ABSTRACT

*Enterococcus faecalis* is gaining importance in the nosocomial environment due to the development of antibiotic resistance. The commonly used method of control against *E. faecalis* is a synergistic combination of a glycopeptide along with an aminoglycoside. The most commonly used aminoglycoside is gentamicin. High level gentamicin resistance (HLGR) (MIC>500mg/ml) is mainly conferred by the fused gene \(aac(6')-Ie-aph(2'')-Ia\) encoding the aminoglycoside modifying enzyme, aminoglycoside acetyltransferase and phosphotransferase. This gene is present in plasmids and/or chromosomes associated with transposable elements which favour horizontal transfer of HLGR. The molecular basis of HLGR in clinical isolates prevalent in India has not yet been analysed.

This study was aimed to determine the genetic factors conferring HLGR, their location and the frequency of transfer using 40 clinical isolates of *E. faecalis*. PCR based screening of the clinical isolates indicated the presence of the fused gene \(aac(6')-Ie-aph(2'')-Ia\) only and not the other genes \(aph(2'')-Ib\, Ic\) and \(Id\) in the clinical isolates. PCR amplification also indicated the presence of insertion sequences IS256 and IS257 in all the clinical isolates. The association of the HLGR gene with the insertion sequences revealed the presence of the transposon Tn5281. Tn5281 was observed to be intact in all the 40 clinical isolates.

Mobility of the HLGR gene was analysed by filter mating technique that indicated the transfer of HLGR gene in 80% of the clinical isolates. Plasmids were also present in all the clinical isolates, but they were
not the carriers of the HLGR gene. However, the role of plasmids favouring mobility of the HLGR gene would be inferred through the sex pheromone responses with the plasmid free recipient strain.

The HLGR clinical isolates showed uniformity with respect to the HLGR gene and the intactness of the transposon Tn5281. However, the factors that influence mobility of resistant traits could create genomic diversity among isolates in a nosocomial situation. Hence, genetic diversity among the isolates was estimated through PFGE and PCR based techniques. Clustering pattern in the dendrogram revealed similarities among a few isolates with respect to the source of origin and antibiogram. Two samples isolated at different time periods showed 100% similarity by both the subtyping methods indicating the possibility of persistence of HLGR within the hospital set up.

Simpson’s diversity index revealed the heterogeneity among the isolates. Heterogeneity could be attributed to the difference in the genome and not with respect to HLGR.