1.0 INTRODUCTION

1.1 Background

_Staphylococcus aureus_ is Gram-positive, spherical coccus having a diameter of 1µm – 1.3µm. On microscopic examination, the organisms appear in clusters, like bunches of grapes. Some strains produce toxins while growing in food. These toxins can cause gastrointestinal disease generally referred to as Staphylococcal food poisoning. The enterotoxin produced by _S. aureus_ is a heat-stable protein that resists heating at 100°C for 30–70 min. _S. aureus_ is thus, also responsible for food-borne infections (Garcia-Alvarez _et al._, 2011). Various disease conditions caused by this organism are: wound infections, septicaemia and toxic shock syndrome. Besides, skin pustules, impetigo, osteomyelitis, renal abscess, pneumonia, endocarditis, meningitis, gastroenteritis and sometimes serious conditions in patients with undergoing hemodialysis, diabetic mellitus etc. such as may also be caused by _S. aureus_ (Lewis and Jorgensen, 2005). Several methods such as Gram’s staining, cell morphology, production of catalase and coagulate enzymes, pigment production, susceptibility to lysostaphin and lysozyme, and anaerobic production of acid from glucose are used for identification of _S. aureus_ (Paul _et al._, 2009). Besides several commercially available systems that allow strains to be biochemically characterized, have also been developed. Other species of _Staphylococcus_ genus are also implicated in similar disease conditions. For example, _S. epidermidis_ is involved in bacterial endocarditis, prosthetic heart valve endocarditis, bacteremia, surgical wound infections, intravascular catheter infections, postoperative endophthalmitis, conjunctivitis and keratitis. Other species of _Staphylococcus_ such as _S. saprophyticus, S. intermedius_ and _S. hyicus_ may sometimes be involved but these can be distinguished from _S. aureus_. The coagulate negative _Staphylococci_ (CoNS) species have been implicated at low incidence in a variety of infections. For example, _S. saprophyticus_ is often regarded as a more important opportunistic pathogen than _S. epidermidis_ in human urinary tract infections (UTIs), especially in young sexually active females. Other staphylococcal species such as _S. haemolyticus, S. hominis_, and _S. lugdunensis_ are usually found as contaminants of blood cultures but these organisms could also be associated with a variety of infections (Martineau _et al._, 2001).

_S. aureus_, the most pathogenic species of Staphylococci is widely distributed and found almost everywhere, particularly on the skin of humans and animals (Mathanraj _et al._, 2009). About 60% of human population is estimated to be colonized by _S. aureus_ and 20% of humans are persistent carriers. The nose is most favourable site (Zorgani _et al._, 2009) but the
organism can also survive on the skin and in the environment for a long time. Colonization of methicillin resistant \textit{S. aureus} (MRSA) also occurs at sites other than the nose e.g. pharynx, axilla, rectum, perineum (Eveillard \textit{et al.}, 2006) which might play an important role in the transmission of infection. Until recently, MRSA has been primarily considered as nosocomial infection, acquired in hospital settings mainly affecting healthcare workers (Zorgani \textit{et al.}, 2009).

1.2 Emergence of drug resistance among \textit{S. aureus} strain

It has become very difficult to treat certain conditions such as wound infections, gonorrhea, tuberculosis, pneumonia, septicaemia and childhood ear infections with the commercially available antibiotics because of the development of resistance in bacteria against these antibiotics. Similarly, MRSA has emerged as a major epidemiological problem in hospitals throughout the world and requires continuous attention. Such strains are often described as \textit{superbug} (Ahmed \textit{et al.}, 2010). Two types of MRSA have been reported i. Hospital acquired methicillin-resistant strains of \textit{S. aureus} (HA-MRSA) and ii. Community acquired methicillin-resistant strains of \textit{S. aureus} (CA-MRSA). The latter is bacteriologically, clinically and epidemiologically, distinct from the former (CDC, 1999), and continues to be prominently involved in nosocomial infections. The major reservoir of \textit{Staphylococci} in hospitals are colonized infected in-patients and colonized hospital workers, with carriers at risk for developing endogenous infection or transmitting infection to healthcare workers and patients. The major mechanism for patient to patient transmission has been through transient carriage of the organism on the hands of healthcare workers (Dar \textit{et al.}, 2006). Animals can also serve as reservoirs of MRSA and serve as source (Milk) for further transmission (Weese \textit{et al.}, 2010, Garcia-Alvarez \textit{et al.}, 2011). The first isolation of clinical MRSA was reported from cow (Devriese \textit{et al.}, 1972). There is evidence that MRSA can infect domestic animals which may pose a zoonotic threat to veterinarians and to the public (Khanna \textit{et al.}, 2008). Different animals have since been reported to be a zoonotic source of MRSA, which can infect their owners or attendants subsequently (Baptiste \textit{et al.}, 2005). The animals could thus, serve as a source of zoonotic MRSA and people in direct contact with animals could contract the MRSA infection. Moreover, humans have been implicated in the passage of MRSA to companion animals and few reports suggest the possibility of transmission (Seguin \textit{et al.}, 1999).

Infections caused by CA-MRSA in particular, have become a major public health threat (Martin and Henry, 2008). The infections due to \textit{S. aureus} in developing countries are perceived as trivial in terms of morbidity and mortality as compared to other infectious
diseases such as malaria, tuberculosis, and HIV infection. This organism is a pathogen of developing country. In South-East Asia, diseases caused by Staphylococcus are prevalent in low-income and lower-middle income countries based on several studies (Nickerson, 2009). The involvement of *S. aureus* in different clinical conditions is independent of methicillin resistance.

**Genetic Basis of CA-MRSA**

CA-MRSA strains appear to be resistant to fewer antimicrobial drugs. They carry a number of different virulence genes, for example, *pvl* gene that encodes panton-valentine leukocidin (*pvl*) toxin, vancomycin resistance gene (*van-A*), also a different type of the gene complex system called ‘Staphylococcal cassette chromosome mecA (SCCmec) encodes for methicillin resistance (Naimi *et al*., 2003). This cassette contains mobile genetic elements (e.g. *mecA* genes) which have been categorized into five different types I to V and it’s identify based on location (Martins & Cunha, 2007).

The genetic diversity among the strains of *S. aureus* has contributed to the emergence of varying degree of antibiotic resistance and virulence patterns (De *et al*., 2007). MRSA has been recognized and considered to be primarily a problem of hospitals (Ahmed *et al*., 2010). In the 1950’s, many strains of *S. aureus* produced penicillinase to overcome penicillin; a β-lactam drug methicillin was introduced which was available in 1950s and become a drug of choice to treat penicillin-resistant staphylococcal infections. One year after the launch of this drug, *S. aureus* resistant strains of *S. aureus* appeared and it emerged as a nosocomial pathogen in the early 1960s (Jorgensen 1986). MRSA emerged as a serious problem in some US hospitals during 1970s and by the 1990s; MRSA became a worldwide problem (Klevens *et al*., 2007).

**1.2.1 Mechanism of methicillin resistance**

Resistance to most β-lactam drugs including methicillin in *Staphylococci* is mediated by an altered penicillin-binding protein, PBP, which is encoded by *mec* gene (Brown *et al*., 2005). The β-lactam including penicillin, methicillin, and even cephalosporin are ineffective if the bacterium possesses *mecA* gene.

**1.2.2 Phenotypes of MRSA**

Many MRSA resistant phenotypes with multi resistance characteristic have been reported globally e.g. MRSA-MLSB (macrolides, lincosamides, and streptogramines B) phenotypes. The MLSB resistance phenotypes (MLSBC and MLSBi phenotypes) confer multiple-resistance to many antibiotic classes including, macrolides, lincosamides, and
streptogramines B (Lewis & Jorgensen, 2005). Such phenotypes have been reported worldwide including developing countries (Siberry et al., 2003; Ahmed et al., 2010).

1.2.3 Resistance to Other antibiotics

Clindamycin is useful drug for treating MRSA infections; however, treatment failures using this drugs in patients with MRSA infections caused by inducible clindamycin resistant (MRSA-MLSBi) strains has been reported by several workers (Siberry et al., 2003, Lewis & Jorgensen 2005). MRSA-MLSBi strains cannot be detected by standard susceptibility tests. However, a test known as D test (Clinical Laboratory Standard 2006) is being use to detect such strains (Ahmed et al., 2010).

Another antibiotic vancomycin was considered as the drug of choice to treat S. aureus infections but Vancomycin resistant S. aureus (VRSA) phenotype has been evolved which confers further resistance to vancomycin (Martins & Cunha, 2007). Many risk factors associated with acquiring MRSA infection have been identified e.g. prolonged hospitalization and antimicrobial therapy. Nasal colonization has also been identified as one of the risk factors for infection and carriage of MRSA in various healthcare settings (Von et al., 2001). MRSA colonization has been identified in people who have had no association with hospitals or other risk factors. Such strains have been identified as community acquired (CA-MRSA), (Martins & Cunha, 2007). Several reports of clinical CA-MRSA have appeared since its first report in 1980s (Fey et al., 2003).

Keeping all the above mentioned facts in mind, the present study has been designed to characterize S. aureus isolates originating from human patients at Indira Gandhi Medical College, Shimla. The isolates originated from blood, pus, urine and catheter associated infections of human patients. Characterization of these isolates would be useful to study the molecular epidemiology of MRSA in the state of Himachal Pradesh. This aim shall be achieved with the following objectives
Objectives

- Confirmation of clinical isolates of *Staphylococcus aureus* recovered from human patients
- To determine the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in the state of Himachal Pradesh.
- Bacteriophage typing and plasmid profiling of the MRSA strains.