6. DISCUSSION

MR-CoNS infections among hospitalized patients, HIV-infected patients, ESRD patients and asymptomatic healthy individuals from various community settings is continuously increasing. Molecular and epidemiological studies on MR-CoNS particularly MRSE from across the world have shown that they differ significantly with geographical location and type of infection in their virulence factors, resistance determinants and their epidemiological types (Lebeaux et al., 2012; Du et al., 2013; Cherifi et al., 2014).

A large proportion of studies performed on CoNS species exclusively on clinical strains, and the role of community-associated strains has been neglected. Consequently, a better characterization of CoNS strains in their commensal lifestyle could give us new insights on how commensal bacteria can turn into dangerous pathogens. Very little is known worldwide regarding CoNS strains isolated from healthy individuals in the community settings (Silva et al., 2001; Barbier et al., 2010; Lebeaux et al., 2012). In the Indian setting, epidemiological studies on various groups at risk of MR-CoNS infections are lacking. The present study was designed to characterize MR-CoNS isolates of community and hospital settings from Chennai, South India among different populations.

6.1. Prevalence of MR-CoNS among various settings

Resistance to antibiotics in CoNS is a major concern for public health. MR-CoNS cause a wide variety of infections and raise high concerns, as few therapeutic
options are available. For the past few decades, the prevalence of MR-CoNS in Indian hospitals is continuously increasing (Pal and Ayyagiri, 1989; Jain et al., 2004; Sharma et al., 2010; Nazir et al., 2014).

In the present study, the prevalence of MR-CoNS among clinical isolates from hospitalized patients was found to be 70.8%. Previous studies from other parts of the world by Koksal et al., (2009), Garza-Gonzalez et al. (2010), Duran et al. (2012) and Talebi et al. (2015) have reported 67.5%, 69% 29.6% and 60% MR-CoNS respectively which was lower than our study. In this study, the prevalence of MR-CoNS among HIV-infected patients was found to be 53.8%. This finding was lower than previous studies from India by Sharma et al. (2011) and Nazir et al. (2014) which reports 88% and 83% respectively.

In this study, nasal carriage rate of MR-CoNS among ESRD patients and hospital personnel was found to be 58.5%. This finding was significantly higher compared to a report by Koziol-Montewka et al. (2006) from Poland which showed a lower carriage rate (38.4%). The nasal carriage rate of MR-CoNS among healthy individuals included in this study was found to be 48.7% which is higher than previous reports (Silva et al., 2001; Jamaluddin et al., 2008; Ruppe et al., 2009). The impact of antibiotic selective pressure could be the reason for this high overall prevalence of carriage of MR-CoNS (Lebeaux et al., 2012).
6.2. Distribution of MR-CoNS species among various groups

CoNS found in the normal skin flora and mucous membranes have recently attracted our attention as a potential pathogen, especially causing nosocomial infections. Further, it also serves as a reservoir for resistance genes. Many laboratories do not identify clinical isolates of CoNS to the species level as they are considered normal inhabitants of healthy skin. The species identification is important in monitoring the distribution of CoNS involved in infection both in the hospital and community settings.

In our study, *S. epidermidis* was the most predominant species 40 (44.4%) from clinical isolates from hospitalized patients followed by *S. haemolyticus* 27 (30%), *S. saprophyticus* 8 (8.8%), *S. hominis* 6 (6.7%), *S. capitis* 5 (5.5%) and *S. warneri* 4 (4.4%). The findings of this study are consistent with the previous reports in India and world-wide (Koksal *et al.*, 2009; Sharma *et al.*, 2010; Zong *et al.*, 2011, Talebi *et al.*, 2015). Among clinical isolates from HIV-infected patients, *S. haemolyticus* 35 (50%) was the predominant species followed by *S. epidermidis* 17 (24.3%), *S. hominis* 3 (4.3%), *S. saprophyticus* 7 (10%), *S. lugdunensis* 5 (7.1%) and *S. cohnii* 3 (4.3%) which was in concordance with the previous study by Jain *et al.* (2004).

In community settings, *S. epidermidis* appears to be a major reservoir of methicillin resistance dissemination within the community. *S. epidermidis* represents 69 to 84% of the MR-CoNS isolates in previous studies among community settings (Ruppe *et al.*, 2009; Barbier *et al.*, 2010). Among nasal carriage
of ESRD patients and hospital personnel in this study, *S. epidermidis* 56 (71%) was
the predominant species followed by *S. haemolyticus* 12 (14.6%), *S. hominis* 6
(7.3%) and *S. capitis* 5 (6.1%). This was in agreement with the previous study by
Koziol-Montewka et al. (2006). Among the 75 MR-CoNS isolates from
asymptomatic healthy individuals, *S. epidermidis* accounted for 41.3% of the
isolates.

Previous studies on community associated CoNS isolates reported similar
results (Lebeaux et al., 2012; Oliveira and Cerca, 2013). Besides *S. epidermidis*,
other MR-CoNS species were isolated: *S. haemolyticus* (26.7%), *S. hominis* (21.3%)
and *S. capitis* (10.7%). Although *S. epidermidis* was the most frequent species
isolated, these results revealed a great diversity of staphylococcal flora from nasal
colonization, which may explain the increasing importance of other CoNS species
in human infections.

6.3. **Prevalence of SCCmec types**

The interest in MR-CoNS and the genetic structure of the SCCmec has
increased in the last decade, which is mainly due to the increase of community-
acquired MRSA (CA-MRSA) found in patients with no contact with MRSA positive
patients or previous contact with hospitals or other health care facilities. Methicillin
resistance conferred by *meca* is carried on the mobile genetic element called
SCCmec.

The prevalence of SCCmec types among MR-CoNS from both hospital and
community settings varies depending on the host species, various environments and
geographical locations. SCCmec elements are more diverse in MR-CoNS, with new variants of ccr genes continuing to be identified (Zong et al., 2011). In this study, 5 different SCCmec types (type I-V) and various combination of SCCmec types (I+V, II+V, III+IV, IV+V) were detected using multiplex PCR and simplex PCR targeting ccr and the mec gene complex.

Varied SCCmec types in MR-CoNS have been distributed and are dominant in different countries. SCCmec type III has been found to be most prevalent in southern Brazil and China (Machado et al., 2007; Zong et al., 2011), whereas SCCmec type IV has been reported to be most common in France (Lebeaux et al., 2012) and Finland (Wisplinghof et al., 2003).

Among 90 clinical isolates from hospitalized patients, 78 had a single SCCmec type including type I (n = 45), type II (n = 2), type III (n = 4), type IV (n = 18, type V (n = 9) and combination type I+V (n = 4) were detected. Eight isolates were found to be non-typeable (NT). Among 70 isolates from HIV-infected patients, type I (n = 38) was predominant type followed by type V (n = 11), type IV (n = 9), type III (n = 7) and type I+V (n = 5). Our finding was found to be in concordance with the previous studies where large number of isolates harbored type I, which indicates the characteristic of MR-CoNS strains that are predominant in healthcare settings (Machado et al., 2007; Ternes et al., 2013).

In this study, eight (8.8%) isolates were found to be NT among hospitalized patients by method of Boye et al. (2007) and Kondo et al. (2007). These eight isolates were found to be positive for the mec gene complex alone, whereas ccr
genes could not be amplified. The ccr regions might be deleted or may contain
mutations in the primer-targeting regions in these NT SCCmec (Bouchami et al.,
2011; Hanssen and Ericson Sollid, 2006).

Among 79 MR-CoNS isolates from nasal carriage of ESRD patients and
hospital personnel, type IV (n = 27) was predominant type followed by type I (n =
18), type V (n = 15), type II (n = 2) and combination type which included I+V (n =
8), III+IV (n = 6) and II+V (n = 3).

Among 75 MR-CoNS isolates from nasal carriage of asymptomatic healthy
individuals, type IV (n = 22) was the predominant type followed by type I (n = 17),
type II (n = 15), type V (n = 10), type III (n = 2) and combination type which
included I+V (n = 1), II+V (n = 2), III+IV (n = 2) and IV+V (n = 4).

Previous studies highlighting the community spread of MR-CoNS have
raised concerns, because of the probable role of MR-CoNS as a source of SCCmec
for CA-MRSA and the increasing prevalence of CoNS in community acquired
diseases, such as native valve endocarditis and late infections of indwelling
prosthetic devices (Chu et al., 2008; Mermel et al., 2009).

In this study, the great genetic diversity of SCCmec types and combination
of two types were found to be in nasal carriage of ESRD patients and hospital
personnel and asymptomatic healthy individuals.

The high number of different SCCmec types present in MR-CoNS can build
up a large reservoir of new SCCmec types for S. aureus and probably facilitate
horizontal transmission between Staphylococcus species. The co-existence of two
SCCmec elements appears to be common in MR-CoNS which strongly suggests that new variants may be present in CoNS and may have a different impact on drug resistance. Due to the various combinations of different SCCmec types in the MR-CoNS observed in this study and reported by others (Bouchami et al., 2011; Zong et al., 2011), there is a clear need to develop a unique typing system for CoNS. The frequent co-existence of SCCmec and the NT elements highlights the great genetic diversity and the need for developing classification schemes for SCCmec in MR-CoNS.

6.4. Prevalence of antibiotic resistance

Antibiotic resistance is a multifactorial phenomenon and high rates of antibiotic consumption along with their misuse are pivotal factors that have created this serious public health issue. Moreover, it is currently known that antibiotic molecules are widely disseminated in a broad range of environmental sources. Hence, their presence in different ecological niches may also account for the local selection of resistant bacteria.

MR-CoNS gained much importance mainly because of its potential multi-drug resistance to antibiotics in addition to β-lactam antibiotics. During the late 1990s and early 2000, significant difference in the antibiotic resistance of MR-CoNS isolates causing hospital-associated and community-associated infections was reported. Recent reports showed that the community acquired MR-CoNS were found to be multi-drug resistant (Lebeaux et al., 2012; Oliveira and Cerca, 2013). The increasing multidrug resistance (MDR) among MR-CoNS poses a great
challenge for the management of hospital-acquired infections and also serves as a reservoir of antibiotic resistance genes.

In this study, the prevalence of antibiotic resistance between MR-CoNS isolates of hospitalized patients, HIV-infected patients, nasal carriage of ESRD patients and hospital personnel and asymptomatic healthy individuals were compared. All the MR-CoNS isolates included in this study were found to be susceptible to glycopeptides. Among MR-CoNS isolates, highest resistance was observed for TMP-SMX (49.4%) followed by ciprofloxacin (38.2%), erythromycin (36%), gentamicin (35.7%), ofloxacin (33.4%), amikacin (26%) and clindamycin (17.2%). Comparatively, low level resistance was observed for tetracycline (17%), mupirocin (16.2%) and rifampicin (11.8%).

6.4a. Aminoglycoside Resistance

Aminoglycosides have broad-spectrum of antibacterial activity used as primary treatment for staphylococcal infections, but are used more frequently in combination with β-lactams or glycopeptides. Resistance has been reported worldwide. All the isolates included in this study were tested for susceptibility to the following aminoglycosides: amikacin, gentamicin and netilmicin. Highest resistance was observed for gentamicin, followed by amikacin and netilmicin.

Among MR-CoNS isolates, the gentamicin resistance was found to be higher among clinical isolates from hospitalized patients (45%) than isolates from HIV-infected patients (41.4%). These findings are found to be higher than previous reports from Indian study by Sharma et al. (2011), while it is low when compared
to the reports from rest of the world (Koksal et al., 2009; Bouchami et al., 2011; Talebi et al., 2015).

25.3% of the nasal carriage isolates from ESRD patients and hospital personnel and 24% of asymptomatic healthy individuals isolates respectively were found to be resistant to gentamicin respectively, which was consistent with previous report by Oliveira and Cerca, (2013), while the resistance was found to be higher than that in community settings in a previous study by Lebeaux et al. (2012). The prevalence of gentamicin resistance was significantly higher among MR-CoNS isolates from hospital settings than community settings (P<0.005).

Among MR-CoNS isolates, amikacin resistance was found to be higher among HIV-infected patients (34.3%) than clinical isolates from hospitalized patients (33.3%) which was lower than previous report by Ardic et al. (2006) and who reported 48% amikacin resistance. This report is higher than the previous report by Talebi et al. (2015). Significant difference in amikacin resistance was observed between MR-CoNS isolates from hospital settings and carrier isolates (p<0.005).

In this study, netilmicin resistance was only observed among MR-CoNS isolates from HIV-infected patients (27.1%) which was higher than the previous report by Ardic et al. (2006). In this study, isolates from group I, group III and group IV were found to be susceptible to netilmicin. This is probably due to their limited use in treating the staphylococcal infections.
6.4b. Erythromycin resistance

Erythromycin is a useful antibacterial against MR-CoNS as it is safe to use in patients with hypersensitivity to penicillins and other β-lactams. In this study, erythromycin resistance was found to be (36.7%) and (45.7%) among MR-CoNS isolates from hospitalized patients and isolates from HIV-infected patients which is lower than the previous reports (Koksal et al., 2009; Bouchami et al., 2011; Talebi et al., 2015).

Among ESRD patients and hospital personnel 35.4% of isolates was found to be resistant to erythromycin. This study was in agreement with the previous report by Lebeaux et al. (2012) who reported 34% of MR-CoNS to be erythromycin resistant. 26.7% of isolates from asymptomatic healthy individuals showed resistance to erythromycin which was comparatively lower than the previous studies by Silva et al. (2001) and Oliveira and Cerca, (2013). There was no significant difference in the prevalence of erythromycin resistance of MR-CoNS between the carrier isolates and the clinical isolates from hospitalized patients (p<0.005).

6.4c. Clindamycin resistance

Clindamycin is effective against both the methicillin resistant and the methicillin sensitive staphylococcal infections. There have been various reports on the pattern of the MLS$_B$ resistance among the staphylococci; some reports indicate a high prevalence of the iMLS$_B$ phenotype, while the others indicate an increasing frequency of the cMLS$_B$ phenotype. There was no significant difference in the
prevalence of clindamycin resistance of MR-CoNS between the carrier isolates and the clinical isolates from hospitalized patients (p<0.005).

Overall, 48% of MR-CoNS isolates showed cMLS$_B$ resistance in our study which was lower than the previous studies, which reported 50%, and 51% by Koziol-Montewka et al. (2006) and Gatermann et al. (2007) respectively. Our study reports 52% of isolates to be iMLS$_B$ phenotype, which is higher than the previous study by Juyal et al. (2013).

6.4d. Quinolone resistance

In this study, ciprofloxacin resistance was second only to TMP-SMX, highest resistance was observed for ciprofloxacin among MR-CoNS isolates from clinical isolates of hospitalized patients (39%), HIV-infected patients (54.3%), nasal carriage of ESRD patients and hospital personnel (35.4%) and asymptomatic healthy individuals (25.3%). There was no statistical difference among various groups. In a study from North India by Sharma et al. (2010), about 47.6% isolates causing infections were found to be ciprofloxacin resistant, which is higher than the ciprofloxacin resistance in the present study among hospitalized patients and lower than in HIV-infected patients. 35.4% of nasal carriage of ESRD patients and hospital personnel and 25.3% of asymptomatic healthy individuals of MR-CoNS carrier isolates were found to be resistant to ciprofloxacin respectively. Our study reported higher resistance than the previous study, which reported 10% and 7% by Koziol-Montewka et al. (2006) and Oliveira and Cerca, (2013) respectively. No
significant difference in ciprofloxacin resistance was observed between MR-CoNS isolates from hospital and community settings (p<0.005).

6.4e. **Trimethoprim-Sulfamethoxazole (TMP-SMX) Resistance**

In this study, 58% of MR-CoNS isolates from hospitalized patients, 67.1% of MR-CoNS from HIV-infected patients, 48% of isolates from nasal carriage of ESRD patients and hospital personnel and 24% of isolates from asymptomatic healthy individuals were found to be resistant to TMP-SMX. The prevalence of resistance is higher among hospitalized patients and HIV-infected patients when compared to the isolates from nasal carriage of ESRD patients and hospital personnel and asymptomatic healthy individuals. Significant difference in TMP-SMX resistance was observed between hospital and community settings of MR-CoNS isolates included in this study (p<0.005).

The highest resistance to TMP-SMX among MR-CoNS isolates from HIV-infected patients may be due to the extensive use of TMP-SMX in HIV-infected patients, administered as a prophylactic therapy for opportunistic infections.

In this study, 58% of isolates from hospitalized patients, 67.1% of isolates from HIV-infected patients were found to be TMP-SMX resistant which was lower than the previous studies, which reported 71% and 78.5% from hospitalized patients by Bouchami et al. (2011) and Talebi et al. (2015) respectively. 48% of nasal carriage of ESRD patients and hospital personnel exhibited resistance to TMP-SMX which was higher than the previous study by Koziol-Montewka et al. (2006) who reported 40%. 24% of isolates from asymptomatic healthy individuals showed
resistance to TMP-SMX. The results were higher than the previous reports by Silva et al. (2001) and Barbier et al. (2010), which reported 27% and 21.6% respectively resistance to TMP-SMX.

**6.4f. Tetracycline resistance**

In this study, 15.5% and 14.3% MR-CoNS isolates exhibited resistance to tetracycline among MR-CoNS isolates of hospitalized patients and HIV-infected patients respectively. Similar results have been reported by Bouchami et al. (2011), but Koksal et al. (2009), Duran et al. (2012) and Talebi et al. (2015) reported much higher tetracycline resistance - 60%, 30.8% and 34% respectively unlike our study from hospital settings. 23% of carrier isolates from ESRD patients and hospital personnel and 16% of isolates from asymptomatic healthy individuals were found to be resistant to tetracycline which was not supported by previous studies by Koziol-Montewka et al. (2006) and Silva et al. (2001) which reported 70% and 62% resistance respectively to tetracycline. However, no significant difference in tetracycline resistance was observed between the hospital and community settings (p<0.005).

**6.4g. Rifampicin resistance**

The prevalence of rifampicin resistance is higher among HIV-infected patients (34.3%) when compared to hospitalized patients (10%) and nasal carriage of ESRD patients and hospital personnel (5%). All the nasal carriage isolates among asymptomatic healthy individuals were found to be susceptible to rifampicin. The high rifampicin resistance among MR-CoNS isolates from HIV-infected patients
may be due to the extensive use of rifampicin in HIV/TB coinfected patients to treat the tuberculosis. The prevalence of rifampicin resistance is significantly high among clinical isolates of MR-CoNS from HIV-infected patients when compared to the clinical isolates from hospitalized patients and asymptomatic healthy individuals (p<0.005).

6.4h. Mupirocin resistance

Mupirocin is a topical antibiotic and has successfully been used to treat various staphylococcal skin infections and plays a key role in the eradication of intranasal staphylococci. It inhibits bacterial protein synthesis by binding to isoleucyl t-RNA synthetase (IleS). With increasing pressure to prevent staphylococcal infection as a part of infection control policy, mupirocin has been used extensively for decolonization and prophylaxis in hospitalized patients.

Mupirocin has also been used for treating the skin and soft tissue infections. Our study reports 33% of MR-CoNS isolates from nasal carriage of ESRD patients and hospital personnel to be positive for high level mupirocin resistance by mupirocin (200µg) disc diffusion test and is higher than previous report from South India (Oomen et al., 2010).

Varying rates of mupirocin resistance has been reported from developed countries. In our study, 16.7% and 7.1% among clinical isolates from hospitalized patients and HIV-infected patients were found to be resistant to mupirocin which is higher than the previous studies from Netherlands and France (Bathoorn et al., 2012; Desroches et al., 2013) respectively. None of the isolates included in this
study showed low-level resistance, which was in agreement with the previous report by Yun et al. (2003) from South Korea. The prevalence of resistance is significantly higher among nasal carriage in ESRD patients and hospital personnel when compared to the MR-CoNS from hospital settings and carrier isolates from asymptomatic healthy individuals (p<0.005).

6.4i. Fusidic acid resistance

Fusidic acid is a bacterial protein synthesis inhibitor used to treat skin, bone and joint infections of staphylococci. Resistance to fusidic acid emerged early during the clinical use of this compound, but resistance rates are considered to be low (Turnidge and Collignon, 1999). Overall, in this study, 8% of isolates were found to be resistant to fusidic acid which is very low when compared with other previous reports from countries, such as Turkey (40%), Tunisia (62.2%) and Iran (26%) by Deveci et al. (2011), Bouchami et al. (2011) and Talebi et al. (2015) respectively.

6.4j. Linezolid resistance

Linezolid, a member of the oxazolidinone class of antibiotics, has been an effective therapeutic option to treat severe infections caused by MDR Gram positive bacteria. However, linezolid resistant strains have been increasingly reported in India and worldwide (Mendes et al., 2008; Rajan et al., 2014; Bender et al., 2015).

In our study, three isolates [HIV-infected patients - one; nasal carriage of ESRD patients and hospital personnel - two] were found to be resistant to linezolid. There have been a few reports of linezolid resistant CoNS from India (Peer et al.,
2011; Gupta et al., 2012; Rajan et al., 2014). The low occurrence of linezolid resistance is mainly attributed to the absolute synthetic nature of this antibiotic for which natural resistance genes are not widely distributed.

Alarming rise in MR-CoNS is limiting the utility of all β-lactam agents, thus considerably limiting the therapeutic options. Linezolid is a viable solution for methicillin resistant staphylococcal isolates. However, emerging resistance to linezolid is a matter of great concern. It is high time that we recognize the far reaching consequences posed by such a great threat and closely monitor and track resistance to linezolid by prospective resistance surveillance studies, particularly where frequent and extended linezolid therapy is used.

6.5. Prevalence of antibiotic resistant determinants of various groups

The acquisition of MDR determinants is a characteristic of MR-CoNS making them dangerous pathogens thereby complicating their successful treatment and control. The accurate and rapid detection of various antibiotic resistance genes is important in preventing the spread of infections caused by MR-CoNS among hospitalized patients. The detection of antibiotic resistant genes among staphylococcal carriage of ESRD patients and hospital personnel and asymptomatic healthy individuals is extremely important for both surveillance and to prevent the spread of bacterial infection. Hence, the present study was aimed to detect various antibiotic resistant genes: AMEs [\(\text{aac(6\text{'})-Ie-aph(2\text{''})-Ia}\), \(\text{aph(3\text{'})- IIIa}\), and \(\text{ant(4\text{'})-Ia}\)], MLS\(_B\) and TMP-SMX resistance [\(\text{msrA, erm(C) erm(A) and dfrA}\)], Tetracycline
[tetK and tetM], mupirocin [mupA], fusidic acid [fusB, fusC and fusD] and linezolid [cfr and 23S rRNA mutation]. Since the ecological niche and the selective pressure involved with each group varies, a difference in the antimicrobial resistant gene content was expected and the same was evaluated using chi square method.

6.5a. Aminoglycoside modifying enzymes (AME)

Aminoglycoside modifying enzyme (AME) genes were investigated using M-PCR:

Aminocyclitol-6’-acetyltransferase-aminocyclitol-2”-phosphotransferase

\[ [aac(6')-Ie-aph(2'')-Ia] \] gene (encoding bifunctional acetyltransferases/phosphotransferases), aminocyclitol-4’-adenyltransferase [ant(4’)-Ia] gene (encoding nucleotidyl transferases) and aminocyclitol-3’ phosphotransferase [aph(3’)-IIIa] gene (encoding phosphotransferases).

In this study, \[ aac(6')-Ie-aph(2'')-Ia \] was the most prevalent AME gene among four groups, group I - 37.8%, group II - 40%, group III - 21.5% and group IV - 13.3% followed by the \[ aph(3')- IIIa \] - 31.1%, 31.4%, 6.3% and 4% respectively. The prevalence of \[ ant (4')-Ia \] gene was found to be group I - 4.4% and group II - 20%, whereas it was negative in group III and group IV.

Similar results were also obtained in a study by Duran et al. (2012) who reported that the prevalence of \[ aac(6')-Ie-aph(2'')-Ia \] (16.4%) was highest followed by \[ aph(3')- IIIa \] (10.1%) and \[ ant(4')-Ia \] (5.7%) gene. In contrast to our findings, Bouchami et al. (2011) reported that the prevalence of the of the \[ aac(6')-Ie-aph(2'')-Ia \]...
The **Ia** (33.3%) gene was much higher than that of the other two AME genes **ant(4')-Ia** (7.1%) and **aph(3')-IIIA** (2.4%).

In contrast to our findings, Ida et al. (2001) reported that in a study they carried out in Japan, the prevalence of the **ant(4')-Ia** (84.5%) gene was much higher than that of the other two AME genes, **aac(6')-Ie-aph(2")-Ia** (61.7%) and **aph(3')-IIIA** (8.9%). There was statistical significance for all the three AME genes among all the four groups included in this study (p<0.005).

### 6.5b. Erythromycin and clindamycin resistance determinants

A large number of erythromycin resistant strains harbored **msrA** gene (24.5%), followed by **erm(C)** and **erm(A)** genes with (11.5%) and (1.27%) respectively. Similar results were also obtained in a study by Bouchami et al. (2011). In contrast to our findings, Duran et al. (2012) reported that the prevalence of the **erm(C)** gene was much higher than that of the other **erm** genes.

The **msrA** gene is widespread in CoNS more than in *S. aureus* (Lina et al., 1999), it is located on large plasmids and may be associated with the **erm(C)** (Barriere et al., 1998). This could explain the diffusion of these two genes in our study. There was no statistical significance for all the three macrolide resistant genes among all the four groups included in this study (p<0.005).

### 6.5c Tetracycline resistance determinants

Among tetracycline resistance genes (**tetK** and **tetM**), all the four groups harbored **tetK** gene [15.5% (14/90), 14.3% (10/70), 23% (18/79) and 16% (12/75)] respectively, whereas **tetM** gene was not detected in our study isolates. Previous
studies reported both tetK and tetM genes (Bouchami et al., 2011; Duran et al., 2012). In fact, the presence of tetK gene on small multicopy plasmids and tetM on conjugative transposons contributes to the spread of these determinants. The isolates which harbored tetK gene are susceptible to minocycline, resistant to tetracycline, whereas minocycline-resistant strains harbor tetM or both tetK and tetM gene. No statistical significance was observed for the prevalence of tetK gene among all the four groups included in this study (p<0.005).

6.5d. Trimethoprim-Sulfamethoxazole (TMP-SMX) Resistance determinant

In this study, 58% of MR-CoNS isolates from hospitalized patients, 67.1% of MR-CoNS from HIV-infected patients, 48% of isolates from nasal carriage of ESRD patients and hospital personnel and 24% of isolates from asymptomatic healthy individuals were found to be positive for dfrA gene. The prevalence of dfrA gene is higher among hospitalized patients and HIV-infected patients when compared to the isolates from nasal carriage of ESRD patients and hospital personnel and asymptomatic healthy individuals. Significant difference in dfrA gene was observed between hospital and community settings of MR-CoNS isolates included in this study (p<0.005).

The highest resistance to TMP-SMX among MR-CoNS isolates from HIV-infected patients may be due to the extensive use of TMP-SMX in HIV-infected patients, administered as a prophylactic therapy for opportunistic infections.
6.5e. Mupirocin resistance determinants

High-level resistance to mupirocin is conferred by the plasmid mediated ileS gene (ileS2) also known as mupA gene. Previous studies suggested that mupirocin resistant CoNS might be an important source of the mupA determinant for MRSA (Bathoorn et al., 2012).

In the present study, 16.2% isolates were found to show high-level mupirocin resistance which was confirmed by mupA gene PCR. Mupirocin resistance was higher among ESRD patients and hospital personnel (33%) than among clinical isolates from hospitalized patients (16.7%) and HIV-infected patients (7.1%). This could be attributed to the fact that mupirocin is used to treat superficial skin infections and for decolonization of anterior nares of staphylococcal carriers thus posing a threat for the use of mupirocin for decolonization of anterior nares of carriers. Statistical significance was observed for mupA gene among all the four groups included in this study (p<0.005).

A nation-wide study conducted in France concluded that the prevalence of mupA harboring clinical isolates of CoNS reached 6.5% (46/708), which was in line with the 8% reported by Bathoorn et al., in 2012 (Desroches et al., 2013). In 2010, Oomen et al., from India reported the presence of 16% mupirocin resistant CoNS among hospital isolates by phenotypic methods which is lower than the findings of the present study.

The present study indicates increasing prevalence of high level mupirocin resistance mediated by mobile elements among CoNS which substantiates their role...
as a putative reservoir of \textit{mupA} resistance gene for MRSA. Prolonged, widespread or uncontrolled use and multiple courses of mupirocin are all associated with the development of mupirocin resistance. Thus, continued surveillance for mupirocin resistance is needed in order to avoid decolonization and treatment failure using mupirocin.

\textbf{6.5f. Fusidic acid resistance determinants}

Fusidic acid is a bacterial protein synthesis inhibitor used to treat skin, bone and joint infections caused by staphylococci. Plasmid mediated resistance has also been described and genes encoding proteins that play a protective role in EF-G were recently identified. Several fusidic acid resistance determinants (mutational and acquired determinants) have been reported. High-level fusidic acid resistance has been associated with the mutational determinants (\textit{fusA} & \textit{fusE}). However, acquired fusidic acid resistance determinants (\textit{fusB}, \textit{fusC} & \textit{fusD}) has been reported to be reason for majority of the fusidic acid resistance (Castanheira \textit{et al.}, 2010). Among fusidic acid determinants, \textit{fusB} was the most prevalent gene in \textit{S. aureus} and \textit{S. epidermidis}.

In this study, all the isolates from group II were found to be susceptible and \textit{fusD} gene was absent in all the study isolates. Our study reports 25/314 (8\%) isolates to be fusidic acid resistant. Among fusidic acid resistant genes, \textit{fusB} gene was prevalent in all the three groups [group I - 9/90; 10\%, group III - 5/79; 6.3\% and group IV - 4/75; 5.3\%], whereas \textit{fusC} gene was harbored by 7 isolates [group I - 3/90; 3.3\%, group III - 2/79; 2.5\% and group IV - 2/75; 2.7\%]. An interesting finding
was that both fusB and fusC was totally absent in group II. Our study report was in agreement with the previous report by Castanheira et al. (2010). There was no statistical significance for fusidic acid resistant genes among all the four groups included in this study (p<0.005).

6.5g. **Linezolid resistance determinants**

Resistance to linezolid is primarily caused by mutations in the domain V of 23S rRNA gene, mutations in the ribosomal proteins L3, L4 and L22 or methylation at C-8 position of A2503 of the 23S rRNA by a methyl transferase encoded by the cfr gene. Co-occurrence of cfr-mediated resistance and mutational resistance has also been documented (Long and Vester, 2012). Although rare (<1% in *Staphylococcus aureus*, and <2% in CoNS), linezolid resistance is currently on the rise and emergence of bacterial strains with multiple mechanisms of linezolid resistance is a cause of concern in antimicrobial chemotherapy.

In this study, linezolid resistance was found in two isolates from ESRD patients and hospital personnel and in one isolate from HIV-infected patients. All the isolates harbored cfr gene and mutations in 23S rRNA gene (G2576T). This is the most frequent mutation (G2576T) in linezolid resistant strains and it has been previously reported (de Almeida et al., 2012). Of these, more worrisome is the presence of cfr gene in the isolate as it is usually located in an unstable genetic environment either on the chromosome or on MDR plasmids. This would facilitate the easy spread of cfr into susceptible population and other pathogenic bacteria.
Furthermore, *cfr*-mediated resistance limits therapeutic options as it encodes resistance to an array of antibiotics.

Although the prevalence of resistance to linezolid remains low, the emergence of linezolid resistant CoNS should prompt increased attention, especially for the horizontal dissemination of *cfr*, and surveillance of linezolid resistance in CoNS is of increasing importance.

6.6. **Prevalence of virulence genes among MRSE isolates from various groups**

**Detection of biofilm formation by phenotypic and genotypic methods**

*S. epidermidis*, although an important commensal, has emerged as the most significant pathogen in infections related to implanted foreign body materials. These infections are often long-lasting, difficult to treat, and involve biofilm formation (Vuong and Otto, 2002). Certain strains of *S. epidermidis* are capable of forming biofilms on such devices which represents the most important virulence determinant which plays a key role in their successful colonization and pathogenesis.

Major genes involved in *S. epidermidis* biofilm formation include autolysin E (*atlE*), accumulation associated protein (*aap*) and intercellular adhesion (*ica*) known as *icaADBC* operon. In the operon, *ica* encodes for N-acetylglucosaminyl transferase, the enzyme for polysaccharide intercellular adhesin (PIA). However, the co-expression of *icaA* and *icaD* leads to significant increase in the activity and is related to the full phenotypic expression of the capsular polysaccharide (Gerke *et al.*, 1998).
In this study, a total of 144 MRSE isolates from four groups [hospitalized patients n = 40, HIV-infected patients n = 17, nasal carriage of ESRD patients and hospital personnel n = 56 and nasal carriage of asymptomatic healthy individuals n = 31] were included and compared for their biofilm production and genetic traits (icaAD, aap and atlE) and the presence of IS256 element.

6.6a. Prevalence of biofilm formation by CRA method

The present study reported that biofilm-producing MRSE isolates was higher among clinical isolates than the carrier isolates. Our study report was in agreement with the other previous studies (Araujo et al., 2006; Du et al., 2013; Hellmark et al., 2013). In this study, the biofilm production by isolates of nasal carriage of ESRD patients and hospital personnel and that of asymptomatic healthy individuals was (57%) and (58%) respectively. Similar results have been reported by other researchers, who observed a significant number of isolates capable of producing biofilm among S. epidermidis obtained from nasal carriers (Farran et al., 2013; Oliveira and Cerca, 2013).

6.6b. PCR based detection of icaAD, aap and atlE genes

Using PCR, all the MRSE isolates were screened for the presence of icaAD, aap and atlE genes and evaluated the association of these genes with biofilm-forming capacity, both in ica+ and ica− backgrounds. In addition to ica gene, other genes have been associated with biofilm formation by S. epidermidis, including aap and atlE genes. The ica operon is considered one of the main genetic determinants of biofilm formation involved in the accumulation phase during biofilm formation.
including *aap* and *atlE* genes and its detection has been suggested as a tool for discriminating invasive from contaminating strains in clinical specimens (Rohde *et al*., 2007; Klingenberg *et al*., 2007).

In the present study, the majority of the biofilm-producing isolates harbored the *icaAD*, *aap* and *atlE* genes. The prevalence of *icaAD* was found to be 77.7%, 64.3%, and 32% from clinical isolates of hospitalized patients, nasal carriage of ESRD patients and hospital personnel and asymptomatic healthy individuals respectively.

The prevalence of *aap* was found to be 65.3%, 48% and 46.6% from clinical isolates of hospitalized patients, nasal carriage of ESRD patients and hospital personnel and nasal carriage of asymptomatic healthy individuals respectively.

The prevalence of *atlE* was found to be 88.8%, 32% and 28.8% from clinical isolates from hospitalized patients, nasal carriage of ESRD patients and hospital personnel and nasal carriage of asymptomatic healthy individuals respectively.

In addition to PIA, Aap and AtlE proteins have also been associated with biofilm formation by *S. epidermidis*. (Hussain *et al*., 1997; Vandecasteele *et al*., 2003). The results of the study indicates that the great majority of biofilm-producing MRSE isolates harbored the *ica* operon and the genes *aap* and *atlE*. With a few exceptions, *icaAD* gene was not detected from five isolates (clinical isolates from hospitalized patients - 1; nasal carriage of ESRD patients and hospital personnel - 4) and they exhibited the *icaAD*aap*atlE* genotype which was in agreement with the previous studies (Petrelli *et al*., 2006; Farran *et al*., 2013).
These results indicate that the isolates exhibiting $icaAD^{aap^+atlE^-}$ genotype seemed to be an important factor for biofilm development independent of $ica$ operon. The presence of $ica$ operon and biofilm-formation makes $S. epidermidis$ among nasal carriage of asymptomatic individuals more capable of producing a chronic infection and surviving adverse conditions. Hence, measures have to be taken to reduce the risk of hospital-acquired $S. epidermidis$ infections.

Previous studies suggested the $ica$ operon as a marker to differentiate the invasive and commensal $S. epidermidis$ isolates (Klingenberg et al., 2007; Monk et al., 2008). In the present study, $ica$ gene was present in 64.3% from nasal carriage of ESRD patients and hospital personnel and 64.5% MRSE isolates from asymptomatic healthy individuals. Thus, these data indicate that despite their role of biofilm production in $S. epidermidis$ infections, both biofilm formation and the genes associated with this phenotype should not be used as markers for clinical significance.

6.6c. Prevalence of IS256 (genetic marker for virulent $S. epidermidis$)

IS (insertion sequence) 256 is a common insertion sequence in Gram-positive cocci. IS256 family of insertion sequences have lengths of between 1298bp (ISRm3) to 1486bp (IS6120). Members carry related inverted terminal repeats of between 24 and 41 bp, and most generate 8 bp direct target repeats, but some 9 bp duplications have been observed for some members. They may also carry internal IRs close to the ends.
The founding member, IS256, was originally isolated as component of the compound transposon Tn4001 which possesses the aminoglycoside modifying enzyme. Some evidence suggests that IS256 generates transposon circles and can form circular copies of the Tn4001 transposon from which it was originally characterized.

Previous studies have reported IS256 as an important factor for *S. epidermidis* infectivity (Gu *et al.*, 2005; Du *et al.*, 2013; Mertens *et al.*, 2013) and marker to differentiate the invasive and commensal *S. epidermidis* isolates. About 80% and 76.4% of clinical isolates from hospitalized patients and HIV-infected patients were IS256 positive, whereas 28.6% of isolates showed presence of IS256 among isolates from nasal carriage of ESRD patients and hospital personnel and 13% of isolates showed presence of IS256 in asymptomatic healthy individuals. Supporting our data, Gu *et al.* (2005) and Du *et al.* (2013) determined similar results and concluded that IS256 is a good marker to differentiate between invasive and non-invasive isolates.

Hence, findings of the present study has clearly indicated that the bacterial insertion sequence element IS256 occurred more significantly in strains of clinical origin indicating that IS256 might thus constitute a molecular marker to discriminate invasive strains from commensal strains of *S. epidermidis*. 
6.6d. Detection of Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs)

*S. epidermidis* infection begins with bacterial adhesion to host tissues. MSCRAMMs refer to a family of cell surface adhesins, which specifically binds to the collagen, enolase, elastin and bone sialoproteins. In this study, all the MRSE isolates were found to be negative for *cna* gene. *eno* was the predominant gene among MSCRAMMs genes screened among the four Groups showing - 67.5% (27/40), 47% (8/17), 57% (32/56), 61.3% (19/31) respectively for group I, group II, group III and group IV followed by *ebps* 20% (8/40), 35.3% (6/17), 21.4% (12/56), 19.4% (6/31) and *bbp* - 12.5% (5/40), 0% (0/17), 5.3% (3/56), 6.4% (2/31) were found to be positive for Group I, Group II, Group III and Group IV respectively. In this study, no significant difference was observed between various groups in the prevalence of *ebps, eno*, and *bbp* (p<0.005).

6.6e. Prevalence of ACME among MRSE isolates from various groups

ACME, a novel genomic island may contribute to the enhanced capacity of this species to colonize the human skin and mucosal surfaces. The ACME, which is integrated into the genome downstream of the SCCmec at the *orfX* site, is located in close proximity to the SCCmec. The mobilisation of ACME is believed to use the same cassette chromosome recombinases (*ccrA* and *ccrB*) as SCCmec.

In this study, ACME was more prevalent in carrier isolates from asymptomatic healthy individuals (61.3%) compared to ESRD patients and hospital personnel (37.5%) and clinical isolates from hospitalized patients (22.5%) and all
the groups harbored ACME type I. This finding supports the notion that the ACME enhances the ability of *S. epidermidis* to colonise and spread within the community (Barbier *et al.*, 2011).

Study by Wisplinghoff *et al.* (2003) has shown a similarity in nucleotide sequence for SCCmec type IV in *S. epidermidis* and *S. aureus* and the gene transfer from *S. epidermidis* to *S. aureus* has also been shown. This similarity might be an indication that *S. epidermidis* functions as a reservoir and donor of genetic materials to *S. aureus*.

The present study also indicates that SCCmec IV contains predominantly ACME type I in community settings and together may act as a reservoir and donor of virulence and antibiotic resistance determinants to *S. aureus*. ACME was more common among the commensal isolates and may represent a survival benefit for *S. epidermidis* colonizing healthy individuals in the community. Within the community settings, the prevalence of ACME was significantly high (p<0.005).

### 6.7. Multi Locus Sequence Typing

Multilocus sequence typing (MLST) which examines the slowly evolving genomic core, is useful for defining ancestry and investigating the long-term global epidemiology and population structure of *S. epidermidis*. Molecular characterization of the representative MRSE isolates (n= 12) from the hospital and community settings showed high level of diversity within *S. epidermidis* which was also observed in other studies (Du *et al.*, 2013; Hellmark *et al.*, 2013). In this study,
*S. epidermidis* strains isolated from the hospital and community settings had a high level of genetic diversity, but the community population was even more diverse than the isolates from hospital settings.

The MLST result showed that the most represented sequence type was ST2 (25%) from clinical isolates of hospitalized patients (n= 2) and HIV-infected patients (n= 1). In our study, all (n= 3) ST2 isolates carried either SCC*mec* type III or IV, a finding that is in accordance with previous studies (Li *et al.*, 2009; Du *et al.*, 2013). ST2 is usually the most prevalent ST in previous epidemiological studies and well-recognized genotype among MRSE that causes nosocomial infections worldwide. It has been the most prevalent genotype reported in several studies especially in Blood stream infections and catheter-related infections (Iorio *et al.*, 2012; Widerstorm *et al.*, 2012; Du *et al.*, 2013).

No ST2 isolate was found in the community setting in the present study; it is likely that ST2 is highly adapted to the hospital environment and differs from commensal *S. epidermidis* in the community. It is possible that patients who are admitted to hospital are soon colonized by these biofilm-forming, MDR *S. epidermidis* isolates and that this newly acquired endogenous micro flora might represent the origin for a later infection.