INTRODUCTION
CHAPTER I
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Enterococci are typically found in the intestinal tract and faeces of man and other animals (Murray, 1990). Some species were also identified from soil, food, water plants, due to their ability to grow and survive under wide range of environmental conditions, (extreme temperature and salt concentrations) which help in their ubiquitous distribution.

There are more than thirty species identified in enterococci of which thirteen are medically important. Species identification of enterococci in clinical samples is important in recent times due to the occurrence of unusual species as well as the different treatment options for the medically important species.

Enterococci are an important cause of nosocomial infections such as bacteraemia, surgical wound infection, urinary tract infection and endocarditis in various regions of the world. Resistance in enterococci can be categorized as tolerance, intrinsic resistance and acquired resistance.

Tolerance is the ability of the organism to survive levels of drugs well in excess of the MIC. Penicillin and vancomycin resistance of enterococci is due to their tolerance upto levels above their MIC and thus, such strains are treated effectively by use of drugs infused above their tolerant range.

All enterococci exhibit decreased susceptibility to penicillin and ampicillin, as well as high-level resistance to most cephalosporins and all semi-
synthetic penicillins, as the result of expression of low-affinity penicillin-binding proteins.

They show different intrinsic susceptibility to Streptogramin family. These are mediated by the chromosomally encoded putative ABC transporter in *E. faecalis* genome, named as *lsa* gene, its mechanism remains poorly understood (Kristich *et al.*, 2014). Disruption of *lsa* gene in *E. faecalis* OG1RF results in a >40-fold decrease in MIC to Quinupristin-Dalfopristin making the drug more susceptible. *E. faecium* strains are highly susceptible to quinupristin-dalfopristin (Pristinomycin) as it does not possess specific resistance determinants, whereas, *E. faecalis* is intrinsically resistant to this combination.

Trimethoprim-sulfamethoxazole appears to be active against enterococci when tested *in vitro* on folate-deficient media, but it fails to be active in animal models. This may be due to the uptake of folate by the bacteria from external environmental sources (Zervos & Schaberg, 1985).

Intrinsic resistance is encoded within the core genome of all members of the *Enterococcus* species and different from acquired resistance, in which it is present only in some members of the same genus. Acquired resistance is obtained via the horizontal exchange of mobile genetic elements or through selection upon antibiotic exposure and pressure.

Since the early 1970s, the synergistic activity of an aminoglycoside with a cell wall-active agent has been predicted by determining the ability of *Enterococcus* to grow in the presence of high levels of the aminoglycoside (>2000 µg/ml) (Swenson *et al.*, 1995).
High level aminoglycoside resistance in enterococci minimizes the synergism with cell wall inhibitor and becomes ineffective. The mechanism of such resistances in these isolates is due to, secretion of enzymes that inactivates the aminoglycoside by various mechanisms such as adenylation and phosphorylation (Randhawa et al., 2004).

Erythromycin was first used for treatment in 1956 and soon resistance has emerged in *Staphylococcus*. Further, erythromycin resistant streptococci were reported from United Kingdom in 1959 and later in other parts of the world. Macrolide and lincosamide resistance was shown to be mediated ribosomal methylation mechanism. *Enterococcus* isolates are found to carry acquired macrolide resistant genes such as *ermA*, *ermB* and *ermC*. *E. faecium* was reported to have more intrinsic resistance to macrolides than the other species (Neu, 1993).

Apart from *erm* genes, low level resistance to macrolides was also found to be encoded by transferrable *mefA* gene through efflux mechanism (Clancy et al., 1996).

Nine distinct gene clusters of Van types had been identified to confer glycopeptide resistance in enterococci. *E. faecalis* V583 was one of the first vancomycin-resistant clinical isolate that carried 25% of acquired DNA in its genome (Polidori et al., 2011). Enterococci are predicted as donors of glycopeptide resistant R-plasmids and had been shown to contribute for the emergence of vancomycin-resistance in *Staphylococcus aureus* (VRSA) (Zhu et al., 2008).
Acquired VanA- and VanB-type glycopeptide resistance are of greater interest as glycopeptides have been considered as the drugs of last resort for the treatment of all multiple drug resistant gram-positive infections (Courvalin, 2006).

Development of multiple drug resistance, high level aminoglycoside resistance and glycopeptide resistance had lead to difficult to treat serious enterococcal infections.

The first examination of enterococcal virulence was reported in 1899 (MacCallum and Hastings., 1899) the same year the organism was discovered (Thiercelin, 1899). The virulence of an organism is regulated with virulence encoding genes present on the genome in special regions which are termed as pathogenicity islands [PAI] (Upadhyaya et al., 2009).

Virulence factors such as Enterococcus surface protein, cytolytic toxin, or aggregation substance or the protease, gelatinase, hyaluronidase, biofilm formation etc., are widely reported among clinical enterococcal isolates.

The identification of these virulence factors which are associated with invasiveness and disease severity has become an important area of research.

Presence or absence of virulence factors in an organism needs to be evaluated with respect to the conditions of its growth since there could be inactive gene product or down regulation of gene expression/silent genes. Hence, in recent times virulence characteristics are analyzed also by genotypic methods (Vankerckhoven et al., 2004).
Mobile genetic elements including plasmids and transposons are important for horizontal transfer of resistance determinants in enterococci (Paulsen et al., 2003; Leavis et al., 2007).

Plasmids are abundant in enterococci and they play a role in adaptation to different environments especially to hospital settings in case of E. faecium (Freitas et al., 2010; Rosvoll et al., 2010).

There are several ways of grouping plasmids viz; replication mechanisms, Inc typing, transferability, resistance gene carriage, etc. Traditionally enterococcal plasmids have been classified into 3 groups namely the Inc 18 group of plasmids which are also called broad host range plasmids (rep group 1, represented by pIP501 and partially rep family 2, represented by pRE25), the rolling circle replication (RCR) plasmids (mostly rep family 4 and 6, represented by pMBB1 and pS86 respectively) and the pheromone responsive plasmids of E. faecalis (rep family 8 and 9, represented by pAM373 and pCF10).

Inc18 group plasmids carry the vancomycin resistance determinants and allow the transfer of vanA gene from E. faecalis to staphylococci (Zhu et al., 2010).

VanA-type vancomycin resistance was transferred from E. faecium to E. faecalis as a result of VanA-encoding pheromone responsive plasmid or through the co-integration of a VanA plasmid and a pheromone-responsive plasmid (Heaton et al., 1996; Hegde et al., 2011). The most studied pheromone responsive plasmids are pAD1 (which secretes cytolysin) and pCF10 (which
encodes tetracycline and minocycline resistance carried by conjugative transposon Tn925).

They secrete small peptides called pheromones which induces the clumping of donor (tet(M) conjugative transposon Tn925) and recipient cells which facilitates the transfer of plasmids (with frequencies as high as $10^{-2}$ - $10^{-1}$/recipient CFU ) between enterococci (Ehrenfeld & Clewell, 1987).

Molecular tools had helped the researchers to easily characterize and classify unknown plasmids using specific replicon probes and primers. Recently a PCR based plasmid typing system for gram positive organisms were developed by Jensen and co-workers based on homology of conserved areas of the replication initiation genes (rep). Altogether 19 plasmid families and 19 unique sequences were defined (Jensen et al., 2010), and 12 of these plasmid families have been identified in *E. faecium* and *E. faecalis*.

The numbers of plasmids are high in enterococci, it is important to monitor the plasmid population in enterococci for better understanding of their changing epidemiology and also the spread of antimicrobial resistance within the species. This would help us to develop strategies for control of infections due to resistant clones in a specific region.

Hence, this study was undertaken to analyze the enterococcal resistance mechanisms, virulence determinants by both phenotypic and genotypic methods and distribution of plasmids by PCR based replicon typing (PBRT) method.