1. INTRODUCTION

The staphylococci are important pathogenic bacteria responsible for a wide range of infections in humans and they are classified as coagulase-positive or coagulase-negative staphylococci (CoNS) depending on their ability to produce the enzyme coagulase, and therefore clot plasma. To date, the genus *Staphylococcus* consists of 51 species and 27 subspecies that are validly published and vividly described (Euzeby, 1997 - http://www.bacterio.net). *Staphylococcus aureus* is one of the most clinically significant pathogens among the coagulase-positive staphylococci.

Amongst CoNS species, *Staphylococcus epidermidis* is the leading cause of infections followed by *Staphylococcus haemolyticus* which is second most frequently isolated from clinical samples (NNIS, 2004; Rogers *et al.*, 2009). Other species such as *Staphylococcus saprophyticus*, *Staphylococcus hominis*, *Staphylococcus lugdunensis*, *Staphylococcus warneri*, and *Staphylococcus capitis* have also been associated in different infections (Piette and Verschraegen, 2009).

The nares are an ecological niche for staphylococci, as 40 to 65% of humans are colonized by CoNS particularly *S. epidermidis*. CoNS are the common bacterial colonizers of the normal human microflora and usually have a benign relationship with the host (Kloos and Bannerman, 1994). Colonization of different parts of the skin and mucous membranes of the host is the key source of endogenous infections. CoNS predominantly cause infections in immunocompromised patients or otherwise healthy individuals with breached skin and mucous barriers (Vuong & Otto, 2002).
Human infections associated with CoNS include meningitis, otitis media, pneumonia, surgical wound infections, urinary tract infections and a series of medical device-related infections. Hospital acquired infections and antibiotic-resistant strains attributed to this group of bacteria have become endemic in many countries and are associated with serious public health issues.

Resistance to antibiotics in CoNS is a major concern for public health. Methicillin-resistant coagulase negative staphylococci (MR-CoNS) cause a wide variety of infections and raise high concerns, as often few therapeutic options are available. Methicillin resistance rates for CoNS ranged globally from 75 to 90% (Diekema et al., 2001). In India, Pal and Ayyagiri, (1989) reported 15% MR-CoNS from hospital settings. Thereafter, several MR-CoNS outbreaks in hospitals were reported from various parts of India (Sharma et al., 2010; Vysakh et al., 2015).

Methicillin resistance is encoded by the mecA gene, which is located in mobile genetic elements called staphylococcal cassette chromosomes (SCCs) (Ito et al., 2001). SCCmec consists of the mec gene and cassette chromosome recombinase (ccr) gene complex. To date, 11 major types of SCCmec (I to XI) and 8 subtypes of SCCmec IV have been assigned based on the classes of the mec gene complex and the ccr gene types (IWG-SCC, 2009). A highly diverse population of SCCmec elements has been discovered for MR-CoNS and it can be assumed that the assortment of SCCmec types and subtypes will be enlarged further. There is evidence of horizontal transfer of SCC cassettes between staphylococcal species (Hanssen and Ericson Sollid, 2006), which implies that CoNS could serve as a reservoir for the spread of antibiotic resistant genes.
contributing to the generation of new strains including Methicillin Resistant Staphylococcus aureus (MRSA). SCCmec typing of CoNS may serve as a useful tool for clinicians and epidemiologists in their effort to prevent and control infections caused by these organisms.

In addition to resistance to all β-lactam agents, MR-CoNS are often resistant to multiple classes of other antimicrobial agents. MR-CoNS show resistance to various groups of antibiotics including macrolides, lincosamides, tetracyclines, aminoglycosides, chloramphenicol, fluoroquinolones, sulphonamides, mupirocin and fusidic acid. Resistance of CoNS to newly developed antimicrobial agents, including streptogramins, tigecycline and linezolid although rare, has also been reported (Cetin et al., 2008; Claesson et al., 2009; Bender et al., 2015). In India, there are a few reports on linezolid resistance among CoNS species (Gupta et al., 2012; Rajan et al., 2014). The increasing multidrug resistance among CoNS poses a great challenge for the management of hospital-acquired infections. In addition, it also serves as a reservoir of antibiotic resistance genes (Sharma et al., 2010).

Besides being multidrug resistant, CoNS such as S. epidermidis has become a leading cause of nosocomial infections with abilities to survive in hospital settings and medical devices with an array of virulence factors (Jamaluddin et al., 2008). It owes its pathogenicity to three major features: its natural niche on human skin, ability to adhere to biomaterials and biofilm production and being multi-drug resistant (Jamaluddin et al., 2008; Otto, 2009). Biofilm formation by S. epidermidis is an important virulence process that begins first with adhesion of bacterial cells to abiotic
or protein-coated biotic surfaces mediated by autolysin (encoded by \textit{atlE} gene), followed by an accumulation process mediated by a polysaccharide intercellular adhesin (PIA) encoded by the \textit{icaADBC} locus and also by proteinaceous factors such as accumulation-associated protein (encoded by \textit{aap} gene) (Otto, 2009).

In addition to biofilm production, \textit{S. epidermidis} has a novel genetic island called arginine catabolic mobile element (ACME) which contains one or two clusters of genes, \textit{arcA} (encoding a secondary arginine deiminase system) and/or \textit{opp-3} (encoding an oligopeptide permease system). It is thought to play an important role in the pathogenicity of \textit{S. epidermidis} and increases the ability to colonize the skin and mucous membranes (Diep et al., 2006 and Diep et al., 2008).

HIV infected patients are vulnerable to opportunistic infections, including those by MR-CoNS because they are repeatedly exposed to the healthcare environment besides being immunocompromised. End stage renal disease (ESRD) patients are highly prone to staphylococcal infections because of their decreased immunity, increased nasal and skin colonization by staphylococci and the multiple needle punctures required for dialysis (Koziol-Montewka et al., 2006).

In community settings, orphanage and old age homes, inmates are at major risk for acquiring CoNS infection, because of nasal colonization, poor hygiene, substance abuse and overcrowding. These factors raised concerns about the community spread of MR-CoNS. Hence, these MR-CoNS from community settings may act as a source of \textit{SCCmec} for Community acquired MRSA (CA-MRSA) thereby increasing prevalence of CoNS in community-acquired diseases (Chu et al., 2008; Lebeaux et al., 2012).
The recognition of CoNS as pathogens has stimulated researchers to develop more accurate methods for its identification and molecular characterization. Several studies of their antibiotic resistance and virulence factors have been carried out in order to establish stringent criteria to discriminate cases of infection from bacterial flora contamination. For the past few decades, the epidemiology of the MR-CoNS infections all over the world has changed drastically, due to the emergence of MR-CoNS with multi-drug resistance causing infections in the hospital and various community settings. Due to the evolution of the advanced molecular and microbiological techniques such as multiplex PCR for specific detection of MR-CoNS and virulence genes, SCCmec typing, pulsed-field gel electrophoresis (PFGE) and Multilocus sequence typing (MLST), the dynamics and molecular basis of the MR-CoNS has been extensively studied. This in turn evoked the research on MR-CoNS all over the world with researchers coming up with new information on its virulence, resistance and its molecular epidemiology leading to confirmation that MR-CoNS is a substantial public health threat.

Compared to MRSA transmission, much less is known regarding the epidemiology of MR-CoNS in health care facilities. Even though there have been extensive studies on MR-CoNS, there are still gaps in understanding the molecular epidemiology of MR-CoNS. The number of serious infections with MR-CoNS is increasing; this is true for both community-acquired and nosocomial infections. This is partially due to the limited data on virulence, resistance determinants and molecular epidemiology of MR-CoNS from developing countries where the prevalence of MR-
CoNS is high. Recently, a few studies from India showed MR-CoNS infections from hospitalized patients and their limited diversity in India (Sharma et al., 2011; Vysakh et al., 2015).

Hence, this study was undertaken to study the virulence and resistance determinants and epidemiological types among the clinical isolates of MR-CoNS from hospitalized patients, HIV-infected patients, carrier isolates from ESRD patients and hospital personnel and asymptomatic population from various closed communities at risk including orphanage and old age home in Chennai, South India. The data generated from this study will serve as a baseline to understand the evolution and epidemiology of different strains of MR-CoNS, their virulence and resistance characteristics both in the hospital and community settings in Chennai, South India.