteria have been of much concern because of a rapid increase of resistance to a variety of antibacterial drugs.

History indicates that the relative importance and the cyclic pathogenicity of various microorganisms have changed and may be expected to continue changing as systemic and topical antibacterial treatment develops. The pattern of bacterial resistance is important for epidemiological and clinical purposes. This increasing emergence and spread of multidrug resistant bacteria in hospitals in general and burn centers in particular is of great concern and continues to challenge infection control and hospital epidemiology practice worldwide (Cheesbrough 2006; Laura *et al.*, 2010).

In the face of high mortality because of bacteraemia in burned patients, it is important to select antibiotics or combination of antibiotics with broad coverage for the usual pathogens. In a large number of patients this has to be empirical pending results of cultures (Wilkinson and Brent, 1981). As the type of bacteria and their sensitivity vary from place to place analysis of burn wound microbial colonization is to be performed so that the prophylactic and therapeutic regimens could be rationalized. Different types of study on burn wound infection have been carried out in different countries of the world.

Increasing antimicrobial resistance among burn wound isolates is a matter of concern, with limited treatment options available for multidrug-resistant strains (Agnihotori *et al.*, 2004). Each isolate was tested by disc diffusion technique for their susceptibility pattern against commonly used antibiotics in Bir hospital

particularly in the burn center. Gram negative organism causing invasive burn wound infection in burn patients. Gram negative organisms were highly resistant to cefixime (91.4%) followed by ceftriaxone (76.5%), gentamycin (74.7%), cotrimoxazole (74.7%), ciprofloxacin (73.5%) and levofloxacin (70.4%). Amikacin (47.5%) was found to be most effective antimicrobial agent for GNB. The result was similar to the other studies in Brazil (Macedo and Santos, 2005) and Iran (Bojary and Hajia, 2012). Gram positive bacteria exhibited high resistant to cefixime (73.6%) and cotrimoxazole (69.8%) while they were highly sensitive to vancomycin. Cefixime and cotrimoxazole were found to be least effective drugs for both GNB and GPB rendering them ineffective for use.

A high level of drug resistance was seen among gram negative isolates especially *P. aeruginosa*. *P. aeruginosa* was highly resistant to cotrimoxazol, chloramphenicol, cefixime, ciprofloxacin, levofloxacin and gentamycin. Polymyxin B was found to be highly sensitive (99%) against *Pseudomonas*. Thus it is considered as multidrug resistant to the commonly used drugs in the burn unit. Similar high resistance of *Pseudomonas* has been reported in other studies (Hunt and Purdue, 1992*). P. aeruginosa* were highly resistant to most antibiotics from 1st week of culture which increased gradually and at the end of 4th week they became completely resistant to cefixime and cotrimoxazole with maximum drugs resistant. Hence, *Pseudomonas* was responsible for delayed in recovery as well as failure of treatment therapy. The subsequent development and use of broad-spectrum antibiotics effective against *Staphylococcus* led to the emergence of gram negative organisms, particularly *P. aeruginosa*, as the predominant organism causing invasive burn wound infections in burn patients (Al-Akayleh 1999).

Also, other non-enterobacteriaceae like *Acinetobacter* spp showed high levels of resistance to most antibiotics, as also shown in another study (Guggenheim *et al.*, 2009). Almost all isolates of *Acinetobacter* spp were completely resistant to most antibiotics used at the fourth week of culture. During the 1st week half of the

antibiotics were effective against *Acinetobacter* spp. Since burn patients require longer hospital stay they become susceptible to hospital acquired infection by Gram negative bacteria which make their infection highly resistant to antibiotics.

S. aureus isolates from burn wounds exhibited high resistant against cefixime (78%), amikacin (65.5%) and cotrimoxazol (63.4%). This was similar to report elsewhere (Kehinde *et al.*, 2003). Vancomycin proved to the most effective antibiotic exhibiting 97.6% sensitivity to *S. aureus*. Other antibiotics least resistant to *S. aureus* were levofloxacin (19.5%), chloramphenicol (19.5%) and ciprofloxacin (39%). CONS were highly resistant to cotrimoxazole (91.7%), gentamycin (83.3%) whereas no isolate of CONS was resistant to vancomycin, this was similar to the previous study (Sloos *et al*., 2000). CONS exhibited higher resistant to oxacillin in compare to *S. aureus*. In addition, they offered least resistant to levofloxacin (33.3%) and ciprofloxacin (41.7%).

Most of the isolates of *S. aureus* were sensitive to majority of antibiotics used throughout the study period. There were not significant changes in the resistant pattern of antibiotics of *S. aureus*. This may be the reason of decreasing the isolation of *S. aureus* from 2nd week to 4th week of culture. However, the resistant pattern of CONS increased rapidly, which resulted in, complete resistant of six antibiotics in second week of culture and then remained fluctuated through the study period. This is why the isolation of CONS throughout the study remains consistent with some increased in 4th week.

CHAPTER-VI
CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Gram negative bacteria were the dominating bacteria all over the study period especially *P. aeruginosa*. It was observed that bacteria, which were isolated from burn patients, were multidrug resistant. Furthermore, *P. aeruginosa* was highly

resistant to most antibiotics used. Amikacin was the drug of choice for most Gram negative bacteria and vancomycin was found to be susceptible drug for Gram positive organisms (*S. aureus* and CONS). In conclusion, present observations seem to be helpful in providing useful guidelines for choosing effective therapy against isolates from burn patients. Regular surveillance of burn wound organisms and their antimicrobial resistance patterns will help in formulating empirical antibiotic therapy and reducing mortality from septic events.

6.2 RECOMMENDATIONS

i. Regular surveillance of bacterial profile and their antimicrobial susceptibilities should be encouraged to help guide first line therapy for burns related sepsis.

ii. Similarly, the pattern of bacterial sensitivities is subject to frequent changes. Its regular assessment is important for clinical and epidemiological purposes.

iii. The pattern of antimicrobial resistance of isolated bacteria observed in this study is very high. This could be an important ground for the development and spread of antimicrobial resistant bacteria that may have danger on their life.

iv. This study focused on the aerobic bacterial isolation of burn wounds. The study of fungi and anaerobic bacteria in burn wounds is also important for the wide coverage of etiological agents and their antibiotic resistant patterns.

v. Serotyping and genotyping of bacterial isolates especially multidrug resistant organisms was not done in the present study. Therefore, serotyping and genotyping analysis of the isolates using suitable techniques is essential in the future.

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APPENDIX-I

PERFORMA

CLINICAL AND MICROBIOLOGICAL PROFILE OF PATIENTS

A. Clinical Profile

Name:

Age:

Sex:

Address:

Bed no.:

Consent:

Questionnaires:

1. **Cause of burn?**

Flame: ………… Scald: ………….. Electrical: ……………

Acid: ………….. Lightening: ………….. Others: ………………

2. **Sites of burn?**

Head and neck: ……….. Extremities: ………….. Trunk: ………………..

Genitilia: ………….. Abdomen: ……………

Extremities and genitilia:………. Other: …………..

3. **Total body surface area of burn (burn %)?** ………………..

4. **Degree of burn?**

First degree: ……… Second degree: …… Third degree: …………

5. **Condition of the patients?**

Conscious: ……… Subconscious: ………… Unconscious: …………

6. **Pain degree?**

Present: ………….. Absent: ……………..

7. **Fever?**

Present: ………….. Absent: ………………

8. **Whether any type of medication used after injury?**

Yes: …………… No: ……………..

9. Observation of burn wound:

Redness	yes: ………	No: ………..
Swelling	yes: ………	No: ……….
Pus discharging	yes: ………	No: ………..

B. Microbiological profile

DAY I

Direct microscopic evaluation

S.N	Gram staining	Results
i.	Gram positive cocci	
ii.	Gram positive bacilli	
iii.	Gram negative bacilli	
iv.	Gram negative cocci	
v.	Pus cells/ WBC	

DAY II

Culture of specimen and colonial Characteristics

S.N	Media used	Characteristics of isolated colonies
i.	NA	
ii.	MA	
iii.	BA	

DAY III

Biochemical Tests

S.N	Biochemical tests employed	Result
i.	Catalase test	
ii.	Oxidase test	
iii.	Coagulase test	
iv.	OF test	
v.	SIM test	
vi.	MR test	
vii.	VP test	
viii.	Citrate test	

ix.	TSI test	
x.	Urease test	

DAY IV

Antibiotic sensitivity profile

S.N	**Antibiotics used**	**Zone of inhibition (ZOI)**	**Remarks**
i.	Amikacin		
ii.	Gentamicin		
iii.	Chloramphenicol		
iv.	Cefixime		
v.	Ceftotaxime		
vi.	Cotrimoxazole		
vii.	Ciprofloxacin		
viii.	Levofloxacin		
ix.	Erythromycin		
x.	Vancomycin		
xi.	Polymyxine B		

Checked by:

C. List of materials

1. Equipments

Autoclave

Burner

Microscope

Hot air oven

Incubator

Refrigerator

Glasswares: petriplates, glass tubes, glass slides, glsss rod etc.

2. Microbiological media (Hi-Media)

Nutrient Agar

Nutrient broth

Mac Conkey Agar

Blood Agar

Muller Hinton Agar

Simmons Citrate agar

TSI Agar

MRVP Broth

Urease Broth

SIM Media

3. Chemicals/Reagents

Catalase reagent (3% H_2O_2)

Oxidase reagent (1% Tetramethyl p-phenylene diamine dihydrochloride)

Kovac's reagent

Barritt's reagent (40% KOH, 5% α-naphthol in a ratio of 1:3)

Crystal violet

Gram's iodine

Acetone-alcohol

Safranine

Blood plasma

Methyl red

4. Antibiotics Discs (Hi-Media)

Amikacin (30mcg)

Gentamicin (30mcg)

Chloramphenicol (30mcg)

Cefixime (5mcg)

Cefotaxime (30mcg)

Cotrimoxazole (25mcg)

Ciprofloxacin (5mcg)

Levofloxacin (5mcg)

Erythromycin (15mcg)

Vancomyin (30mcg)

Polymyxine(300mcg)

5. Miscellaneous

Cotton swabs, Distilled water, Lysol, Inoculating loop, Inoculating wire, Immersion oil, dropper, etc.

APPENDIX-II

A. Composition and preparation of different types of culture media (Hi-Media)

(Note: All compositions are given in grams per liter and at 25°C temperature.)

1. Nutrient Agar (NA)

	Gram
Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Agar	1.5
Final P^H	7.4=0.2

2.8 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121°C for 15 minutes.

2. Nutrient broth (NA)

Peptone	5.0
Sodium chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Final P^H	7.4=0.2

1.3 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121°C for 15 minutes.

3. MacConkey Agar (MA)

Pancreatic digest of gelatin	17.0
Peptone	3.0
Lactose	10.0
Sodium Chloride	5.0
Bile salt	1.5
Agar	13.5
Neutral red	0.03
Crystal Violet	0.001
Final P^H	6.9-7.3

5.5gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121°C for 15 minutes.

4. Blood Agar (BA)

Composition of Blood Agar base	15.0	
Protease peptone		2.5

Liver digest	5.0
Yeast Extract	5.0
Sodium Chloride Agar	15.0
Final P^H	7.4

Blood agar base medium was prepared and autoclaved at 121°C for 10 minutes. It is then cooled down to 48°C and blood (7-10%) is added aseptically and mixed thoroughly. About 18-20 ml. of the media was then poured on Petri-plates. If bubbles appear in the poured plates, a flame is passed over the bubbled before the media sets.

5. Muller Hinton Agar

Beef infusion Broth	300.0
Casein Acid Hydrolysate	17.0
Starch	1.0
Agar	17.0
Final P^H	7.0=0.2

3.8 gms of media was suspended in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121°C for 15 minutes. It was then poured while at 45-48°C into sterile petriplates in 25 ml quantity.

6. Peptone water

Peptone	10
NaCl	5
Final P^H	7.2=0.2

15 gm was dissolved in 1000 ml distilled water and was sterilized by autoclaving at 15 lbs pressure at 121° C for 15 minutes.

B. Composition and preparation of different types of biochemical media

1. MR-VP medium

Buffered peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0
Final P^H	6.9

1.7 gms of media was dissolved in 100 ml distilled water and heated to dissolve the media. The media was distributed in the amount of 5ml each into several test tubes and sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes.

2. Simmons Citrate Agar

Magnesium sulphate	0.2
Mono-ammonium phosphate	1.0
Dipotassium phosphate	1.0

Sodium Citrate	2.0
Sodium Chloride	5.0
Bromothymol Blue	0.08
Agar	15.0
Final P^H	6.8=0.2

2.42 gms of media was dissolved in 100 ml distilled water and heated to dissolve the media. The media was distributed in the amount of 5ml each into several test tubes and sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was then allowed to cool in standing position to prepare slant.

3. Triple Sugar Iron (TSI) Agar

Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Beef Extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium chloride	5.0
Sodium Thiosulphate	0.3
Phenol Red	0.024
Agar	12.0
Final P^H	7.4=0.2

6.5 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was distributed in the amount of 5 ml each into several test tubes and sterilized by autoclaving at 15 Ibs pressure at 121°C for 15 minutes. The media was allowed to set in slope form to from a slant with butt 1 inch long.

4. Urea Broth Base

Monopotassium Phosphate	9.1
Dipotassium Phosphate	9.5
Yeast Extract	0.1
Phenol red	0.01
Final P^H	6.8=0.2

1.85 gms of media was dissolved in 95 of distilled water and hence to dissolve the media. The media was sterilized by autoclaving at 15 Ibs pressure at at 121°C for 15 minutes. It was then cooled to 55°C and 5ml of sterile 40% urea solution was aseptically added. The contents were then mixed well and distributed into sterile test tube.

5. Sulphide Indole Motility medium (SIM)

Beef extract	3
Peptone	30
Peptonized iron	0.2
Sodium thiosulphate	0.025
Agar	3
Final P^H	7.3=0.2

36 gm of the medium was dissolved in 1000 ml distilled water and distributed into tubes. The medium was sterilized by autoclaving at 15 lbs pressure at 121° C for 15 minutes.

C. Composition and preparation of different reagents

1. Gram staining reagents

I. Crystal violet Gram stain

Crystal violet	20g
Ammonium oxalate	9g
Ethanol or methanol, absolute	95ml
Distilled water	1 liter

Preparation:

i. Crystal violet is weighed and transferred to a clean bottle and absolute ethanol is added and mixed until dye is completely dissolved.

ii. Ammonium oxalate is weighed and dissolved in about 200 ml of distilled water. Then it was added to the stain and total volume is made 1 liter by adding distilled water and mixed well.

II. Iodine Solution

Potassium iodide	1.5g
Iodine	1.0g
Distilled water	150ml

Preparation:

i. Potassium iodide is weighed and transferred to a clean bottle

ii. 30-40 ml of distilled water is added to Potassium iodide and mixed until it is fully dissolved.

iii. Iodine is weighed and added to potassium iodide solution and mixed well.

iv. Final volume is made 150ml by adding distilled water and mixed well.

III. Acetone-alcohol decolorizer

Acetone	500ml

Ethanol (absolute)	475ml
Distilled water	25ml

To 25 ml distilled water, 475 ml of absolute alcohol was added, mixed and transferred into a clean bottle. Then 500 ml acetone was added and mixed well.

IV. Counterstain solution

Safranine	10gm
Distilled water	1 lit

In a piece of clean paper, 10 gm of safranine was weighed and transferred to a clean bottle. Then 1 lit. distilled water was added to the bottle and mixed well until safranine dissolves completely.

2. Test reagents

I. Kovac's Reagent

Para-Dimethylaminobenzaldehyde	2g
Isoamyl alcohol	30ml
Hydrochloric acid	10ml

Preparation:

i. Para-Dimethylaminobenzaldehyde is weigh and dissolved in Isoamyl alcohol.
ii. Concentrated Hydrochloric acid is added and mixed well.

II. Methyl Red Solution

Methyl red	0.05g
Ethanol, absolute	28ml
Distilled water	22ml

Preparation:

Methyl red was weigh and dissolved in ethanol and water.

III. Voges-Proskauer reagent (Baritt's reagent)

i. VP reagent A (To make 100 ml)

α-napthol	5gm
Ethanol, absolute	100ml

Preparation:

To 25 ml distilled water 5 gm α-napthol was dissolved and transferred to a clean brown bottle. Then the final volume was made 100ml by adding distilled water.

ii. VP reagent B (To make 100 ml)

Potassium hydroxide	40gm
Distilled water	100ml

Preparation:

To 25 ml distilled water, 40 gm of KOH was dissolved and transferred to a clean brown bottle and final volume was made 100 ml by adding distilled water.

IV. Catalase reagent (To make 100 ml)

Hydrogen peroxide solution	3ml
Distilled water	97ml

Preparation:

To 97 ml distilled water, 3 ml of hydrogen peroxide solution was added and mixed well.

V. Oxidize reagent (To make 100 ml)

Tetramethyl P-Phenylenediamine dihydrochloride (TPD)	0.1gm
Distilled water	10ml

Preparation:

This reagent was made by dissolving 0.1 gm TPD in 10 ml distilled water. To that solution strips of Whatman's no. 1 filter paper were soaked and drained for 30 seconds. Then these stripes were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

VI. Hydrochloric acid (1M/L HCL) solution (To make 100 ml)

HCL (conc.)	8.6ml
Distilled water	100ml

Preparation:

8.6 ml of conc. HCL was added to 25 ml distilled water into a clean bottle. Then the final volume was made 100 ml by adding distilled water.

D. Procedure of different biochemical tests

1. Catalase test

i. A small amount of isolated colony from pure culture was transferred to the surface of clean dry glass slide.
ii. A drop of 3% H_2O_2 was placed onto the inoculum.
iii. The evolution of oxygen bubbles was recorded immediately.
iv. The slide was then discarded into a disinfectant.

2. Oxidase test

i. A piece of filter paper was placed in a clean petridish and 2-3 drops of freshly prepared oxidase reagent was added.
ii. Using a glass rod, a colony of test organism was smeared on the filter paper.
iii. It was observed for the development of blue purple colour within a few seconds.

3. Coagulase test

I. Slide test (to detect bound coagulase)

i. A drop of physiological saline was placed on end of a slide and colony of test organism was emulsified in each of the drops to make two thick suspensions.
ii. A drop of plasma was added to one of the suspensions and mixed gently. It was looked for clumping of the organism within 10 seconds. But no plasma was added to second suspension. This is used to differentiate any granular appearance of the organism from the coagulase clumping.

II. Tube test (to detect free coagulase)

i. The plasma was diluted 1 in 10 physiological saline (mixing 0.2 ml of plasma with 1.8 ml of saline)
ii. 3 tubes were taken and labeled as:
    a. T = test organism (18-24 hour broth culture),
    b. P = Positive control (*S. aureus* broth culture),
    c. N = Ne gative control (sterile broth).
iii. 0.5 ml of diluted plasma was pipetted into each tube.
iv. About 5-5 drops each of test organism, S. aureus culture, and sterile broth was added to the tubes labeled 'T', 'P' and 'N' respectively.
v. After mixing gently, 3 tubes were incubated at 37°C. It was examined for clotting after 1 hour. If no clotting occurs tubes were examined at 30 minutes intervals for up to 6 hours.

4. Motility test

i. The test organism was inoculated by stabbing in the medium with a sterile straight wire.
ii. Then the medium was incubated at 37^oC for 24 hours.
iii. After incubation, the appearance of spreading turbidity from the stab-line or the turbidity throughout the medium is the indication of the positive motility test.

5. Hydrogen sulphide (H_2S) production test

i. The test organism was inoculated by stabbing in the medium with a sterile straight wire and the medium was incubated at 37^oC for 24 hours.
ii. After incubation, a black coloured iron-containing precipitate in the medium indicates the production of H_2S gas.

6. Indole test

i. Using a sterile straight wire, sterile SIM medium was inoculated with pure culture of test organism and incubated at 37^oC for 24 hours.
ii. Then few drops of Kovac's reagent was added into the medium and observed for the development of pink red colour.

7. Methyl red (MR) test

i. A colony of test organism was inoculated in 0.5 ml of sterile MR-VP broth.
ii. After overnight incubation at 37^oC, a drop of methyl red solution is added.
iii. A positive MR test is shown by the appearance of a bright red colour, indicating acidity, while a yellow or orange colour is indicative of negative test.

8. Voges-Proskauer (V-P) test

i. The test organism was inoculated in the broth medium and incubated at 37^oC for 24 hours.
ii. After incubation, 1 ml of 40% potassium hydroxide and 3 ml of 0.5% solution of α-naphthol in absolute ethanol was added.
iii. A positive reaction is indicated by the development of a pink colour in 2-5 minutes, becoming crimson in 30 minutes.

9. Citrate utilization test

i. Using a sterile straight wire, the test organism was inoculated by streaking on the slope of the medium and incubated at 37°C for 24 hours.
ii. The appearance of growth on the streaked line and change in colour of the medium from green to blue was the indicator of the positive citrate test.

10. Triple Sugar Iron (TSI) test

i. Using a sterile straight wire, the test organism was first stabbed down the centre of the agar butt and then streaked on the slanted surface.
ii. The TSI agar tubes were incubated at 37°C for 24 hours.
iii. The tubes were observed for gas formation, fermentation of sugars, and H_2S gas production.

Interpretation of results

From this test three different results can be drawn.

Production of gas: indicated by the cracking of the media.

Production of H2S: indicated by the formation of black coloured iron containing precipitate in the butt.

Fermentation of sugars: Fermentation pattern is shown in table 1:

Table 1: Interpretation of TSI agar test

Slant/butt	Colour	Utilization
Alkline/acid	Red/Yellow	Glucose only fermented; peptone utilized
Acid/acid	Yellow/Yellow	Glucose fermented; lactose and/or sucrose fermented
Alkaline/alkaline	Red/Red	No fermentation of glucose, lactose or sucrose; peptone utilized

(Source: Collee *et al*, 1996)

11. Urea hydrolysis test (Urease test)

i. Heavy inoculums of test organism was inoculated into Christensen's urea broth and incubated at 37°C for 24 hours.
ii. The change in the colour of the medium into pink indicates urease positive test.

APPENDIX-III

A. Antibiotic disc used and procedure of susceptibility test

1. Antibiotic disc used

Antibiotics used	symbol	Disc Content (mcg)	Diameter of Zone of inhibition (mm)		
			Resistant	Intermediate	Sensitive
Amikacin	AK	30	14	15-16	17
Gentamicin	GEN	30	12	13-14	15
Chloramphenicol	C	30	12	13-17	18
Cefixime	CFM	5	15	16-18	19
Cefotaxime	CTX	30	14	15-22	23
Cotrimoxazole	COT	25	10	11-15	16
Ciprofloxacin	CIP	5	15	16-20	21
Levofloxacin	LE	5	13	14-16	17
Erythromycin	E	15	13	14-22	23
Vancomycin	VA	30	14	15-16	17
Polymyxine B	PB	300	11	-	12
Oxacillin	OX	1	10	11-12	13

2. Procedure of sensitivity test (Kirby-Bauer's Disc Diffusion Method)

i. Preparation of inoculum: For inoculum preparation, 3-4 pure culture colonies were transferred into nutrient broth and incubated at 37°C for 2-4 hours to obtain turbidity.

ii. Inoculation: A sterile cotton swab was dipped into the inoculum and rotated by pressing it against the upper inside wall of tube to remove excess inoculum. Then it was allowed to dry for 10 minutes.

iii. Application of discs: With the help of flamed forceps, disc were carefully placed on the agar surface at least 15 mm away from the edge and pressed lightly to make contact with the surface of the medium and plates were allowed to stand at room temperature for 30 minutes. (prediffusion time).

iv. Incubation: The plates were incubated at 37°C for 24 hrs.

v. Interpretation of result: After incubation, diameter of ZOI was measured.

APPENDIX-IV

A. Antibiotic resistant pattern of isolated bacteria from burn wound infections

1. Antibiotic resistance pattern of *Klebsiella* spp.

Cotrimoxazol was the most effective drug for *Klebsiella* spp. during the first week of the culture however complete resistant developed on onward weeks. *Klebsiella* spp. were completely resistant to all antibiotics except amikacin in the third week. Amikacin was found to be the most effective drug for the whole period.

Table 20: Antibiotic resistance pattern of *Klebsiella* spp.

Antibiotics	Time of sampling (week)				Total N=8
	First, n=3	Second, n=3	Third, n=1	Fourth, n=1	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
AK	2 (66.7)	0 (0)	0 (0)	0 (0)	**2 (25)**
GEN	1 (33.3)	3 (100)	1 (100)	0 (0)	**5 (62.5)**
CFM	2 (66.7)	3 (100)	1 (100)	1 (100)	**7 (87.5)**
CTX	1 (33.3)	3 (100)	1 (100)	1 (100)	**6 (75)**
C	2 (66.7)	3 (100)	1 (100)	1 (100)	**7 (87.5)**
COT	0 (0)	3 (100)	1 (100)	1 (100)	**5 (62.5)**
CIP	1 (33.3)	2 (66.7)	1 (100)	1 (100)	**5 (62.5)**
LE	1 (33.3)	2 (66.7)	1 (100)	1 (100)	**5 (62.5)**

2. Antibiotic resistance pattern of *E. coli*

All isolates of *E. coli* were completely resistant to cefotaxime, ceftriaxone and chloramphenicol for whole periods of culture except in the third week which were 66.7% resistant each. Amikacin 1 (14.3%) and levofloxacin 2 (28.6%) were found to be least resistant to the organism.

Table 21: Antibiotic resistance pattern of *E. coli*

Antibiotics	Time of sampling (week)				Total N=7
	First, n=2	Second, n=1	Third, n=3	Fourth, n=1	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
AK	1 (50)	0 (0)	0 (0)	0 (0)	**1 (14.3)**
GEN	1 (50)	1 (100)	3 (100)	0 (0)	**5 (71.4)**
CFM	2 (100)	1 (100)	2 (66.7)	1 (100)	**6 (85.7)**
CTX	2 (100)	1 (100)	2 (66.7)	1 (100)	**6 (85.7)**
C	2 (100)	1 (100)	2 (66.7)	1 (100)	**6 (85.7)**
COT	1 (50)	1 (100)	1 (33.3)	1 (100)	**4 (57.1)**
CIP	1 (50)	0 (0)	1 (33.3)	1 (100)	**3 (42.9)**
LE	1 (50)	0 (0)	0 (0)	1 (100)	**2 (28.6)**

3. Antibiotic resistance pattern of *Citrobacter* spp.

No *Citrobacter* spp. were isolated in third and fourth week of cultures. All three isolates of *Citrobacter* spp. during first and second week of culture were completely sensitive to amikacin and gentamycin. Miscellaneous resistant patterns were observed in other antibiotics during first and second week of culture.

Table 22: Antibiotic resistance pattern of *Citrobacter* spp.

Antibiotics	Time of sampling (week)				Total N=3
	First, n=1	Second, n=2	Third, n=0	Fourth, n=0	
	No. (%)	No. (%)	No. (%)	No. (%)	
AK	0 (0)	0 (0)	-	-	**0 (0)**
GEN	0 (0)	0 (0)	-	-	**0 (0)**
CFM	1 (100)	1 (50)	-	-	**2 (66.6)**
CTX	1 (100)	0 (0)	-	-	**1 (33.3)**
C	0 (0)	1 (50)	-	-	**1 (33.3)**
COT	1 (100)	1 (50)	-	-	**2 (66.7)**
CIP	1 (100)	0 (0)	-	-	**1 (33.3)**
LE	0 (0)	1 (50)	-	-	**1 (33.3)**

4. Antibiotic resistance pattern of *Enterobacter* spp.

The two *Enterobacter* spp. were isolated in first and second week of culture. The most effective antibiotics were found to be amikacin and chloramphenicol whereas they were completely resistant to cefixime. No *Enterobacter* spp. was isolated in third and fourth weeks of culture.

Table 23: Antibiotic resistance pattern of *Enterobacter* spp.

Antibiotics	Time of sampling (week)				Total N=2
	First, n=1 No. (%)	Second, n=1 No. (%)	Third, n=0 No. (%)	Fourth, n=0 No. (%)	
AK	0 (0)	0 (0)	-	-	**0 (0)**
GEN	0 (0)	1 (100)	-	-	**1 (50)**
CFM	1 (100)	1 (100)	-	-	**2 (100)**
CTX	1 (100)	0 (0)	-	-	**1 (50)**
C	0 (0)	0 (0)	-	-	**0 (0)**
COT	0 (0)	1 (100)	-	-	**1 (50)**
CIP	1 (100)	0 (0)	-	-	**1 (50)**
LE	0 (0)	1 (100)	-	-	**1 (50)**

5. Antibiotic resistance pattern of *Proteus* spp.

Proteus spp. was completely sensitive to most of the antibiotics like amikacin, gentamycin, ciprofloxacin and levofloxacin. Resistant pattern gradually increased to cefixime in which the organism became completely at the third week of the culture. No *Proteus* spp. was isolated in the fourth week.

Table 24: Antibiotic resistance pattern of *Proteus* spp.

Antibiotics	Time of sampling (week)				Total N=6
	First, n=2 No. (%)	Second, n=3 No. (%)	Third, n=1 No. (%)	Fourth, n=0 No. (%)	No. (%)
AK	0 (0)	0 (0)	0 (0)	-	**0 (0)**
GEN	0 (0)	0 (0)	0 (0)	-	**0 (0)**
CFM	1 (50)	2 (66.7)	1 (100)	-	**4 (66.7)**
CTX	1 (50)	0 (0)	0 (0)	-	**1 (16.7)**
C	1 (50)	0 (0)	0 (0)	-	**1 (16.7)**
COT	0 (0)	2 (66.7)	0 (0)	-	**2 (33.3)**

CIP	0 (0)	0 (0)	0 (0)	-	**0 (0)**
LE	0 (0)	0 (0)	0 (0)	-	**0 (0)**