Chapter -1

Introduction
Burn causes damage to intact skin which is extremely painful and takes a very long time to heal (Shakespeare, 2001). Different burns come from a variety of sources such as fire, heat, electricity, radiation or caustic chemicals. Thermal burns are the most common type of burns (Groohi et al., 2002). The level of burns varies according to the severity of injury. The factors that contribute to wound complications are both the size and depth of the wound (Alaghehbandan et al., 2001; Panjusthahin et al., 2001). Burn depth is usually categorized into first-degree (superficial, involving only the epidermis), second-degree (partial thickness, involving both epidermis and dermis) and third-degree (full thickness, through the epidermis, dermis and into fat) (Palmieri and Greenhalgh, 2002). In India, about 600,000 people suffer from burns annually. More than 50,000 are treated in hospitals and approximately 10,000 succumb to thermal injury (Subramanyan, 1998).

Skin is the first line of defense against invading microbes capable of recruiting inflammatory cells to enable neutralization and clearance of bacteria and fungi (Steinstraesser et al., 2005). The skin serves as an effective physical barrier because of its laminar structure which renders it relatively resistant to abrasion, puncture, and percutaneous absorption (Montagna, 1981; Jablonski, 2004). However, burn results in damage and destruction of skin layers. Severe injury, particularly thermal injury impairs host defense mechanism against invading microorganisms as it leads to suppression of nearly all aspects of immune responses (O’ Sullivan and O’ Connor, 1997; Steinstraesser et al., 2002). Post burn serum levels of immunoglobulins, complement and fibronectin are reduced. Chemotaxis, phagocytic and killing functions of neutrophils, monocytes and macrophages are impaired. Granulocytopenia is common following burn injury Lymphocyte proliferation and antibody production is impaired (Patenaude et al., 2005). Hyperproduction of inflammatory cytokines and overstimulation of immune cells during inflammatory responses are potential mechanisms involved in immune suppression (Gennari and Alexander, 1995;
The most common organisms isolated from burn wound culture and biopsies of total invasive burn wound infections include *Pseudomonas aeruginosa* (21.6%), *Klebsiella pneumoniae* (15.2%), *Escherichia coli* (13.6%), *Staphylococcus aureus* (13.2%), coagulase negative Staphylococci (11.6%), *Streptococcus pyogenes* (8.3%), *Enterobacter* spp. (6.6%), *Enterococcus faecalis* (5.9%), *Proteus* spp. (3.19%) *Acinetobacter* spp. (1.1%), *Salmonella senftenberg* (0.8%) and others (3%) (Nasser et al., 2003). Gram negative organisms are the most common isolates from burn wound infections and have greater motility, possess many antibiotic resistance mechanisms, have the ability to secrete collagenases, proteinases, lipases and elastases, enabling them to proliferate and penetrate into the subeschar space. Among gram-negative bacteria, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are the two most important organisms (Rumbaugh et al., 1999; Livemore, 2004). *Pseudomonas aeruginosa* is a gram negative, versatile, opportunistic pathogen found along with other *Pseudomonas* spp. as part of the normal flora of the human skin (Larson et al., 2002; Mooij et al., 2007; Moreau-Marquis et al., 2008). *P. aeruginosa* is an opportunistic human pathogen that infects individuals with weakened immune system such as hospitalized patients and those suffering from severe burns or other traumatic skin damage (Stieritz and Holder, 1975; Vasishta et al., 1991; Richard et al., 1994; Budzik et al., 2004; McVay et al., 2007). *Klebsiella pneumoniae*, a facultative anaerobe, non-motile, non-sporing, gram-negative bacilli, are ubiquitous and may colonize the skin, pharynx, or gastrointestinal tract in humans. Nosocomial *Klebsiella* infections are caused mainly by *Klebsiella pneumoniae*, a successful opportunistic pathogen, is among the most common gram-negative bacteria encountered by physicians worldwide (Podschun and Ullmann, 1998, Ko et al., 2002). The immunocompromised people who are hospitalized and have been associated with various ailments such as urinary tract infections, septicaemia, respiratory tract infections and diarrhea are the main target of these bacteria (Subha and Ananthan, 2002, Wu et al., 2007). It
institute Paris, concluded that some mysterious agent was capable of killing bacteria (Summers, 1999; Summers, 2001). Scientists harnessed nature's way of tackling antibiotic resistant bacteria. However, the use of bacteriophages as therapeutic agents was abandoned during 1940's and 1950's due to certain limitations inherent in phage physiology (e.g. narrow host range, rapid clearance from the body), technological limitations in the era (e.g. poor understanding of host pathogenesis, lysogeny not yet discovered) and inadequate scientific methodologies used by workers at that time (Soothill and Barrow, 1997; Bull et al., 2002). Moreover, in 1940, success with antibiotics was so tremendous that phage therapy was discarded at that time. Phage therapy remained mostly inactive until the early 1980 when increasing concerns over the antibiotic resistant bacteria prompted researchers to reconsider bacteriophage therapy as an alternative to antibacterial agents (Lopez et al., 2004).

The ability of bacteriophages to rapidly kill or lyse infected bacteria, their specificity and their proven clinical safety makes them ideal, robust, safe, and effective self-replicating antibacterial drugs of the future (Carlton et al., 2005; Hanlon, 2007). Lytic phages, which kill their host, may offer an alternative strategy to combat bacterial infections (Westwater et al., 2003). Phages are amongst the most abundant living entities on earth playing important role in maintaining the natural abundance and distribution of microorganisms (Dabrowska et al., 2005). Phages are considered to be very safe for therapeutic use as they possess some unique advantages over antibiotics (Clark and March, 2006; Skurnik and Strauch, 2006). Phages are capable of replicating by themselves whereas antibiotics decrease in concentration from the time they are administered. Unlike antibiotics, which disrupt the normal microflora, leading to serious secondary infections, phages are highly specific and do not harm normal flora. Phages replicate at the site of infection and are thus, available where they are needed most. No side effects have been described with phages; while with antibiotics, multiple side effects have been reported (Inal, 2003; Theil, 2004;
The present study was planned with the following aims and objectives:

1. Isolation, purification and selection of bacteriophages showing lytic activity against *Klebsiella pneumoniae* B5055 and *Pseudomonas aeruginosa* PAO.

2. Characterization of selected phages.

3. Classification of selected phages on the basis of morphology, structural proteins and genetic material.

4. Establishment of burn wound infection in mice with selected strains of *Klebsiella pneumoniae* B5055 and *Pseudomonas aeruginosa* PAO.

5. To evaluate the efficacy of selected phages alone or in cocktail for treating post burn bacterial infection.

6. Topical treatment of burn wound using different variety of ointment/creams impregnated with lytic phage(s).

7. Comparison of phage treatment with currently available treatment strategies for trauma cases.