Chapter VI

SUMMARY AND CONCLUSIONS
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*S. aureus* has always been one of the major human pathogens and is the cause of most nosocomial organisms. It is an opportunistic bacterium frequently part of the human micro flora causing disease when the immune system becomes compromised. Anterior nares form the primary sources of *S. aureus*, nevertheless it can be found in many other parts of the human body as well.

Initially MRSA was considered to be associated with the hospital environment but in recent years MRSA infections have been encountered in the community. Due to this changing epidemiology, it is important to assess the carriage rate of MRSA in the community amongst healthy individuals who have not been hospitalized nor had antibiotic therapy in the recent past.

The rising incident of *S. aureus* infections is due to the increase in antibiotic resistant strains called Methicillin Resistant *Staphylococcus aureus* (MRSA). Bacterial, genetic and microbiological adaptive changes and properties gave rise to the emergence of antibiotic resistance of the organism. The genetic basis of methicillin resistance of MRSA isolates is the presence of *mecA* encoding PBP2a, that is located on a mobile genetic element called the Staphylococcal Cassette Chromosome (SCCmec).

Since, medical staff can be a vehicle in the spread of MRSA within a hospital, as direct person to person contact contributes to the transmission of MRSA, we also screened hospital personnel for carriage of *S. aureus*. 
For the present study samples have been collected from K.C General Hospital, Wockhard Hospital, Fortis Hospital and St John’s medical college and Hospital, Institute of Preventive Medicine and Public Health Laboratories in Bangalore, which receives clinical samples from both public and private hospitals in the city of Bangalore.

The present investigation is undertaken to isolate *S. aureus* from various clinical samples, healthy individuals of hospital personnel and healthy children and adults of the community and identify the frequency of MRSA among them. Antibiogram was carried out for all the isolates of *S. aureus*. SCCmec typing and PVL analysis were performed for selected MRSA strains isolated from different types of samples.

The main aim of this study was to know the distribution of SCCmec types and the presence of PVL exotoxin among the *S. aureus* isolates and distinguish *S. aureus* isolates from hospitals and community by phenotypic and molecular methods.

A total of 210 clinical samples, 28 hospital personnel and 110 healthy individuals were screened for isolation of *S. aureus*. All the isolates obtained were confirmed as *S. aureus* by various biochemical and physiological tests. All the *S. aureus* isolates thus obtained were screened for methicillin resistance using various phenotypic methods like oxacillin disc diffusion using 1μg oxacillin, oxacillin salt agar method, cefoxitin disc test using 30 μg disc and oxacillin MIC.

All the 177 *S. aureus* isolates were further subjected to antimicrobial susceptibility testing using Kirby-Bauers disc diffusion method. Various
antibiotics like penicillin-G, cloxacillin, gentamicin, erythromycin, ampicillin, ciprofloxacin, amoxycylav, methicillin, cefotaxime, amikacin and vancomycin were used.

For molecular characterization, 38 isolates of MRSA were randomly selected from clinical samples, hospital personnel and healthy individuals. The selected isolates were subjected to SCC\textit{mec} typing by multiplex PCR. Eight sets of primers were used in this multiplex PCR to amplify the different genes to determine the MRSA isolates into 4 SCC\textit{mec} types and their variants (subtypes).

The MRSA isolates were further subjected to PVL analysis by simple PCR. PVL is an exotoxin mostly associated with community acquired \textit{S. aureus}. Thus PVL was used as a marker in our study to differentiate the hospital acquired MRSA and community acquired MRSA.

\textbf{Following are the important observations in our study}

A total of 104 \textit{S. aureus} from clinical samples, 23 \textit{S. aureus} from hospital personnel and 50 \textit{S. aureus} from healthy individuals were obtained. Among clinical isolates, most of the isolates were from pus, obtained from burns ward. Significant number of isolates were also obtained from sputum, urine and catheter tips. Among hospital personnel \textit{S. aureus} isolation was highest in nursing assistants followed by nurses and doctors. \textit{S. aureus} isolation among healthy individuals was maximum in school children followed by adults.

In all 177 \textit{S. aureus} isolates were obtained from hospitals and community. Among 177 \textit{S. aureus}, 88 isolates were confirmed as MRSA by various phenotypic
methods. We observed 100% concordance in the results obtained from various phenotypic methods. Remaining 89 *S. aureus* isolates were identified as MSSA. Overall *S. aureus* isolation rate was found to be 50.86% (177/348) and incidence of MRSA was 25.28% (88/348) incidence of MRSA carriage rate in healthy individuals of community was very low 6.3% (7/110) and was high (32.85%) (Table-9) in the clinical samples.

The *S. aureus* isolates from clinical samples and hospital personnel showed higher MIC values ranging from 32-512 µg/ml, whereas *S. aureus* isolates from healthy individuals had lower oxacillin MIC ranging from 2 - 64 µg/ml.

*S. aureus* isolates from clinical samples and hospital personnel showed high resistance to most of the antibiotics tested. *S. aureus* isolates from clinical specimens and hospital personnel (hospital acquired MRSA) were resistant to most of the antibiotics and were multi-drug resistant isolates. Whereas, MRSA isolates from healthy individuals were resistant to 7 to 8 antibiotics. Only 1 isolate was resistant to 10 antibiotics and all isolates were susceptible to vancomycin.

Among the MRSA isolates (38) from all types of samples screened for the SCCmec typing, *S. aureus* isolates from clinical and hospital personnel possessed SCCmec type III or its variants and isolates from community had type IV SCCmec. None of the isolates in our study belonged to either SCCmec type I or II.

PVL was associated with SCCmec type IV which was present in all the community isolates. PVL was absent in clinical isolates having SCCmec type III.
A good correlation was observed between MICs, SCC\textit{mec} and PVL typing. SCC\textit{mec} type III isolates showed the oxacillin MIC range from 8-512 µg/ml. The number of isolates having type III increased with higher MIC values. The isolates with highest oxacillin MIC 512 µg/ml had SCC\textit{mec} III. Isolates having SCC\textit{mec} type IV had low oxacillin MIC ranging from 8 to 64 µg/ml and the same isolates were positive for PVL.

**CONCLUSIONS**

MRSA isolates from clinical samples and hospital personnel belonged to SCC\textit{mec} type III and variants and MRSA from community belonged to type IV. MRSA from hospitals showed higher drug resistance patterns compared to those from the community. Thus, MRSA isolates from hospitals and community differed significantly in their phenotypic and genotypic characteristics. We conclude that SCC\textit{mec} typing and PVL analysis are valuable adjunctive tools for distinguishing \textit{S. aureus} isolates from the hospitals and community.

**FUTURE PROSPECTS**

One of the important tasks for physicians and public health department is to control the spread of MRSA strains in the hospital and in the community. Microbiologists and genetists worry about the acquisition of resistance to newly discovered drugs and it becomes an imminent task for them to understand two significant trends in recent MRSA evolution; acquisition of vancomycin resistance in hospitals and prevalence of highly virulent MRSA clones in the community.
To overcome these tasks one should need to study systematically on Genomic Islands (GIs) especially SCC\textit{mec}, bacteriophages and conjugation transposons of \textit{S. aureus} isolates from clinical samples, healthy individuals and hospital personnel. Studies focusing on the SCC\textit{mec} of different methicillin resistant staphylococcal species would be of interest for the elucidation of the origin of this mobile genetic element. Continuous surveillance of a particular geographical area for long term may help in solving these problems.

In this direction, we made little efforts in this study to identify and characterize the \textit{S. aureus} isolates from healthy individuals, hospital personnel and clinical samples by applying both conventional and molecular methods. Our results suggest that CA-MRSA and HA-MRSA evolved independently and have different SCC\textit{mec} types and CA-MRSA also possessed PVL toxin. This study needs further investigation on the mode of transmission, acquisition of \textit{mecA} and PVL gene. This can be possible by taking large populations and conducting long term continuous surveillance using latest molecular techniques.