Chapter 6

DISCUSSION

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The present study gives MRSA prevalence rate of 24 %. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) gives a MRSA prevalence rate of 41 % which is higher when compared to the present study [50].

In this study hVISA prevalence rate was 7 % and this was consistent with a study by Chaudhari et al. 2015, from India that shows hVISA prevalence rate of 6.9 % and Asian Network for Surveillance of Resistant Pathogens (ANSORP) gives a prevalence rate of 6.3 % [51,52]. However, in the present study none of the isolates showed homogenous vancomycin intermediate resistance and this is concordant with other studies [53, 54].

Brain heart infusion agar containing 6µg vancomycin (BHIV6) detected 16.4 % of the isolates as hVISA/VISA but PAP-AUC confirmed only 7 % of the isolates as hVISA/VISA. This clearly depicts that BHIV6 has got high sensitivity but less specificity and thus PAP-AUC remains as one of the gold standard reference method for the detection of hVISA/VISA isolates [55].

The proportion of MRSA and MSSA strains in the analyzed 468 *S.aureus* isolates were 24 % and and 76 % respectively. Of the total 468 *S.aureus* strains used in the present study, 2.3 % of the hVISA isolates were MRSA and 4.7 % of the hVISA isolates were MSSA. A similar study done by Jain Hu etal, gives 10 % prevalence rate of hVISA. Out of which 4.1 % of the isolates were MSSA and also summarizes that the prevalence rate of hVISA among MSSA strains has significantly increased through the years and has reached almost to 7.2 % [34]. This highlights the fact that reduction in vancomycin susceptibility has become common among

methicillin susceptible isolates of S.aureus[53].Therefore treatment of MSSA isolates with vancomycin can lead to treatment failures [3,4].

Of the 23 blood, 24 urine, 13 respiratory and 408 exudate *S.aureus* strains, 21.7 %, 4.2 %, 0 % and & 6.6 % of them were hVISA strains respectively. hVISA strains were prevalent among invasive(blood) isolates. This was found to be consistent with Hu *et al*., which gives a higher hVISA prevalence rate from blood isolates [35].

In this study, 13 % of the MRSA isolates were negative for DNase [54]. Another study by Kateete David P et al., gives 25 % negativity for DNase test [56].

Hemolysins play a major role in host cell damage. In the present study 64 % of the MRSA isolates showed beta hemolysis which was higher when compared to the study done by Desouky, et al. [57]. Another study by Suheyla et. al., shows 58.9 % of *S.aureus* strains were found to be hemolytic[14].

S.aureus strains that possess hemagglutination property have the ability to adhere to prosthetic devices to establish infection. About 43.8 % of MRSA strains were found to possess hemagglutinating property[58]. This was high when compared to the study done by Mark. et al., which shows that only 13 % of *S.aureus* strains were positive for hemagglutination [59].

Slime producing strains of *S.aureus* has the ability to form intact biofilm and also have higher rate of colonization in host tissues [60]. About 57.8 % of the MRSA strains were found to be slime producers. This report was consistent with another study done by Podbielska et al., in which 69 % of S.aureus strains were slime producers [61].

Strains of *S.aureus* which has the property of biofilm formation confer antibiotic resistance and colonize most of the indwelling medical devices [62]. In this study most of the MRSA strains were biofilm producers. Only 3.5% of the strains were found to be non-adherent. About 74% were weakly adherent, 20% were moderately adherent and 1.7% were found to be strongly adherent. A biofilm prevalence study in MRSA done by Maryam Rezaei, et al., reports that 100 % of the MRSA isolates were biofilm producers out of which 15.4 %, 19.2 % and 65.4 % were strong, medium and weak respectively. This report was concordant with this study results on biofilm formation [63]. These biofilm producing MRSA isolates were found to confer high degree of resistance to various antibiotics [64].

In this study all the hVISA isolates showed reduced autolytic activity with Triton- X induced lysis, TEM revealed the presence of thickened cell-wall and about 39% of these isolates did not show colony spreading. This explains that these isolates had thickened cell wall and therefore non-susceptible to triton-X induced lysis [2].

About 6(18%) were negative for DNase, 2(6%) were weakly positive for coagulase, 45% were biofilm producers and only 25(76%) were positive for hemolysin production. Only (14) 42% and 13(39%) of the hVISA isolates were strong hemolysin and DNAse producers as detected by quantitative assay. Quantitative detection of hemolysin and DNAase were found to be superior detection methods when compared to direct colony spot inoculum method.

Accessory gene regulator was found to up regulate extracellular virulence factors like DNase , coagulase and also responsible for colony spreading on soft agar surface. Thus reduced colony spreading, reduction in DNase, coagulase suggests *agr*

dysfunction in these isolates [13,66]. Late coagulase production can be due to the thickened cell wall and this can lead to misidentification of the *S.aureus* isolates. A similar study by Sirichoat et al., has reported about 11.8% of the hVISA did not show proper coagulase production even after 24 hours of incubation[42].

Murein hydrolase that is upregulated by *agr* is responsible for the cell wall turn over. Due to some defect in the *agr* dysfunction and reduction in the synthesis of murein hydrolase had resulted in thickened cell wall in hVISA isolates as demonstrated by TEM and Triton –X 100 autolytic assay.

In the present study out of the 33 hVISA strains 14(42.4 %) belonged to *agr* I, 8(24.2 %) belonged to *agr* II, 10 (30 %) belonged to *agr* III and 1(3 %) belonged to *agr* IV. Thus in this study *agr* type I was predominant followed by *agr* type III and this was consistent the study done by Singh etal, 2017 shows that 82.8 % were *agr* type I, 10.3 % were type III and 6.9 % were non-typeable [67]. However in the present study all the strains were typeable by *agr* - duplex PCR. Among MR-hVISA *agr* type II was found to be the predominant type and this was concordant with many previous studies [68,69].

In this study the antibiotics resistance pattern of MRSA isolates includes 63 %, 60 % and 56 % for ciprofloxacin, erythromycin and co-trimoxazole respectively. INSAR report gives a prevalence rate of 79 %, 70 % and 55 % for ciprofloxacin, erythromycin and co-trimoxazole respectively. Similarly the resistance rate for gentamicin, clindamycin includes 33 % and 22 % respectively.

INSAR gives a prevalence rate of 58 % for gentamicin and 46 % for clindamycin which was higher when compared to this report [50]. Asian Network for

Surveillance of resistant Pathogens (ANSORP) gives a prevalence rate of 77 % for ciprofloxacin, 78 % for gentamicin, 64 % for clindamycin, 90 % for erythromycin and 43 % for cotrimoxazole [52].

The hVISA/VISA isolates showed better susceptibility pattern to other group of antibiotics like aminoglycosides, macrolides, quinolones, cotrimoxazole and tetracycline when compared with MRSA isolates.

Hence judicious use of antibiotics is recommended in healthcare settings in order to avoid the emergence of multidrug resistant strains. Because overuse of vancomycin can create selective pressure and may lead to the emergence of strains with reduced vancomycin susceptibility.

About 8.7 % of the MRSA strains and 12 % of the hVISA/VISA strains were HLMR and 0.8 % of the MRSA strains were LLMR. Another study by Chaturvedi et al., gives a mupirocin prevalence rate of 15 % for MRSA [70].

All the mupirocin resistant isolates were found to be susceptible to fusidic acid. Another study by Solmaz et al., gives 100 % fusidic susceptibility towards mupirocin resistant MRSA isolates [64].