2. INTRODUCTION
INTRODUCTION

In the past few decades, methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as an important nosocomial pathogen worldwide (Townsend *et al.*, 1987; Panlilio *et al.*, 1992). The emergence and rapid spread of this organism has created important new challenges for infection prevention and control services in hospitals and other health care facilities. Interestingly, there appears to be significant variability in the epidemiology and prevalence of MRSA in different parts of the world and even in different regions of a country (Voss *et al.*, 1994).

Patients in the intensive care units (ICU) are at a higher risk of acquiring nosocomial infections compared with patients in general wards (Donowitz *et al.*, 1982; Wenzel *et al.*, 1983). This is partly because of the severity of the underlying illnesses and partly because of iatrogenic factors related to the high frequency of invasive procedures required for monitoring and treatment (Edgeworth *et al.*, 1999). Bacteremia continues to be a major cause of morbidity and mortality in hospitals (Vincent *et al.*, 1995). The overall or crude rate of death does not distinguish between the contribution of the patients' underlying diseases and the contribution of bloodstream infections (Wenzel *et al.*, 2001). The prognosis of postbacteraemia infection (true bacteremia or fungaemia) is very variable. In recent years, many studies have analyzed the mortality rates in relation to pathogens, source of infection, patient age, and underlying diseases.
In the preantibiotic era *S. aureus* bacteremia resulted in 80% mortality (Smith *et al.*, 1960) however, with the advent of antibiotics, the organism was reported to be susceptible to the earliest antimicrobial agents, the sulfonamides and penicillin. The widespread use of these antibiotics in the 1950s induced the predominance of β-lactamase-producing resistant strains. To solve the problem, the β-lactamase-resistant penicillins were developed, but reports of resistance to this new group started to appear in the 1960s in Europe (Jessen *et al.*, 1969) and in the 1970s in the United States (Peacock *et al.*, 1980). The emergence of antibiotic-resistant strains of *S. aureus* is now considered to be a major problem in most hospitals. Virtually, all nosocomial strains produce a β-lactamase and thus are resistant to penicillins. Moreover, data from the Centers for Disease Control and Prevention indicate that throughout the United States there has been an increase in the frequency of methicillin-resistant *S. aureus* (MRSA) strains resistant to multiple antibiotics in both large and small hospitals (Hughes, 1987). Thus far, all strains of MRSA have been susceptible to vancomycin, although certain strains have exhibited tolerance. It is possible, however, that vancomycin resistant grampositive cocci such as *Enterococcus* spp. (Al-Obeid *et al.*, 1990; Uttley *et al.*, 1989) and *Staphylococcus haemolyticus* (Schwalbe *et al.*, 1987) may transmit the gene(s) responsible for this phenotype to *S. aureus*, leaving few if any options for antimicrobial chemotherapy of infections caused by the organism.
Recent studies suggest that the infection due to MRSA is not only hospital-acquired but community acquired as well (Salmenlinna et al., 2002). MRSA now represent a global problem (WHO, 1996). Some large outbreaks have been reported from different parts of the world, where it had caused severe infections including septicemia, endocarditis and meningitis (WHO, 1996). A study by Dickinson in England and Wales has concluded an increase in the trend of death due to MRSA infection (Dickinson et al., 2002). Infections caused by MRSA can be expensive in terms of antibiotic therapy, isolation facilities and materials and length of hospital stay. According to a World Health Organization literature, the global financial burden because of MRSA infection has been worked out to be $20,000 to $114,000 for outbreaks and from $28,000 to $1600,000 for endemic infections per year. The common sources of these infections are human patients and carriers (Collier et al., 1998). The risk factors that contribute to MRSA are antibiotics abuse, prolonged hospitalization, intravascular instrumentation and hospitalization in an intensive care unit. There is considerable variation in numbers of clinical infections among units, hospitals and countries.

The analyses of the data showed a higher prevalence of S. aureus in nursing staff and attendants compared to the doctors. Age, sex, health status could not be correlated with the rate of infection, however, it could be due to the nature of job and place of work of the individuals. The prevalence of S. aureus was found higher in surgical wards than the general wards. Our study shows that the risk of infection is higher in individuals occupationally exposed to such microbes. MRSA has been
reported earlier from hospitals in various parts of the world (Panlilio et al., 1992). There is a need to screen individuals in hospitals for risk exposures and infections, to avoid outbreak and cross infections.

Other factors including prolonged hospitalization, multiple antibiotic therapy sessions, and intravenous catheterization also increase the risk of nosocomial infections in burn patients. MRSA is an important causative agent of nosocomial infections in India. According to an Indian study, the prevalence of infections caused by MRSA has increased from 12 percent in 1992 to 80.03 percent in 1999 (Verma et al., 2000). Many of these MRSA isolates are becoming multidrug-resistant, and are susceptible only to glycopeptides (Mehta et al., 1998).

In the 1980s, due to the widespread occurrence of MRSA, empiric therapy for staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health-care institutions. Vancomycin use in many countries also increased during this period because of the growing numbers of infections with Clostridium difficile and coagulase-negative staphylococci in health-care facilities (Ena et al., 1993). Thus, the early 1990s saw a discernible increase in vancomycin use. As a consequence, selective pressure was established that eventually led to the emergence of strains of Staphylococcus aureus and other species of staphylococci with decreased susceptibility to vancomycin, but in 1997 the first clinical isolate of Staphylococcus aureus with reduced susceptibility to vancomycin was reported from Japan (Hiramatsu et al., 1997). Data from the December 2000 report of the National Nosocomial Infection Surveillance (NNIS) System indicated that about 75% of
coagulase-negative *staphylococci* and 47% of *S. aureus* isolates from intensive care units were resistant to methicillin ([www.cdc.gov/ncidod/hip/NNIS/DEC2000sar.PDF](http://www.cdc.gov/ncidod/hip/NNIS/DEC2000sar.PDF)). Vancomycin remains the drug of choice for these infections. Vancomycin resistance among *staphylococci* was developed in laboratories even before the drug was in use clinically (Geraci *et al.*, 1956; Ziegler *et al.*, 1956). However, this resistance was so difficult to induce that many felt it would be unlikely to occur in a clinical setting (Moellering *et al.*, 1998). That no vancomycin-resistant *staphylococci* were reported in the first 20 years the drug was used only strengthened this assumption. Unfortunately, this confidence was shattered by the first reports of vancomycin resistance in coagulase-negative *staphylococci* in 1979 and 1983 (Siebert *et al.*, 1979; Tuazon *et al.*, 1983).

*Staphylococcus aureus* is a major cause of hospital acquired infections, causing high morbidity and mortality throughout the world. Vancomycin has been the drug of choice for 30 years for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA). Over the last decade, methicillin resistant *Staphylococcus aureus* (MRSA) strains have become endemic in hospitals worldwide. In addition, it is now incipient community pathogen in many geographical regions. The emergence of high levels of penicillin resistance followed by development and spread of strains resistant to the semi synthetic penicillins (methicillin, nafcillin and oxacillin), macrolides, tetracyclins, and amino glycosides has made therapy of *Staphylococcal* disease a global challenge. By the 1990s, resistance to semi synthetic penicillins had spread throughout the world, compromising the use of these drugs for empiric
therapy for *Staphylococcal* infections in a number of regions. This had lead to increased reliance on vancomycin for treatment on documented MRSA infections. As a consequence, selective pressure was established that eventually lead to the emergence of strains of *Staphylococcus aureus* and other species of *Staphylococci* with decreased susceptibility to vancomycin and other glycopeptides. The first report of the *Staphylococcus aureus* with reduced susceptibility to vancomycin from Japan (Hiramatsu *et al.*, 1997). This report was quickly followed by similar ones from other countries, including United States (Smith *et al.*, 1999), Belgium (Denis *et al.*, 2002), and India (Assadullah *et al.*, 2003). The extensive longitudinal study of current situation of vancomycin resistance and have reported the first incidence of VRSA emergence from Northern part of India (Hare Krishna *et al.*, 2006). The first clinical infection with vancomycin resistant *Staphylococcus aureus* was reported from Michigan with second case in Pennsylvania. Further, the second confirmed VRSA from Pennsylvania was reported which represents the VRSA isolate from patient in United States. Emergence of decreased Vancomycin susceptibility in MRSA strains presents a significant clinical problem with few therapeutic options. The rapid evolution of antibiotic resistance is of considerable concern. Considering high prevalence of MRSA and increased use of vancomycin, the development of vancomycin resistance (VRSA) in clinical strains seems likely to occur. In 1996, the documented infection caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin (vancomycin-intermidiate S. aureus [VISA]) was reported in Japan (Hiramatsu *et al.*, 1997). Thereafter about 20 cases (Walsh *et al.*, 2001) of VISA
infections have been reported in several countries, including Korea (Kim et al., 2002).

In addition to VISA and VRSA, another type of vancomycin resistance called hetero-VISA (hVISA), has been described (Hiramatsu et al., 1997). This strain is susceptible to vancomycin but contains a sub population, at a frequency of $10^6$ or higher with MIC of vancomycin of more than 4 $\mu$g/ml. The potential importance of hVISA is that it may be associated with treatment failure (Hiramatsu et al., 1997, Wong et al., 1999 and 2000) and a precursor of VISA (Hussain et al., 2002; Sieradzki et al., 1999). Although a number of studies have been undertaken to determine the prevalence of hVISA, reported frequencies have ranged from 0-20% (Aucken et al., 2000, Geisel et al., 1999, Hiramatsu et al., 1997, Hubert et al., 1999, Ike et al., 2001, Kim et al., 2002, Marchese et al., 2000, Reverdy et al., 2001, and Woodford et al., 2000), depending on the definitions and methods employed for screening and confirmation. Four isolates of VRSA have been reported in the USA, with isolates found in June 2002 and February 2005 in Michigan (Severin et al., 2003, Chang et al., 2003 and Rudrik et al., 2005) in September 2002 in Pennsylvania (MMWR Morb Mortal Wkly Rep., 2002, Bozdogan et al., 2003 and Tenover et al., 2004), and in March 2004 in New York. Common features of these four patients were a history of chronic underlying diseases (diabetes, morbid obesity, residence in chronic care facility, peripheral vascular insufficiency with skin ulceration) and isolation of VRSA from skin ulcers or urine. These isolates have been highly resistant to vancomycin (MICs $\geq$32 $\mu$g/mL), although resistance requires induction in some
isolates and may be missed by some automated susceptibility test systems. The first two isolates have been shown to have plasmid-mediated vancomycin resistance due to \textit{vanA}, which was probably acquired from VRE present in the lame lesion in the first Michigan case (Tenover \textit{et al.}, 2004; Weigel \textit{et al.}, 2003). VRSA strains are resistant to glycopeptides, but are susceptible to lipopeptides such as daptomycin (Appelbaum \textit{et al.}, 2004). The first Michigan VRSA was highly resistant to vancomycin, with MICs of 1024 \(\mu\text{g/mL}\) by broth microdilution and \(>256 \mu\text{g/mL}\) by E-test. The phenotypic and genotypic characteristics of \textit{Staphylococcus aureus} isolates were analyzed in order to gain new insights into the evolution of MRSA, to elaborate the MRSA typing scheme, analyze trends of MRSA/VRSA occurrence and to analyze molecular characteristics of isolates in order to identify those linked to epidemics.

Therefore the present investigation was undertaken to study the molecular characteristics and epidemiology of \textit{Staphylococcus aureus} from clinical isolates of Hubli-Dharwad region, Karnataka State.