CHAPTER 6

Conclusion
6.0 CONCLUSION

The introduction of penicillin was followed by an extraordinary period of discovery and exuberant use of different antibiotics. Resistant bacterial strains have emerged and have spread throughout the world because of the remarkable genetic plasticity of the microorganisms, heavy selective pressure of antibiotic use, and mobility of the world population. The problem of resistance to antimicrobial drugs is particularly troublesome in developing countries. An urgent need exists for more appropriate selection and use of antimicrobial drugs in the developed and developing countries. The impact of antimicrobial resistance in a particular region ranges from failure in an individual patient to respond to therapy, to serious implications for prescribing, to hospital costs and the choice of optimal empirical therapy.

Oxacillin-resistant staphylococci are major nosocomial pathogens with frequent multiple resistance, led to the use of glycopeptides in therapy. One of the priority measures is to decrease the strong antibiotic pressure and to optimize the detection of oxacillin resistance in clinical laboratories. Different phenotypic methods used to detect oxacillin susceptibility in staphylococci suffer from various drawbacks like the
heterogeneous phenotypic expression of oxacillin resistance. In addition, oxacillin resistance is frequently influenced by culture conditions such as inoculum size, incubation time, temperature, pH and salt concentration of the medium. These factors complicated the detection, especially for strains with low resistance, and emphasize the need to use a rapid, accurate and sensitive method for detection of oxacillin resistance that is not dependent on growth conditions. In present study, the detection of mecA gene, responsible for methicillin resistance in all clinical methicillin-resistant staphylococcal strains, was considered as the reference test.

Out of 1272 Staphylococcal clinical isolates from five different cities of Vidarbha region, 78.4% of S.aureus clinical isolates and 84% of CoNS isolates were found to harbor mecA gene. Majority of the strains of eight species of CoNS studied S.epidermidis, S.capitis, S.warneri, S.haemolyticus, S.hominis, S.saprophyticus, S.cohnii and S.simulans amplified mecA gene indicating the wide distribution of the gene among coagulase-negative Staphylococcus species.
Comparing the results of various phenotypic methods of detection of oxacillin resistance in clinical staphylococcal isolates, it was found that “E” test was most sensitive method for detecting oxacillin resistance in both \textit{S.aureus} and CoNS strains. Oxacillin disk diffusion had lowest sensitivity for \textit{S.aureus} and CoNS clinical isolates. Oxacillin agar dilution lacked sensitivity in detecting \textit{meca} positive isolates compared to “E” test. Oxacillin agar screen test was found to be sensitive for \textit{S.aureus} except for four strains that exhibited discrepancy with PCR \textit{meca} detection, and for CoNS species, the highest sensitivity was observed at 4\textmu g/ml oxacillin concentration.

\textit{bla-Z} gene was detected by PCR amplification in \textit{meca} negative isolates of \textit{S.aureus} and \textit{S.saprophyticus} that had discrepancy with “E” test MIC. Further \beta-lactamase hyperproduction was also observed by amoxicillin-clavulanic acid disk test in these isolates. Therefore \beta-lactamase hyperproduction explained the borderline resistance of these \textit{meca} negative staphylococcal clinical isolates.

\textit{meca} and \textit{bla-Z} amplicons were confirmed by Southern hybridization with biotinylated internal probe. No false positive
signal was observed and thus confirmed the amplification of targeted \( mecA \) and \( bla-Z \) genes. Sequencing of \( mecA \) and \( bla-Z \) amplicons was performed for randomly selected samples and sequence of amplicon was compared with the internal sequence of \( mecA \) and \( bla-Z \) gene. The sequence of amplicon matched with the internal gene sequence of \( mecA \) and \( bla-Z \) genes.

Dot blot hybridization of \( mecA \) and \( bla-Z \) genes was performed with biotinylated \( mecA \) and \( bla-Z \) gene probe. Results were compared with the PCR \( mecA \) and \( bla-Z \) detection results. Sensitivity and specificity of 100% was observed, further substantiating the fact that direct detection of gene involved in resistance mechanism was unarguably the best method for detecting antimicrobial resistance in clinical isolates.

In conclusion, molecular methods for detection of antimicrobial resistance should be used routinely in clinical laboratories to know the resistance genotype of the clinical isolate without any discrepancy. This practice will help the physician in taking early and correct decision regarding antibiotic therapy for the patient. The use of appropriate antibiotic therapy could reduce the selective antibiotic pressure in the nosocomial flora and could reduce the spread of resistance in community.