7. SUMMARY AND CONCLUSION

- Methicillin resistant coagulase-negative staphylococci (MR-CoNS) are strains of CoNS which are resistant to penicillinase stable penicillins (oxacillin, methicillin) and pose a major health threat in both hospitalized patients as well as in healthy individuals all over the world.

- MR-CoNS isolates from four different populations, viz., hospitalized patients, HIV-infected patients, nasal swabs from ESRD patients and hospital personnel from a nephrology unit, and asymptomatic healthy individuals from various communities at risk, collected from Chennai, South India were studied for virulence and resistance determinants and epidemiological types by molecular methods.

- A total of 546 CoNS isolates including 127 from hospitalized patients, 130 from HIV-infected patients, 135 carrier isolates from ESRD patients and hospital personnel and 154 carrier isolates from asymptomatic healthy individuals in closed communities at high risk with no previous hospitalization were included for the study.

- The overall prevalence of MR-CoNS in this study was found to be 57.5%. The prevalence of MR-CoNS among hospitalized patients, HIV-infected patients, nasal carriage of ESRD patients and healthy personnel and nasal carriage from asymptomatic healthy individuals was found to be 70.8%, 53.8%, 58.5% and 48.7% respectively.
Overall (n=314), 8 different MR-CoNS species were identified from the four different groups. Species distribution showed S. epidermidis to be the predominant species in group I (44.4%), group III (71%) and group IV (41.3%), whereas S. haemolyticus was the predominant species from group II (50%), followed by S. epidermidis (24.3%).

SCCmec typing showed that overall, 5 types (I-V), combination types (type I+V, II+V, III+IV and IV+V) and NT elements of SCCmec were detected among MR-CoNS (n=314) isolates from four groups. SCCmec type I was the predominant type in group I (50%) and group II (54.3%), whereas type IV was predominant in group III (34%) and group IV (29.3%).

Among type IV subtypes, type IVa was found to be the predominant subtype among MR-CoNS from various groups included in this study.

All the tested isolates were sensitive to vancomycin. Highest resistance was observed towards TMP-SMX (49.4%).

About 28 (8.9%) and 26 (8.3%) MR-CoNS isolates were found to exhibit constitutive and inducible clindamycin resistance respectively (D-test positive, erm(A), erm(C) and msrA genes positive).

The overall prevalence of mupirocin resistance (mupA gene) in this study was found to be 16.2%. Mupirocin resistance was found to be significantly higher among group III (33%) compared to group I (16.7%), group II (7.1%) and group IV (6.7%).

About 8% of MR-CoNS isolates were found to be fusidic acid resistant and showed the presence of fusB/fusC.
- Overall, three isolates were found to be linezolid resistant and positive for cfr gene and mutation in domain V of 23S rRNA gene (G2576T).
- Multi-drug resistance was found to be significantly higher among clinical isolates (group I and group II) compared to carrier isolates (group III and group IV).
- All the MRSE isolates from various groups were subjected to screening for biofilm formation by CRA method and detection of associated genes (icaAD, aap and atlE genes).
- By CRA, 29/40 (72.5%), 10/17 (58.8%), 32/56 (57%) and 18/31 (58%) were found to be positive for biofilm formation among group I, group II, group III and group IV respectively.
- 19/40 (47.5%), 7/17 (41.2%), 22/56 (39.3%) and 11/31 (35.5%) from group I, group II, group III and group IV respectively were found to be positive for all the three genes tested, viz., icaAD⁺, aap⁺, atlE⁺. Overall, five isolates showed biofilm production even in the absence of ica genes and exhibited icaADatlE⁺ aap⁺ genotype.
- The present study showed that the prevalence of IS256 among clinical isolates was significantly higher among the carrier isolates from group III and group IV.
- The ACME type I was significantly higher among carrier isolates from group IV (61.3%) and group III (37.5%) compared to the clinical isolates of group I (22.5%) and group II (0%).
- A total of 12 representative MRSE isolates (3 isolates from each group) were analysed for MLST. In group I, 2 STs were identified, with two isolates belonging
to ST2 and one to ST5. The isolates of group II belonged to ST2, ST23 and ST243. Group III isolates belonged to ST23, ST36 and ST16 and group IV isolates belonged to ST28, ST85 and ST120.

To conclude, this study compares the molecular characteristics of resistance and virulence determinants of MR-CoNS from four different populations including a group of ESRD patients who neither belong to the community nor to the hospital owing to their treatment modality. Antibiotic resistance and virulence factors were significantly high among clinical isolates compared to carrier isolates from ESRD patients and hospital personnel and asymptomatic healthy individuals. Greater genetic diversity of SCCmec was observed among carrier isolates than clinical isolates from hospital settings.

The species identification of MR-CoNS could help in determining the contribution of each species to antibiotic resistance and SCCmec types in the hospital and community settings and help in designing effective surveillance and control strategies. This study brings new insights on the biodiversity and dynamics of MR-CoNS carriage in the ESRD patients and hospital personnel and asymptomatic healthy individuals provides further evidence of their role as facilitator for genetic diversity of SCCmec in this settings.

Furthermore, this study may partially explain why CoNS are becoming important nosocomial pathogens, despite very few recognized virulence factors viz., biofilm formation, ACME production and presence of IS256. 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.

This study substantiates the usefulness of investigating staphylococcal colonization of the nasal mucosa – the primary ecological niche for these microorganisms – in order to not only better understand the epidemiology of this phenomenon, but also to develop prevention measures and treatment strategies in case of established infections among predisposed patients such as those suffering from end-stage renal disease and undergoing hemodialysis treatment.
HIGHLIGHTS OF THIS DOCTORAL STUDY

셜 This is the first Indian report to study the virulence factors, resistance determinants and epidemiological typing of MR-CoNS isolates from hospitalized patients, HIV-infected patients, nasal carriage from ESRD patients and hospital personnel and asymptomatic healthy individuals from various communities at high risk.

셜 This is the first reported study from India to compare the resistance determinants, virulence factors and epidemiological typing among large number of MR-CoNS isolates from hospital and community settings.

셜 This is the first Indian study to report the great genetic diversity of SCCmec types (type I-V) and combinations types (type I+V, II+V, III+IV and IV+V) among MR-CoNS isolates from hospital and community settings.

셜 First study to report the high prevalence of mupirocin resistance (33%) among nasal carriage of ESRD patients and hospital personnel.

셜 This could be the first study to report fusB, fusC genes responsible for fusidic acid resistance among MR-CoNS from different groups.

셜 In our study three isolates were found to be linezolid resistant by phenotypic and genotypic methods (cfr and 23S rRNA gene mutation).

셜 This is the first study in world to detect the prevalence of various resistance and virulence genes among MRSE isolates from HIV-infected patients and nasal carriage of ESRD patients and hospital personnel.
Virulence factors and resistance determinants were found to be high among the clinical isolates than the carrier isolates.

To the best of our knowledge this could be the first study to compare virulence genes associated with biofilm production among four different groups which includes, clinical isolates from hospitalized patients, HIV-infected patients, carrier isolates from ESRD patients and hospital personnel and carrier isolates from asymptomatic healthy individuals.