5. RESULTS

5.1. Source of CoNS isolates

A total of 546 isolates of CoNS were collected between September 2011 to January 2015 from four different populations viz; hospitalized patients (n=127), HIV infected patients (n=130), ESRD patients and hospital personnel (n=135) and asymptomatic healthy individuals from closed communities (n=154).

5.1.1. Source of CoNS from clinical isolates from hospitalized patients (Group I) and HIV-infected patients (Group II)

Hospitalized patients (group I) included CoNS isolates from pus (n=33), intravenous catheters (n=32), blood (n=24), urine (n=22), throat swab (n=7), ascetic fluid (n=6) and synovial fluid (n=3) (Fig 5.1a).

CoNS isolates from HIV-infected patients (group II) were collected from the inpatient and outpatient settings. Majority of the isolates were obtained from the blood (n=45), followed by pus (n=34), catheters (n=18), sputum (n=16), broncho alveolar lavage (n=14) and synovial fluid (n=3) (Fig 5.1b).
5.1.2. Source of CoNS isolates from nasal carriage of ESRD patients and hospital personnel (Group III)

The study participants included 115 ESRD patients and 30 hospital personnel of the Nephrology unit, Billroth Hospital, Chennai. Of the 145 nasal swabs from group III, 135 (93.1%) were found to be carriers of CoNS isolates. Of the 135 carrier isolates ESRD patients and hospital personnel, 72 were from males and 63 were from females [Fig 5.1c].

Details of Study Participants

Details of ESRD Patients: (n=115)

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<th>No. of Patients</th>
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<td>22 – 84</td>
</tr>
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<td>Females</td>
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<td>20 – 78</td>
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Details of Hospital Personnel: (n=30)

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<tr>
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<td>22 – 46</td>
</tr>
<tr>
<td>Females</td>
<td>20</td>
<td>19 – 60</td>
</tr>
</tbody>
</table>

5.1.3. Source of CoNS isolates from nasal carriage of asymptomatic healthy individuals from closed communities (Group IV)

Out of 195 nasal swabs from group IV, 154 (78.9%) were found to be carriers of CoNS isolates. Carrier isolates of CoNS included isolates from healthy individuals of two different closed communities at risk viz; old age homes 110/126 (87.3%) and orphanages 44/69 (63.8%) [Fig 5.1c].
Of the 110 carrier isolates from old age homes, 35 were from male and 75 were from female. The median age of the participants with CoNS nasal carriage was found to be 71 years (range- 63 to 93 years). Of the 44 carrier isolates from orphanages; 28 were from female and 16 were from male. The median age of the participants with CoNS nasal carriage was found to be 6 years (range-3 to 16 years).

5.2. Prevalence of MR-CoNS among various settings

The prevalence of MR-CoNS among all CoNS isolates included in this study was found to be 314/546 (57.5%). Of the 127 CoNS isolates from group I of various samples, 90 (70.8%) isolates were found to be MR-CoNS and 37 (29.2%) isolates were found to be Methicillin Sensitive CoNS (MS-CoNS). Out of 130 CoNS isolates from group II, 70 (53.8%) isolates were found to be MR-CoNS and 60 (46.2%) isolates were found to be MS-CoNS. Of the 135 carrier isolates of CoNS from group III, 79 (58.5%) were found to be MR-CoNS and 56 (41.5%) isolates were found to be MS-CoNS. Among 154 CoNS isolates from group IV, 75 (48.7%) were found to be MR-CoNS and 79 (51.3%) isolates were found to be MS-CoNS [Plate 5.2 & Fig 5.2].

5.3. Species distribution among MR-CoNS from various settings

Overall, 8 different MR-CoNS (n= 314) species were identified from four different study groups. Amongst the identified species, S. epidermidis was the predominant species. Among 90 MR-CoNS isolates from group I, S. epidermidis 40 (44.4%) was the predominant species 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%). Of the 70 MR-CoNS isolates from group II, *S. haemolyticus* 35 (50%) was the predominant species followed by *S. epidermidis* 17 (24.3%), *S. saprophyticus* 7 (10%), *S. lugdunensis* 5 (7.1%) and *S. cohnii* 3 (4.3%). Of the 79 MR-CoNS isolates from group III, *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%). Of the 75 MR-CoNS isolates from group IV, *S. epidermidis* 31 (41.3%) was the predominant species followed by *S. haemolyticus* 20 (26.7%), *S. hominis* 16 (21.3%) and *S. capitis* 8 (10.7%) [Table 5.3].

**5.4. SCCmeC typing and type IV subtyping of MR-CoNS**

Overall, 5 types (I-V), combination types (type I+V, II+V, III+IV and IV+V) and non-typeable (NT) elements of SCCmeC were detected among MR-CoNS (n=314) isolates from four groups [Table 5.4a & 5.4b].

**Group I:**

All the five types and combination type I+V were present among the MR-CoNS isolates from group I, SCCmeC type I (n=45; 50%) was predominant followed by SCCmeC type IV (n= 18; 20%), V (n= 9; 10%), III (n= 4; 4.4%), I+V (n= 4; 4.4%), and II (n= 2; 2.2%). Eight isolates were found to be NT by Boye *et al.*, 2007 and Kondo *et al.*, 2007. Among type IV (n= 18), IVa subtype (n= 9; 50%) was predominant followed by IVc & IVE subtype (n= 4; 22.2%), IVg subtype (n= 2 isolates; 11.1%) and NT (n= 3; 16.7%).
Group II:

Four SCCmec types and combination (I+V) were present among the group II, SCCmec type I (n= 38; 54.3%) was predominant followed by type V (n= 11; 15.6%), type IV (n= 9; 13%), type III (n= 7; 10%) and type I+V (n= 5; 7.1%). Among type IV [n= 9 isolates; IVa subtype = 7; 77.8% and NT= 2; 22.2%].

Group III:

Among group III (n= 79), SCCmec type IV (n= 27; 34%) was predominant followed by type I (n= 18; 22.8%), type V (n= 15; 18.3%), type I+V (n= 8; 9.8%), type III+ IV (n= 6; 7.2%), type II+V (n= 3; 3.6%) and type II (n= 2; 2.4%). Among type IV (n= 27), IVa subtype (n= 21; 77.8%) was predominant followed by IVg subtype (n= 2 isolates, 7.4%), IVd subtype n= 1; 3.7%) and NT (n= 3; 11.1%).

Group IV:

Among group IV (n= 75), SCCmec type IV (n= 22; 29.3%) was predominant followed by type I (n= 17; 22.7%), type II (n= 15; 20%) type V (n= 10; 13.3%), type III (n= 2; 2.7%), type IV+V (4; 5.4%), type III+ IV (n= 2; 2.7%), type II+V (n= 2; 2.7%) type I+V (n= 1; 1.3%). Among type IV (n= 22), IVa subtype (n= 15; 68.2%) was predominant followed by IVg subtype (n= 4; 18.1%) and NT (n= 3; 13.7%) [Plate 5.4a & 5.4c]; [Fig 5.4a & 5.4b].

In our study, eight (8.8%) isolates were found to be NT among hospitalized patients by Boye et al. (2007) and Kondo et al. (2007). These eight isolates were found to be positive for the mec gene complex alone [Plate 5.4b].
5.5. Antibiotic resistance among MR-CoNS isolates

All the study isolates from the four different groups were found to be susceptible to vancomycin and teicoplanin.

5.5.1. Aminoglycoside resistance

Among the tested aminoglycosides, highest resistance was observed for gentamicin followed by amikacin and netilmicin [Table 5.5].

Among MR-CoNS isolates, the prevalence of gentamicin resistance was as follows: group I - 50% (45/90), group II - 41.4% (29/70), group III - 25.3% (20/79) and group IV - 24% (18/75).

Amikacin resistance was observed among MR-CoNS isolates from all the four different settings. Of the 90 isolates from group I, 33.3% (30) were found to be resistant, whereas 34.3% (24/70) of MR-CoNS isolates from group II, 17.7% (14/79) and 13.3% (10/75) were found to be resistant in group III and group IV respectively.

Netilmicin resistance was observed among MR-CoNS isolates from group II - 27.1% (19/70) were found to be resistant whereas all the isolates from group I, group III and group IV were found to be susceptible.

5.5.2. Fluoroquinolone resistance

Resistance was observed for both the tested fluoroquinolones (ciprofloxacin and ofloxacin) among MR-CoNS isolates from all the groups included [Table 5.5].
Among the tested isolates, prevalence of ciprofloxacin resistance was as follows; group I - 39% (35/90), group II - 54.3% (38/70), group III - 35.4% (28/79) and group IV - 25.3% (19/75).

Ofloxacin resistance among MR-CoNS was found in 33% (30/90) from group I, 47% (33/70) from group II, 35.4% (28/79) from group III and 18.7% (14/75) from group IV.

5.5.3. Erythromycin and clindamycin resistance

Erythromycin resistance was observed among MR-CoNS isolates from four different groups [Table 5.5]. Of the 90 isolates from group I, 36.7% (33) were found to be resistant, whereas 45.7% (32/70) of MR-CoNS isolates from group II, 35.4% (28/79) and 26.7% (20/75) were found to be resistant in both group III and IV respectively. Constitutive clindamycin resistance (cMLS\textsubscript{B}) was detected among 8.9% (8/90), 14.3% (10/70), 6.3% (5/79) and 4% (3/75) isolates from four groups respectively.

A total of 28 (52%) MR-CoNS isolates including 7 from group I, 7 from group II, 9 from group III and 5 from group IV were found to show inducible clindamycin resistance (iMLS\textsubscript{B})

5.5.4. Trimethoprim-Sulfamethoxazole (TMP-SMX) Resistance

Among the 314 isolates, highest resistance was observed in group II - 67.1% (47/70), followed by group I - 58% (52/90), group III - 48% (38/79) and group IV- 24% (18/75) [Table 5.5].
5.5.5. Fusidic acid resistance

Among the tested isolates, prevalence of fusidic acid resistance was as follows; group I - 13.3% (12/90), group III - 9% (7/79) and group IV - 8% (6/75). All the isolates from group II were found to be susceptible to fusidic acid [Table 5.5].

5.5.6. Linezolid resistance

Overall, 3 isolates [S. haemolyticus - 2; S. epidermidis - 1; group II - 1/70; group III - 2/79] were found to be resistant to linezolid. All the isolates from group I and group IV were found to be susceptible to linezolid [Table 5.5].

5.5.7. Mupirocin resistance

High level mupirocin resistance was seen in 33% (26/79), 16.7% (15/90), 7.1% (5/70) and 6.7% (5/75) among group III, group I, group II and group IV respectively [Table 5.5].

5.5.8. Rifampicin resistance

Among the four groups, highest resistance towards rifampicin was seen in group II - 34.3% (24/70), followed by group I - 10% (9/90) and group III - 5% (4/79). All the isolates of group IV were found to be susceptible to rifampicin [Table 5.5].

5.5.9. Tetracycline resistance

Among the isolates tested, prevalence of tetracycline resistance was as follows; group I - 15.5% (14/90), group II - 14.3% (10/70), group III - 23% (18/79) and group IV - 16% (12/75) [Table 5.5].
5.6.  PCR based screening of antibiotic resistant determinants

5.6.1. Detection of aminoglycoside modifying enzymes (AMEs): \([\text{aac}(6')-\text{Ie-aph}(2'')-\text{Ia, aph}(3')-\text{IIIa, and ant}(4')-\text{Ia genes}]\)

Amongst the genes for aminoglycoside resistance from group I, the \(\text{aac}(6')-\text{Ie-aph}(2')-\text{Ia}\) was the most prevalent gene among aminoglycoside-resistant isolates, detected singly in 34 isolates (37.8%) and in combination with \(\text{aph}(3')-\text{IIIa}\) in 14 (15.5%) and \(\text{ant}(4')-\text{Ia}\) in 2 (2.2%) isolates. The \(\text{aph}(3')-\text{IIIa}\) was detected in 28 (31.1%) isolates and the \(\text{ant}(4')-\text{Ia}\) in four (4.4%) isolates.

Of the 70 isolates from group II, \(\text{aac}(6')-\text{Ie-aph}(2')-\text{Ia}\) was the most prevalent gene among aminoglycoside-resistant isolates, detected singly in 28 (40%) isolates and in combination with \(\text{aph}(3')-\text{IIIa}\) in 18 (25.7%) isolates and with \(\text{ant}(4')-\text{Ia}\) in 8 isolates (11.4%). The \(\text{aph}(3')-\text{IIIa}\) was detected singly in 22 (31.4%) isolates and along with \(\text{ant}(4')-\text{Ia}\) in 14 (20%) isolates.

Of the 79 isolates from group III, \(\text{aac}(6')-\text{Ie-aph}(2')-\text{Ia}\) was the most prevalent gene among aminoglycoside-resistant isolates, detected singly in 17 isolates (21.5%) and in combination with \(\text{aph}(3')-\text{IIIa}\) in 3 (3.8%) isolates. The \(\text{aph}(3')-\text{IIIa}\) was detected singly in 5 (6.3%) isolates. The \(\text{ant}(4')-\text{Ia}\) gene was not detected in any isolate in this group.

Among the 75 isolates from group IV, \(\text{aac}(6')-\text{Ie-aph}(2')-\text{Ia}\) was the most prevalent gene among aminoglycoside-resistant isolates, detected singly in 10 (13.3%) isolates. The \(\text{aph}(3')-\text{IIIa}\) was detected singly in 3 (4%) isolates, while all the isolates were negative for \(\text{ant}(4')-\text{Ia}\) gene [Table 5.6; Plate 5.6a].
5.6.2. Detection of macrolide, lincosamide resistant genes: \([erm (A), erm (C), mrrA\) genes]

Amongst the erythromycin-resistant isolates screened for MLS\(_B\) resistant genes of group I, 31 (34.4\%) isolates harbored \(mrrA\). The \(erm(C)\) and \(erm(A)\) gene were found singly in 10 (11.1\%) and 2 (2.2\%) isolates respectively and in combination with both \(erm(A)\) and \(mrrA\) in 3 (3.3\%) isolates.

Of the group II, \(mrrA\) 27\% (19/70) was the most predominant gene followed by \(erm(C)\) 15.7\% (11/70). The combination of \(mrrA\), \(erm(C)\) and \(erm(A)\) was found in 4.3\% (3/70) isolates.

Among MLS\(_B\) resistant isolates of group III, \(mrrA\) - 28\% (22/79) was the most predominant gene followed by \(erm(C)\) - 17.7\% (14/79).

Among MLS\(_B\) resistant isolates of group IV, \(mrrA\) - 14.7\% (11/75) was the predominant gene followed by \(erm(C)\) - 9.3\% (7/75) and \(erm(A)\) - 2.7\% (2/75) genes [Table 5.6; Plate 5.6b & 5.6c].

5.6.3. Tetracycline resistance: [\(tetK\) and \(tetM\) genes]

Among tetracycline resistance genes, all the four groups harbored \(tetK\) gene -[15.5\% (14/90), 14.3\% (10/70), 23\% (18/79) and 16\% (12/70)] respectively, whereas \(tetM\) gene was not detected in all the study isolates [Table 5.6; Plate 5.6b].

5.6.4. Detection of TMP-SMX gene: [\(dfrA\) gene]

TMP-SMX resistant gene was highest in group II - 67.1\% (47/70), followed by group I - 58\% (52/90), group III - 48\% (38/79) and group IV - 24\% (18/75) [Table 5.6; Plate 5.6c].
5.6.5. Mupirocin resistance: \(mupA\) gene

High-level mupirocin resistant determinant - \(mupA\) gene was highest in group - III 33% (26/79) followed by group I - 16.7% (15/90), group II - 7.1% (5/70) and group IV - 6.7% (5/75) [Table 5.6; Plate 5.6d].

5.6.6. Fusidic acid resistance: \(fusB, fusC\) and \(fusD\) genes

Overall, 25/314 (8%) isolates were found to be fusidic acid resistant. All the isolates from group II were found to be negative for \(fus\) genes. Among fusidic acid resistant genes, \(fusB\) gene was prevalent in 36% (9/25), 20% (5/25) and 16% (4/25) in group I, III and IV respectively, whereas only 7 isolates from group I, III and IV harbored 12% (3/25), 8% (2/25) and 8% (2/25) \(fusC\) gene respectively [Table 5.6; Plate 5.6e].

5.6.7. Linezolid resistance: \(cfr\) and 23S rRNA gene

Linezolid resistance was found in 2 isolates from group III and 1 isolate from group II. All the 3 isolates harbored \(cfr\) gene and also possessed resistance-conferring mutations such as G2576T in domain V of 23S rRNA gene mutation [Table 5.6; Plate 5.6f].

5.7. Prevalence of virulence factors among MRSE isolates from various Groups

5.7.1. Detection of biofilm production by Congo red agar method and its associated genes

All the MRSE isolates from four groups were subjected to CRA method for the detection of biofilm formation and associated genes (\(icaAD, aap\) & \(atlE\) genes).
72.5% (29/40), 58.8% (10/17), 57% (32/56) and 58% (18/31) were found to be positive for biofilm formation among group I, group II, group III and group IV respectively [Table 5.7; Fig 5.7a; Plate 5.7a].

19/40 (47.5%), 7/17 (41.2%), 22/56 (39.3%) and 11/31 (35.5%) from group I, group II, group III & group IV were found to be positive for all the three genes tested, viz., icaAD+, aap+, atlE+ genotypes respectively. Overall, five isolates showed biofilm formation even in the absence of ica genes and exhibited icaAD- atlE+ aap+ genotype [Table 5.7a, 5.7b, 5.7c & 5.7d; Fig 5.7b]

### 5.7.2. Detection of IS256 (genetic marker for virulent S. epidermidis)

IS256 was found in 80% (32/40), 76.4% (13/17), 39.3% (22/56) and 13% (4/31) among the four different groups respectively [Table 5.7; Plate 5.7b].

### 5.7.3. Detection of Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs)

All the MRSE isolates were found to be negative for cna gene [Table 5.7.3]. 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 gene was detected in - 12.5% (5/40), 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 [Plate 5.7c].
5.7.4. Prevalence of Arginine catabolic mobile element among MRSE from various Groups

The ACME type I was more common among carrier isolates from group IV - 61.3% (19/31) and group III - 37.5% (21/56) than among group I - 22.5% (9/40) [Table 5.7.4; Fig 5.7c; Plate 5.7d]

5.8. Multi locus sequence typing of representative MRSE isolates

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 [Table 5.8].