6.0 DISCUSSION

6.1 Isolation of *Staphylococcus aureus* and phenotypically characterize MRSA strains.

In the present study the average antibiotic susceptibility patterns for the major antibiotics tested were: Erythromycin (17.4%), Gentamycin (23.6%), Ciprofloxacin (45.6%), Clindamycin (59.6%) and Linezolid (100%). This is in comparison to study conducted by Joshi et al., 2013 in which Erythromycin susceptibility was seen in 29.2% of patients, Gentamycin in 41.7%, Ciprofloxacin in 20.3%, Clindamycin in 53.4% and Linezolid susceptibility in all the patients. Another study conducted by Kitara et al., 2011 showed Erthromycin susceptibility as 7.8%, Gentamycin as 0% and Ciprofloxacin as 1.6%.

6.2 Comparison of conventional and rapid phenotype methods for detection of MRSA (n=48).

6.2.1 Conventional method

In recent studies, disc diffusion test using cefoxitin disc is considered superior to other phenotypic methods and is accepted method now for the detection of MRSA by many reference groups such as CLSI (Skov et al., 2003). In the present study, the cefoxitin disk diffusion test showed all the 48 (100%) strains as resistant. This result is in comparison to the previous study conducted by Velasco et al., 2005, in which also cefoxitin disc diffusion test showed 100% sensitivity. In the study conducted by Anand et al., 2009, the sensitivity and specificity were optimal (100%) in the 50 strains tested.
6.2.2 Rapid methods

6.2.2.1 PBP2’ Detection

Detection of product of mecA gene (PBP2’ protein) is a rapid and easier method of predicting resistance to methicillin. In the present study, 45 out of 48 (93.75%) clinical isolates of MRSA tested, showed agglutination by the slide latex agglutination test and three (6.25%) were negative.

This data is in comparison to the study conducted by Mohanasundaram and Lalitha, 2008, in which all the 33 (100%) MRSA tested showed positive in latex agglutination test but they have reported false positive reaction in one which has been discussed as may be due to incorrect inoculum or rotating the test card for more than 3mins.

The present study can be compared to the study conducted by Cavassini et al., 1999 in which MRSA-Screen, slide latex agglutination test, detected all the 80 (100%) MRSA isolates. The present study can also be compared to the study conducted by Velasco et al. 2005, in which, PBP2’ detection by the kit showed optimal sensitivity.

The sample data also in comparison to other published literature (Griethuysen et al., 1999), in which out of 267 strains which were mecA positive by PCR; 4 tested negative by the kit which resulted in a sensitivity of 98.5%. Louie et al. 2000, in their study reported a sensitivity ranging in the range of 97 to 100%. Even in studies which included borderline isolates the sensitivity was 98.5%.

The reason for reduced sensitivity of the MRSA-Screen kit used in the present study is unknown. The reduced beta-lactam resistance relies on the down-regulation of mecA transcription (Mempel et al., 1994) and is influenced by auxiliary genes such as mecR, mecI (Kuwohara-Arai et al., 1996), and the fem genes (de Lencastre et al.,
However, these cryptic methicillin-resistant strains, also called preMRSA (Hiramatsu et al., 1992; Kuwohara-Arai, 1996) are potentially highly resistant, since they can generate highly resistant subclones in vitro (Hiramatsu et al., 1992; Tokue et al., 1992).

Therefore, their detection appears to determine the choice of antibiotic therapy and relies mainly on the detection of the gene encoding for methicillin resistance (meca).

6.2.2.2 ORSAB

The ORSAB medium showed a sensitivity of 95.8% which is low compared to the data of Velasco et al., 2005, in whose study ORSAB medium showed a high sensitivity. Accurate determination of methicillin resistance in Staphylococci in a clinical laboratory by conventional method is subject to many environmental conditions as mentioned earlier such as temperature, pH, salt concentration and incubation time. Difficulties in detection occur when the organisms have their MICs near break point. In such instances that molecular techniques are useful in accurately diagnosing the infection.

6.2.2.3 MIC by E-test

MIC by E-test is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. Of the penicillinase-stable penicillins, Oxacillin is preferred in in-vitro testing as it is more likely to detect heteroresistant strains of Staphylococci.

In the present study MIC by E-test for oxacillin showed an optimal sensitivity (100%). This sample data is higher than that of Velasco et al. 2005, in whose study E-test for oxacillin showed a lower sensitivity in that 5.9% (three out of 51) clinical strains tested, which were positive for meca gene, showed false negative results.
In another study conducted by Cavassini et al.1999, out of the 80 MRSA isolates, 20 were resistant heterogeneously to oxacillin with MICs ranging from 0.25 to 3.0µg/ml by E-test. The false negative results in previous study may be explained as due to growth conditions.

6.3 To standardize a multiplex PCR for rapid and simultaneous detection of Hospital associated MRSA (HA-MRSA) and Community acquired MRSA (CA-MRSA) from clinical isolates. The present study showed 100% sensitivity with 48 strains positive for mecA gene. One (2.08%) strain was negative for PVL which has been classified as hospital acquired MRSA.

This data is in comparison to the sample data of McClure et al., in 2006 targeting PVL genes and simultaneous discrimination of methicillin-susceptible strains of S. aureus from methicillin-resistant S. aureus.

The multiplex PCR was found to be highly sensitive. In 34 strains correlation was seen with uniplex PCR and phenotypic susceptibility. In the 14 strains which were phenotypically heteroresistant as the amount of isolates with the mecA gene were less the correlation was week. On whole the above standardized method is reliable.

The sensitivity of present study can also be compared to the study conducted by Zhang et al., 2008, who developed a novel multiplex PCR for rapid detection and discrimination of the USA300 strains from USA400 strains and concomitant discrimination of PVL genes, detection of mecA gene for simultaneous discrimination of methicillin-resistant strains of S. arueus (MRSA) from methicillin susceptible (MSSA), 16sRNA and nuc genes to discriminate S. aureus from the coagulase negative Staphylococci (CONS).
The study showed 100% sensitivity and the results were in concordance to the control strains.

Studies reveal, despite increases in the proportion of CA-MRSA strains among inpatients, the persisting high level of HA-MRSA among them in contrast to reports from around the world (Popvich et al., 2008) suggests that CA-MRSA strains are only adding to the problem of MRSA rather than replacing the HA-MRSA strains in the health-care settings.

The fact that the frequency of HA-MRSA has decreased suggests that there could be some crowding out of HA-MRSA strains within the hospital.

However, lack of a decrease in their rate within the health-care settings shows that HA-MRSA strains may be fit there and therefore CA-MRSA strains are unable to replace them completely. The result is a coexistence of both strains in the health-care settings and maintenance of CA-MRSA because of the large inflow of colonized and infected patients.

6.4 To characterize MRSA strains by staphylococcal cassette chromosome mec (SCCmec) typing.

In the present study, the predominant types of SCCmec were type V with 13 out of 48 (27.08%). SCCmec type III was harboured by 9 (18.75%), SCCmec type IV was one in 48 (2.08%) among the CA-MRSA strains. These results are in comparison to that of the study carried out by Susan Boyle-Vavra, 2005 on pediatric patients with skin and soft tissue infections (SSTI) from Taipei, Taiwan in which 76% (13 out of 17) CAMRSA isolates (multilocus sequence type 59) did not carry SCCmec types I to IV.
but DNA sequence analysis to determined that these nontypeable isolates is a novel subtype of SCCmec V.

SCCmec V was also present in 14.7% (5 out of 34) CAMRSA colonization isolates collected from healthy children from Taipei. 79.4% (27 of 34) harbored SCCmec IV. One (2.9%) harbored SCCmec III which is lower than the data in the present study. The values of the present study were also in comparison to the study conducted by Boye et al., 2007 in which out of 143 MRSA isolates recorded during 2003 and 2004, 70% were CA-MRSA and 30% were HA-MRSA.

In the current study, nine (18.75%) CA-MRSA strains harboured SCCmec III and 4 (8.33%) CA-MRSA harboured SCCmec I. The study conducted by Boye et al., 2007, all SCCmec types were represented in both categories, except for SCCmec type V, which was detected only among the CA-MRSA isolates. SCCmec type IV was found in 86% of the CA-MRSA isolates and in 84% of the HA-MRSA isolates.

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