1.0 INTRODUCTION

1.1 General features of the genus *Staphylococcus*

*Staphylococci* are gram positive bacteria, 0.5-1.5 µm in diameter and appear as individual coccius, in pairs, tetrads or in grape like clusters. The genus *Staphylococcus* has 41 species many of which colonize human body (Resch *et al.*, 2008). *S. aureus* and *S. epidermidis* are two species which are well characterized and have been studied in detail. These organisms are non-motile, non-spore forming, facultative anaerobes that grow by aerobic respiration or by fermentation. Their growth is optimum at 37°C but yellow pigment formation can be seen at room temperature (20-25°C). Colonies of *S. aureus* are round, smooth with entire margin, opaque, raised and glistening on solid media. Most species of this genus require complex nutrition, but in general, they require nitrogen as organic source, which is supplied by amino acids (Wilkinson, 1997). *Staphylococci* have the ability to tolerate high salt concentration (Kloos and Lambe, 1991). Members of the genus *Staphylococcus* are catalase positive and oxidase negative. The catalase test differentiates *Staphylococci* from *Streptococci*. These genera also differ in the composition of their cell walls. Pathogenic *Staphylococci* such as *S. aureus* can generally be identified by their ability to produce coagulase enzyme. The coagulase negative strains of *Staphylococcus* genus (CoNS) are commensals or saprophytic but some of them can cause opportunistic infections (Murray *et al.*, 2002).

1.2 Disease conditions caused by *Staphylococcus aureus*

*S. aureus* produces a wide range of clinical conditions in humans such as impetigo, *Staphylococcal* food poisoning, Staphylococcal scalded skin syndrome (SSSS) and toxic shock syndrome (TSS). Other conditions may result from the proliferation of the bacteria leading to abscess formation and tissue destruction (Fischetti *et al.*, 2000). Disease conditions due to *S. aureus* range from minor infections of skin to serious post-operative wound infections, and necrotizing pneumonia. The antibiotic penicillin was first used to treat *S. aureus* infections but resistant strains appeared in 1942 first in the hospital and later in the community. Since 1960, 80% of *S. aureus* strains have developed resistance to this antibiotic (Lowy, 2003). *S. aureus* is now considered to be a major pathogen which colonises and infects both hospitalized patients as well as healthy individuals in the community. The bacterium is found on skin and nasopharynx of the human body as
commensal and has the ability to cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract. The skin and mucous membrane act as excellent barriers against local tissue invasion by *S. aureus*. These organisms enter the underlying tissues, due to trauma or surgery when these surfaces are breached, producing localized abscess. The organism may spread through lymphatics or blood stream causing septicemia (Waldvogel, 1990).

*S. aureus* produces wide range of extracellular toxins and exfoliative toxins (Projan and Novick, 1997). Ingestion of enterotoxin produced by *S. aureus* in contaminated food can cause *Staphylococcal* food poisoning (Dinges *et al*., 2000). Toxic shock syndrome is caused by some strains of *S. aureus* that produce a toxin known as toxic shock syndrome toxin-1 (TSST-1). The production of this toxin mediated through the *tst* gene (Waldvogel, 1990). This syndrome is relatively a rare condition characterized by fever, rash, diarrhoea and inability to maintain proper haemostasis. In severe cases, the disease may involve multiple organs and there may be desquamation of the skin over the entire body and death in some cases (Todd *et al*., 1978). The young menstruating women who use high absorbency tampons constitute high risk group. Cases are also seen in men and non-menstruating woman (Davis *et al*., 1980). Another syndrome, Staphylococcal scalded skin syndrome (SSSS) is associated with an exfoliative toxin of *S. aureus* strains. This toxin causes epidermal necrosis, erythema and impetigo. This syndrome is prominent in children and is characterized by acute onset of local perioral erythema. Bullous impetigo is a localised blister caused by *S. aureus* (Hanakawa *et al*., 2002).

*S. aureus* sometimes causes serious disease conditions with mortality rate as high as 50% due to bacteremia and acute infective endocarditis. The symptoms of endocarditis due to *S. aureus* initially are non-specific influenza-like illness which deteriorates rapidly leading to irregular cardiac output and septic embolization. The bacteremia due to methicillin resistant *S. aureus* (MRSA) is a worldwide problem and is responsible for high mortality rate (Murray *et al*., 2002).

Besides humans, *S. aureus* can infect different animal species also, leading to a number of important conditions such as dermatitis, pneumonia, septicemia, osteomyelitis and meningitis. *S. aureus* is one of the most important causes of bovine mastitis in dairy cattle and buffaloes. Mastitis is an inflammatory response of mammary gland tissues causing
chronic and deep infections in the mammary glands which is very difficult to treat (Hassan et al., 2010). Consequently, this condition is responsible for huge economic losses to the dairy industry world over due to reduced milk yield, deterioration of milk quality, and increased labour cost (Beck et al., 1992). Not only clinical but subclinical mastitis in dairy herds is also responsible for such losses (Kavitha et al., 2009). In poultry, S. aureus causes bumble foot disease (Quinn et al., 2000). S. intermedius is commensal bacterium found on skin and mucosal membrane of wide range of animals like dogs, cats, pigeons and horses. The organism is associated with pyoderma and otitis in dogs and has the ability to cause sporadic infections in humans (Sasaki et al., 2007). S. aureus, S. intermedius and S. hyicus are some of the important pathogens of domestic animals. The coagulase negative Staphylococci (CoNS) are sometimes also associated with animal diseases (Quinn et al., 2002).

1.3 Emergence of multidrug resistant (MDR) and Methicillin resistant Staphylococcus aureus (MRSA) strains

Being an important pathogen of man and animals, S. aureus poses a serious threat to the human health due to emergence of multidrug resistance among S. aureus strains because the infections due to resistant strains are difficult to treat. An additional therapeutic challenge has been presented by MRSA strains which appeared during 1960’s and 1970’s. Among all S. aureus isolates, more than 50% account for causing clinical disease in the US (Drago et al., 2007) while a lower proportion has been reported from other countries; France (15.5%) (Lamey et al., 2012), and Netherlands (3.1%) (Wassenbrg et al., 2012). The emergence of resistance to multiple antibiotics has also been reported in a number of other pathogens such as Streptococcus pneumoniae, Pseudomonas aeruginosa and Mycobacterium tuberculosis. A number of factors are responsible for the emergence of antimicrobial resistance in various bacterial species. Indiscriminate use of antibiotics, addition of antibiotics as growth enhancers in animal feeds, increase in regional and international travel, antibiotic resistant bacteria can cross geographical barriers etc. are some of the important factors (Tomasz, 1994; Swartz, 1997). S. aureus has the ability to adapt to variable environmental conditions and it is for this reason that it is implicated in a variety of clinical conditions (Waldvogel, 2000). The antibiotic methicillin was used to treat S. aureus infections due to penicillin resistant strains but resistance to this antibiotic
was observed soon after its introduction into clinical use. The methicillin-resistance in *S. aureus* infections is mediated through the *mecA* gene, which is present on the *Staphylococcus* chromosome cassette (SCCmec) (De Zhi *et al.*, 2013). SCCmec is a novel genetic element composed of the *mec* gene complex which is responsible for methicillin resistance and cassette chromosome recombinase (*ccr*) gene complex responsible for mobility (Ito *et al.*, 2001). The first methicillin resistant *S. aureus* (MRSA) strain (NCTC 10442) was isolated in UK during the year 1961 which had SCCmec type I. MRSA strain (N315) with SCCmec type II was discovered in Japan in 1982. This New York/Japan clone spread worldwide. MRSA strain (85/2082) with SCCmec type III was reported from New Zealand in the year 1985. MRSA strains having SCCmec IV spread all over the world in 1990s and at the beginning of 21st century, first MRSA strain (WIS) with SCCmec type V has been described in Australia (Ito *et al.*, 2004; Vandenesh *et al.*, 2003). Hospital acquired MRSA spread from hospital to community in 1961. Initially, MRSA strains affected hospitalized patients and those with chronic illness. The emergence of community acquired methicillin-resistant *S. aureus* (CA-MRSA) strains primarily caused the skin and soft tissue infections (SSTIs) in healthy persons, generally children. These strains immediately led to epidemic of CA-MRSA infections with serious consequences (Francis *et al.*, 2005). The impact of overall morbidity and mortality of MRSA is higher as compared to that produced by methicillin sensitive *S. aureus* (MSSA) (Cosgrove *et al.*, 2003). MRSA infections have been associated with long hospital stay and cost effective healthcare system than MSSA (Cosgrove *et al.*, 2005). HA-MRSA and CA-MRSA strains cause different clinical syndromes and affect different patient populations. HA-MRSA commonly causes pneumonia, bacteraemia and invasive infections but CA-MRSA in contrast, are usually associated with skin and soft tissue infections in children. More severe infections include: necrotizing pneumonia (Kreienbuehl *et al.*, 2011), pyomyositis (Burdette *et al.*, 2012), sepsis (Bassetti *et al.*, 2011), osteomyelitis (Kreienbuehl *et al.*, 2011) and necrotizing fasciitis (Changchein *et al.*, 2011). The characterization of MSSA and MRSA (CA-MRSA and HA-MRSA) is therefore, essential for effectively managing infections due to these organisms. The present study has been designed with a view to characterize *S. aureus* strains recovered from different clinical cases from the outdoor as
well as indoor patients at Indira Gandhi Medical College (IGMC), Shimla in Himachal Pradesh. The proposed study has been planned with the following objectives.

Objectives:

- Screening of *Staphylococcus aureus* isolates for multidrug resistance.
- Molecular characterization of selective methicillin sensitive *Staphylococcus aureus* (MSSA) and methicillin resistant *Staphylococcus aureus* (MRSA) strains based on PCR amplification of segments of virulence genes; coagulase (*coa*), staphylococcal protein A (*spa*) and toxic shock syndrome toxin-1 (*tst*) genes.
- Bacteriophage typing of methicillin sensitive *S. aureus* strains.