CHAPTER 6

SUMMARY AND CONCLUSION

6.1 SUMMARY

*Enterococcus faecalis*, a frequently identified commensal in the human intestine is gaining importance in clinical environments due to its increasing virulence to cause infections that could even lead to death. Resistance to antimicrobials is one major cause of concern that is making control of this bacterium difficult. The commonly used control measure against *E. faecalis* is a synergistic combination of a cell wall mediating glycopeptide along with an aminoglycoside. Gentamicin is the most commonly used aminoglycoside against enterococci. High level resistance to gentamicin has been widely reported from various parts of the world. The occurrence of *E. faecalis* in many clinical set ups and other environmental niches have been noted in India. Hence an attempt was made to understand the molecular mechanism associated with the HLGR gene(s), its mobility to related strains and the genetic diversity. Forty HLGR clinical isolates were obtained from a multi-speciality hospital and was investigated to comprehend the above objectives. The salient findings of the present study could be summarised as follows

- Genotypic analysis of the clinical isolates revealed the presence of the fused gene *aac(6')-Ie-aph(3'″)la* coding for the
bifunctional enzyme aminoglycoside acetyltransferase – phosphotransferase. The other genes, \(\textit{aph}(2''\prime)-lb\), \(\textit{aph}(2''\prime)-Ic\) and \(\textit{aph}(2''\prime)-Id\) coding for phosphotransferase also reported to confer HLGR were absent in all the clinical isolates.

- Sequence analysis of the PCR amplified HLGR gene fragment confirmed 100% identity to the already available gene sequence (M13771) and implied that the conserved motifs in the aminoglycoside-modifying enzymes play an important role in resistance determination.

- Presence of insertion sequences IS256 and IS257 was detected in all the HLGR clinical isolates as these two insertion elements are found commonly associated with HLGR in \textit{E. faecalis}. Isoforms of IS257 were detected in the both the HLGR and the sensitive isolate taken for study.

- All the 40 HLGR clinical isolates were found to carry the transposon Tn5281. Presence of Tn5281 was confirmed with two different amplification approaches and also by hybridisation using the PCR amplified HLGR gene fragment as probe.

- All the 40 HLGR isolates were found to carry the transposon Tn5281 in intact form, as against various truncated forms identified in \textit{E. faecalis}. Modified forms of Tn5281 involving the association of IS257 with the HLGR gene were not detected.

- Conjugative transfer of the HLGR gene was observed in 80% of the HLGR isolates used for the study. The conjugation frequency was observed to lie between \(2.32\times10^{-7}\) to \(9.58\times10^{-13}\).

- Plasmids of sizes as small as 1.2kb to sizes above 21kb was observed in all the HLGR clinical isolates and few of them were found to be transferred to the recipient strain through filter
mating. However these plasmids were not the carriers of the HLGR gene in all the clinical isolates taken for study.

- HLGR clinical isolates that did not respond to conjugation was also found to carry the transposon Tn5281. Plasmids inducing responses to the sex pheromone secreted by the plasmid free recipient strain could influence conjugative transfer of the HLGR gene. Thus plasmids carried by the HLGR clinical isolates may hold a significant role in disseminating the resistance gene.

- The lower conjugation frequencies shown by the isolates could be correlated to the chromosomal location and the intactness of the transposon housing the HLGR gene.

- Molecular typing of the HLGR clinical isolates analysed through PFGE showed that the clinical isolates are more related than diverse. The dendrogram generated using PFGE profiles depicted relatedness among isolates with respect to source of infection, antiogram and year of isolation. 65% of the isolates had shown more than 75% similarity coefficient in the dendrogram generated.

- The dendrogram was able to identify isolates M 15 and M 34 that seemed persistent for a period of three years in the clinical set up. Clustering together of clinical isolates like M 264 and M 264(T) showed the relapse of the infection in individuals.

- Molecular subtyping of the HLGR clinical isolates was also estimated by PCR using the insertion sequence IS256. The differences in the PCR banding pattern due to the presence of multiple copies of IS256 was utilised to assess the diversity of the isolates taken for study. The diversity analyses produced results comparable with the PFGE based subtyping.
• Simpson’s diversity index was calculated using the clustering pattern obtained from the dendrogram generated through both PFGE profiles and IS256 based PCR amplified profile. The scores projected heterogeneity among the isolates taken for study.

• Relatedness of the clinical isolates could be attributed to the HLGR phenotype used as the selection marker. The genotypic screening had implied uniformity to be pronounced among the clinical isolates with respect to the HLGR gene. The genome accounts a good degree of stability with respect to the HLGR gene.

• Diversity among the clinical isolates could be reflected due to the presence of insertion sequences in the isolates. Determination of other virulence features harboured by the clinical isolates could aid in understanding the heterogeneity among the isolates.

• Simpson’s diversity index calculated using PFGE based subtyping and IS256 based subtyping gave “D” scores that showed only less variation. This could imply the similarity in clusters established by the two techniques. Hence the clustering ability of the IS256 based subtyping could be comparable with the well establish technique of PFGE for *E. faecalis*.

### 6.2 CONCLUSION
The following conclusions could be possible as a result of the investigation made in the HLGR clinical isolates taken for this study,

- The most common genetic determinant of HLGR in clinical isolates of *E. faecalis* is the bifunctional gene, *aac(6')-Ie-aph(2'')-Ia*.
- The resistance gene exists in association with an insertion sequence favouring mobility of the resistance gene among related isolates.
- HLGR resistance is capable of conjugative transfers within related strains of *E. faecalis* due to the involvement of transposons.
- Chromosomal location and intact transposons favours stability of the genetic mechanism of resistance. However, mobility could be enhanced if located in plasmids.
- Diversity measurements can highlight the genome wide variation among the isolates that carry significant genetic relatedness with respect to single phenotype of HLGR.
- IS256 based subtyping could be used as a potential tool for analysing diversity in *E. faecalis*.

6.3 **FUTURE DIRECTIONS**

All the clinical isolates screened from the PSG hospitals showed high similarity with respect to the transposon Tn5281 and the location of the HLGR gene. This is a peculiar finding as against reports from other countries, of truncations in Tn5281 and the location of the HLGR gene to be common in both plasmids and chromosomes. Extension of a similar investigation to isolates from other hospitals in Coimbatore and also from other locations within India could enable a clear understanding of HLGR and its spread.
Assessment of other virulence factors carried by these clinical isolates and analyses regarding their transfer through conjugation will help in understanding the dissemination of virulence traits in a nosocomial situation.

Antibiotic resistant *E. faecalis* has been isolated from a private poultry farm near Coimbatore. Relatedness of those isolates with the clinical isolates can be established by PFGE profiling. Estimating the relatedness could give an indication of alternative sources for the acquisition of antibiotic resistance in *E. faecalis*. 