Chapter-I

INTRODUCTION
Staphylococci are Gram-positive spherical shaped bacteria that occur in microscopic clusters resembling looks like grapes. Bacteriological culture of the nose and skin of normal humans invariably yields staphylococci. *Staphylococcus aureus* is frequent and very important human pathogens that causes in two ways like hospital and community acquired infections (Chambers, 1997; Deresinski, 2005; Friedman et al., 2002; Naimi et al., 2003). It is marvelously equipped as a pathogen containing biochemical machinery (John and Sheagren, 1984). *S. aureus* is an opportunistic bacterium frequently part of the human micro flora, causing disease when the immune system becomes compromised. Although *S. aureus* can be found in different parts of the body, anterior nares are the primary ecological niche in humans. Nasal carriage differs between individuals and is one of the major risk factors for *S. aureus* infections (Kluytmans, 1997).

*S. aureus* is one of the most successful and adaptable human pathogen. The remarkable ability of the bacteria to acquire antibiotic-resistance mechanisms and advantageous pathogenic determinants has contributed to its emergence in both nosocomial and community settings (Nicola Zetola et al., 2005). The bacterium has a versatile arsenal of secreted and surface molecules which contributes to its ability to cause disease. Infections are initiated when a breach of the skin or mucosal membrane allows staphylococci to enter adjoining tissues or blood stream. Whether an infection is contained or spreads depends on a complex interplay between *S. aureus*
virulence determinants and host defense mechanisms (Franklin and Lowy, 1998). S. aureus can cause a wide variety of infections showing 3 general types: i) Superficial lesions such as wound infections ii) Systemic and life threatening conditions such as osteomyelitis, pneumonia, brain abscesses, meningitis and bacteremia and iii) Toxins such as food poisoning, scalded skin syndrome and toxic shock syndrome (Tenover and Gaynes, 2000).

Risk factors most likely to result in colonization or infection with multi-drug resistant species include advanced age, severity of illness, inter institutional transfer, prolonged hospital stay, gastro-intestinal surgery, transplantation, exposure to medical devices and exposure to broad spectrum antibiotics (Safdar and Maki, 2002). The virulence of S. aureus is due, in large part, to a host of extracellular products it generates, including cytotoxins that result in pore formation and pro-inflammatory changes. The cellular damage caused by these toxins may contribute to the manifestations of sepsis syndrome. Enterotoxins are responsible for toxic shock syndrome and food poisoning. Exfoliative toxins, including epidermolytic toxins A and B, cause skin erythema and separation, as seen in staphylococcal scalded skin syndrome. Panton-Valentine leukocidin is a leukocytolytic toxin and has been associated with severe cutaneous infections and necrotizing pneumonia. In addition to toxins (Volturo et al 2006). The gastrointestinal tract is the main habitat of Enterococcus spp., but they are occasionally found in oropharyngeal secretions, vaginal secretions, and on the skin, especially in the perineal area. They can cause endocarditis, urinary tract infections, intraabdominal pelvic and wound infections, peritonitis, nosocomial bacteremia, central
nervous system infections, osteomyelitis, neonatal sepsis, and pneumonia (Zeliha et al 2010).

The incidence of community-acquired and hospital-acquired *S. aureus* infections has been on the rise with the emergence of drug resistant strains called Methicillin Resistant *Staphylococcus aureus* (MRSA). Bacterial, genetic and microbiological adaptive changes and properties gave rise to the emergence of antibiotic resistance of the organism (Santhosh et al., 2007). The drug of choice for staphylococcal infections was penicillin, but indiscriminate use and genetic manipulations on the part of the organism slowly led to penicillin resistance. In order to replace penicillin, methicillin was used. A year after the discovery of methicillin in 1959 staphylococci started to show resistance even to methicillin, which led to the emergence of the ‘super bug’ MRSA (Santhosh et al., 2007). However, MRSA was first reported in 1961 (Jevons, 1961). The genetic basis of methicillin resistance of MRSA isolates is the presence of *mecA* encoding PBP2a (Hartman and Tomasz, 1981) that is located on a mobile genetic element called the Staphylococcal Cassette Chromosome (SCCmec) (Katayama et al., 2000) and incorporated in the chromosome of *S. aureus* at a site specific location near the origin of replication (Ito, et al., 1999). Two years later, *Staphylococcus aureus* showed resistance to other β-lactam antibiotics like oxacillin, nafcillin, and the cephalosporins, that invariably contributed to a multiple drug resistance (MDR) pattern in this organism (Chambers, 2001). There were only sporadic outbreaks of MRSA, and this became a major problem only during the late 1970s and in the early 1980s. Many outbreaks were reported after that from different parts of the world (Grubb, 1989). Geographically, MRSA is distributed worldwide (Lee, 2003).
S. aureus is transmitted through aerosol or direct contact with fomites, infected animals, or infected people. Approximately 30% of healthy humans carry S. aureus in their nasopharynx or on their skin. Human staphylococcal infections are frequent, but usually remain localized at the portal of entry by the normal host defenses. The portal may be a hair follicle, but usually it is a break in the skin which may be a minute needle-stick or a surgical wound. In a laboratory rodent setting, it is more likely for humans to infect animals rather than vice versa (Fox et al. 2007).

The interest in S. aureus infections has been heightened by the difficulties encountered in the treatment and eradication of S. aureus resistant to large number of antibiotics. More serious infections of the skin may occur, such as furuncles or impetigo. Localized infection of the bone is called osteomyelitis. Serious consequences of staphylococcal infections occur when the bacteria invade the blood stream. S. aureus has great adaptive power to antimicrobial agents and little by little it has been acquiring resistance to all antibiotics available in clinical practice. Due to an increasing number of infections caused by multi-drug resistant MRSA strains, therapy has become problematic. Many MRSA are susceptible only to glycopeptide antibiotics and investigational drugs (Aires Marta de Sousa et al. 2004). The inexorable spread of antimicrobial resistance is now of concern to agencies of numerous government and health agencies worldwide including the World Health Organisation (WHO), which has attempted to bring order to chaos and provide rational solution to the problem (Martin Wood et al., 2003).

*Staphylococcus aureus* is a commensal and pathogen of several mammalian species, particularly humans and cattle. We aimed to (i) identify S. aureus genes
associated with host specificity, (ii) determine the relatedness of human and animal isolates, and (iii) identify whether human and animal isolates typically exchanged mobile genetic elements encoding virulence and resistance genes (Julia et al., 2008).

The epidemiology of infections caused by MRSA is rapidly changing (Cynthia et al., 2007). Prevalence of MRSA had been previously confined or limited to hospital settings, but as of late, incidences of MRSA infections in the community have also been reported in epidemiological surveys and studies (Santhosh et al., 2007). The increasing prevalence of community acquired MRSA in multiple countries and the substantial morbidity and mortality associated with these infections suggest that community acquired MRSA will continue to develop into a challenging public health problem.

Meticillin-resistant Staphylococcus aureus (MRSA) is endemic in many hospitals worldwide. Previously, MRSA infections were nearly always associated with hospital or health-care contact, but new strains of MRSA have emerged that cause community infection in patients without previous health-care contact (Jonathan et al., 2010).

Staphylococcus aureus is a major human pathogen responsible for a variety of nosocomial and community-acquired infections ranging from mild to life-threatening diseases. Along with the spread of this bacterium, an increase of antibiotic resistance has been reported over the last decades. Since the early sixties, meticillin-resistant S. aureus (MRSA) has caused large, life-threatening nosocomial outbreaks worldwide (Jean et al., 2009).

In a recent and dramatic evolutionary development, however infection with novel community acquired strains of MRSA in previously healthy individuals without either direct or indirect association with health care facilities has emerged as new and
important public health problem (CDC, 2003; Naimi, 2003). Supplicative skin infections and severe necrotizing pneumonias are the most well known clinical syndromes caused by these new strains. The ability of a new community acquired MRSA strains to colonise hosts in the community and cause clinical syndromes is mediated by unique combination of traditional and newly described virulence factors. The most well known community acquired MRSA virulence factor is Panton-Valentine Leucocidin (PVL) (Nicola Zetola et al., 2005), an exotoxin often associated with severe skin infections and necrotizing pneumonia (Dufour et al., 2002). The expression of Panton-Valentine leukocidin (PVL) has been implicated as a virulence factor for community-acquired Staphylococcus aureus pneumonia with most reported cases involving methicillin-resistant strains. Here we describe a case of community-acquired, PVL-positive methicillin-sensitive Staphylococcus aureus (MSSA) sufficiently virulent to cause rapidly progressive necrotizing pneumonia. (Narimon et al. 2007).

Clinical isolates from invasive infections can only focus on the severity of the disease, but does not give an estimate or prevalence of carriers among the healthy population (Santhosh et al., 2007). Although nasal carriage of S. aureus is harmless in healthy individuals (Lee, 2003), they can become carriers (Foster, 2004), who could pose the risk of spreading infections to the community at large. Various hospital-based studies have described the incidence of MRSA and carriage of this organism in health care workers (Salaria and Singh, 2001). Recently, even community acquired S. aureus strains have shown resistance to methicillin. Due to this changing epidemiology, it is important to assess the carriage rate of MRSA in the community amongst healthy individuals who have not been hospitalized nor had antibiotic therapy in the recent past. Methicillin-resistant Staphylococcus aureus (MRSA) has traditionally been considered a healthcare-associated pathogen in patients with established risk factors. More recently, however, MRSA infections have been described in community-dwelling patients without established risk factors for the acquisition of MRSA. The
emergence of this community-acquired MRSA (CA-MRSA) as a clinically significant pathogen necessitates reconsideration of current empirical treatment with β-lactam antibiotics for community acquired S. aureus infections and current control strategies for MRSA in hospitals (Kluytmans et al 2006).

Staphylococcal infections cause significant morbidity and mortality in both the community and hospital settings. Treatment of infection caused by S. aureus has become more problematic since the development of antimicrobial resistant Staphylococcus aureus (MRSA). Since MRSA strain are resistant to all β-lactam antibiotics and the treatment options are limited significantly. The incidence of nosocomial infection caused by MRSA continues to increase worldwide. Infections caused by MRSA strains are associated with longer hospital stay (Somayeh Karami et al 2011).

Thus, this formed the basis for our study and its screening for healthy carriers of MRSA in healthy individuals and hospital personnel, since medical staff can be a vehicle in the spread of MRSA within a hospital, as direct person to person contact contributes to the transmission of MRSA.

For this present study we have selected Bangalore as another geographical location in South India. Bangalore is one of the cosmopolitan cities in south India with varied environment having large number of hospitals and health care centers. For the present study samples have been collected from K.C General Hospital, Wockhard Hospital, Fortis Hospital and St John’s medical college and Hospital which receives clinical samples from both public and private hospitals in the city of Bangalore. Many of
these *S. aureus* strains are multi-drug resistant and they are characterized only phenotypically at present and molecular genotyping is not done.

A thorough understanding of the molecular epidemiology and evolution of MRSA is required to help detect, track, control and prevent human diseases due to this organism. The discriminate power of most of phenotypic methods is restricted and ambiguous (Ip, 2003; Schlichting *et al*., 1993). Molecular typing methods have in the last few years paved the way, for sophisticated technique to track the source and transmission route of bacterial pathogens. Full characterization of MRSA requires definition of not only the putative bacterial genetic background but also of the complex and heterologous SCC*mec* elements. Antimicrobial resistance profile of each strain of MRSA, therefore can be evaluated by detecting the resistance genes identified on SCC*mec*. The overall effectiveness of empiric therapy can be improved and infections can be treated in their early stages before the pathogen overwhelms the patient. It has been reported that PVL determinant is common in *S. aureus* isolated from community but is rare among the isolates of hospitals. And therefore PVL can be used as a useful marker for the identification of community acquired MRSA worldwide. There have been very few reports from India where *S. aureus* strains have been characterized by SCC*mec* and PVL analysis (Nicola Zetola *et al*., 2005)

Therefore, the present study was undertaken to distinguish *S. aureus* isolates from hospitals and community by phenotypic and molecular methods. *S. aureus* were isolated from various clinical samples, hospital personnel and healthy individuals from the community. All *S. aureus* isolates were screened for MRSA isolates by various phenotypic methods. Antibiotic susceptibility testing of *S. aureus* was performed to
analyze the resistance profiles. MRSA isolates were randomly selected from hospital and community sources for molecular characterization. SCCmec typing by multiplex PCR was performed to determine the predominant SCCmec types among the isolates obtained from hospital and community. Presence of PVL exotoxin was determined by simple PCR since it was one of the markers used in our study to distinguish *S. aureus* isolates obtained from hospitals and community.