CHAPTER 2

REVIEW OF LITERATURE

2.1. HISTORICAL PERSPECTIVE

Enterococcus species are Gram positive cocci inhabiting the alimentary tract of humans and animals. Though they were believed to be harmless commensal for many years with no medical significance, they have emerged recently as one of the most common nosocomial pathogen (Lopes et al., 2006).

To emphasize the intestinal origin of Gram-positive diplococcus, Thiercelin in 1899 described the group as Enterococcus. Thiercelin and Jouhaud proposed the genus Enterococcus in 1903 (Thiercelin and Jouhaud, 1903). Subsequent members of this genus was described by Andrews and Horder in 1906 (S.faecalis), Orla-Jensen in1919 (S. faecium), Sherman in 1937 (S.durans).

In 1930s, Lancefield recommended the division of Streptococci into four groups based on their reaction with A-G antisera and Enterococcus reacted with group D antisera were classified as group D streptococci. Sherman, (1937) proposed a new classification scheme for the genus Streptococcus and separated it into four divisions designated: pyogenic, viridans, lactic and enterococci.

In 1970, a separate genus of Enterococcus including S. faecalis and S. faecium, was recommended (Kalina, 1970). Though the recommendation was not accepted, it was informally named. In 1984, the bacteria under genus Streptococcus was reallocated into three genera, Enterococcus, Lactococcus,
and *Streptococcus* (Schliefer and Killper-Baltz, 1984). Valid *Enterococcus* species identified by DNA-DNA hybridization studies are following: *E. avium*, *E. casseliflavus*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, and *E. mundtii*, *E.asini*, *E.aquimarinus*, *E.caccae*, *E. canis*, *E.cecorum*, *E.columbae*, *E.sulfureus* *E.dispar*, *E.gilvus*, *E.hemoperoxidus*, *E.italicus*, *E.moraviensis*, *E.pallens*, *E.phoeniculicola*, *E.ratti*, *E.pseudoavium*, *E.termitis* and *E.villorum* (Patel et al., 1998; Tsiodras et al., 2000).

According to Facklam and Collins typing schemes based on biochemical reactions the genus has different groups. Group I (*E. avium*, *E. raffinosus*, *E. malodoratus*, *E. pseudoavium*), Group II (*E. faecalis*, *E. faecium*, *E. casseliflavus*, *E. mundtii*, *E. gallinarum*) and Group III (*E. durans*, *E. faecalis*, *E. hirae*).

### 2.2. HABITAT AND DISTRIBUTION

A major habitat of enterococci is the gastrointestinal tract of humans and of other animals, where they frame a significant portion of the normal gut flora (Klein, 2003). Small numbers of enterococci are occasionally found in oropharyngeal secretions, vaginal secretions and on the skin, especially in the perineal area.

Other than human sources they are also isolated from animal sources, plant sources and environmental sources (Klein, 2003). There are studies with information of isolation from different food items such as cheese, fish, sausages, minced beef and pork. *E. mundtii* and *E. sulfureus* have been isolated from fish and meat (Klein, 2003). Enterococci that have been isolated from dogs and cats include *E. avium*, *E. durans*, *E. faecalis*, *E. faecium*, *E. raffinosus* and *E. hirae* (Devriese et al., 1992). The rarely
isolated species *E. cecorum* and others like *E. dispers* and *E. malodoratus* have been isolated from faeces from bovine and poultry sources (Devriese *et al.*, 1991) and have been connected with spoilage of sausage (Ong *et al.*, 2002).

*E. avium, E. casseliflavus, E. durans, E. faecalis, E. faecium, E. gallinarum, E. hirae, and E. mundtii* have been isolated from surface waters (Rice *et al.*, 1995).

### 2.3. MORPHOLOGICAL FEATURES

*Enterococcus* species are Gram-positive cocci arranged in short chains or in pairs. Under certain growth conditions they can elongate and appear coccobacillary form. Ovoid cells are elongated in direction of the chains and are arranged in pairs and in chains. They are 0.5 to 1.5 micrometer in diameter. Though generally nonmotile, *E. casseliflavus* and *E. gallinarum* are motile species (Murray, 2000). There are three main components that make up its cell wall: peptidoglycan, teichoic acid, and polysaccharide. 40% of the cell wall is made up of peptidoglycan, while the rest of the cell wall is made up of a “rhamnose-containing polysaccharide and a ribitol-containing teichoic acid”. *E. faecalis* is generally considered a non capsulated organism (De la Maza *et al.*, 2004). However, few *E. faecalis* isolates possess a capsular polysaccharide (Murray, 1998). Surface proteins called the aggregation substances that recognize a specific ligand on recipient cells” ensure successful connections for conjugation (Thurlow *et al.*, 2009). *E. faecalis* also have the capability to make surface pili which help in the formation of a biofilm. Nallapareddy *et al.*, (2006) has reported that the *E. faecalis* strains that cause endocarditis contain large amounts of these pili.
2.4. **GROWTH REQUIREMENT AND CULTURAL CHARACTERS**

Enterococci are facultatively anaerobic and non fastidious bacteria. For growth they require a carbon source, usually glucose, vitamin B, and nucleotide bases. They have an absolute requirement of histidine, methionine, isoleucine and tryptophan and some may require arginine, glutamate, glycine, leucine and valine.

KF agar (Kenner Faecal Agar) medium is a selective differential agar that contains sodium azide that inhibits catalase positive organisms. Other media used are the enterococcus selective agar, and the kanamycin Aesculin Azide (KAA). BEA (Bile Aesculin Azide agar) is known by several synonyms-Pfizer selective enterococcus (PSE) agar, Enterococcosel (ECSA) agar, and D-CoccoSel, the frequently recommended selective Enterococcus agar because of its ability to discriminate enterococci from specimen containing multiple microbial components (Murray, 1990).

Enterococcal colonies on solid media are smooth, cream or white coloured and entire. On blood agar media with 10% (v/v) blood, enterococci produce beta, alpha or non hemolytic colonies. On Brain Heart Infusion agar medium, colonies are non pigmented but *E. mundtii, E. casselilavus & E. sulfurosus* produce yellow pigment (Murray, 1990). On Bile Esculin Agar these colonies are grey surrounded by a black halo due to the hydrolysis of esculin to escultin which reacts with ferric ammonium citrate to give a black colour due to production of insoluble iron salts.

2.5. **IDENTIFICATION AND TYPING**

Enterococci are presumptively identified for years by their cultural, morphological and biochemical characters. The genus can be identified
based on grams staining. To differentiate them further from other Gram positive catalase negative cocci, serogrouping with group D Ag according to Lancefield were suggested (Murray, 1990). The demonstration of group D antigen is not specific for *Enterococcus* as *S. bovis* and *S. equinus* have the group D antigen and some strains of Leuconostoc and most strains of Pediococci also have the D antigen. These streptococci can be differentiated from enterococci by the lack of growth in 6.5% NaCl and at 10°C. The tests like PYR and LAP can differentiate enterococci species from other genera like *Leuconostoc, Pediococcus* and *Weissella* (Murray, 2000). *Lactococci* and *Aerococci* are PYR positive and LAP negative. A combination of Gram stain, positive PYR & LAP test, and negative group A serological test can be used as a useful screening test for presumptive identification of genus enterococci since group A streptococci are the only streptococci that are PYR positive.

2.6. SPECIES IDENTIFICATION

Enterococcal isolates can be identified to the species level by using a conventional physiological tests devised by Facklam and Collins, (1989). A number of biochemical differences are utilized for the differentitation of various species. These were performed in heart infusion broth base with 1% sugars like mannitol, sorbitol, sorbose, inulin, arabinose, melibiose, sucrose, raffinose, trehalose, lactose, glycerol, salicine or maltose. Deamination of arginine is tested in Moellers decarboxylation broth. *E. faecalis* grows on medium containing 0.04% tellurite and has the ability to reduce tetrazolium to formazan and to produce acid from glycerol (Facklam & Collin, 1989). *E. faecium* produces acid from melibiose and L-arabinose. *E. casseliflavus* and *E. gallinarum* are motile. *E. casseliflavus* and *E. mundtii* produce yellow pigment (Collins *et al*., 1986).
Commercial systems for identification of Enterococci are API20S, GPI system, and the rapid strep System or RapID STR system. Most of these tests require only 4 h.

2.7. VIRULENCE FACTORS

Enterococci exist as commensal, in harmony with the host and with other gut flora. Perturbation in the dynamics of the host commensal relationship like antibiotic treatment, host injury, diminished immunity, allow these intestinal bacteria to gain access to extra intestinal host sites to cause infection (Sekirov, et al., 2010). The nosocomial enterococci might have extra capabilities to colonize, overgrow and invade host tissues. These capabilities might be supported by the use of antibiotics without significant anti-enterococcal activity, thus overcoming the competition of indigenous flora and creating niches which can be readily colonized by the nosocomial enterococci.

An in vitro study (Guzman et al., 1989) provided evidence for the role of adherence in the pathogenesis of E. faecalis urinary tract infection and endocarditis (Guzmàn et al., 1989). Following colonization of the GI tract, bacteria may be able to translocate and evidence of translocation of E. faecalis across the intact intestinal epithelium was given by Wells et al., (1990), who cultured E. faecalis from liver, spleen and lymph nodes of mice (Wells et al., 1990). Followed by phagocyte uptake, the gastrointestinal bacteria exit at the apical side or migrate in phagocytes to the mesenteric lymph nodes, proliferate and spread to distant sites (Berg, 1995). Although antibodies to enterococci are found in humans with enterococcal infections (Sulaiman et al., 1996), efficacy it in the prevention of infections is quite contradicting.
(PAIs) pathogenicity islands, where virulence genes often clustered on the genome in distinct regions has been recognized in *E. faecalis* (Shankar *et al*., 2002). Most prominent among these virulence determinants are the surface adhesins Esp and aggregation substance (AS), Ace, a MSCRAMM (microbial surface components recognizing adhesive matrix molecules), secreted toxin cytolysin, gelatinase and serine protease, enterococcal capsule, cell wall polysaccharides and extracellular superoxide (Upadhyaya *et al*., 2009). Each of these virulence factors will be discussed briefly in the following sections.

2.7.1. **Cytolysin:**

Hemolysin also called cytolysin. It occurs in majority of *E. faecalis* isolated from clinical infections (Gilmore *et al*., 1994). The encoding operon is arried on a plasmid or integrated into bacterial chromosome (Ike and Clewell, 1992). Several animal models were used to establish the virulence potential of cytolysin in enterococcal infection like rabbit endophthalmitis model and rabbit endocarditis model. Cytolysin producing strains were found to be more virulent and they aggravate the clinical course of human infection (Ike *et al*., 1984). Cytolysin possesses toxic activities and bacteriocin like properties. Furthermore, bacteraemia by cytolytic strains has been determined to be associated with a fivefold increased risk for death compared to the infections caused by non-cytolytic strains (Huycke *et al*., 1991). This toxins cause a beta lytic reaction on human on sheep blood agar, which is frequently used in clinical microbiology laboratory (Mundy *et al*., 2000) for identification.
Chapter 2

The eight genes concerned with production of the cytolysin are organized in a cyl operon produced by products of eight genes, designated cylR1, cylR2, cylLL, cylLS, cylM, cylB, cylA, and cylI (Koch et al., 2004).

2.7.2. Enterococcal surface protein (Esp)

Esp is a large bacterial cell wall surface associated protein found to be a part of a pathogenicity island. (Mundy et al., 2000). Esp is encoded by the esp gene. The frequency of the gene has been found to be significantly higher among isolates recovered from infected patients than among other isolates and stool isolates from healthy individuals (Kayaoglu and Orstavik, 2004). It promotes adhesion, colonization and evasion of the immune system. In addition, the contribution of the surface protein Esp to colonization and persistence of *E. faecalis* in urinary tract infections has been shown in animal models (Shankar et al., 1999). Furthermore Toledo-Arana et al., (2001) found a significant correlation between the presence of Esp and the ability of *E. faecalis* to form biofilms on polystyrene.

2.7.3. *E. faecalis* antigen A (efaA)

The *E. faecalis* antigen A (EfaA) was identified through screening of different surface proteins with sera from endocarditis patients (Xu et al., 1997). However, the contribution of EfaA to pathogenesis could not be confirmed though there is a higher incidence in infection derived isolates, the gene was found in all *E. faecalis* isolates from both infection and healthy volunteers (Singh et al., 1998).

2.7.4. Ace

Adhesion of bacteria to host cells or extracellular matrix proteins is the initial step in infection. Ace, which is a collagen binding protein, belongs to MSCRAMM (Microbial surface component recognizing adhesive matrix
molecules) family. Ace has a role in the adhesion of bacteria to dentin (Hubble et al., 2003). Results from Nallapareddy et al., (2000) indicate that Ace might be produced during infection because antibodies to Ace were found in 90% of sera from endocarditis patients.

2.7.5. Aggregation substance (AS)

Aggregation substance (AS) is a pheromone-responsive, plasmid-encoded bacterial surface protein that promotes aggregate formation between bacteria which mediates plasmid transfer during bacterial conjugation & has a role in the pathogenesis (Galli and Wirth, 1991; Koch et al., 2004; Kayaoglu and Orstavik, 2004).

2.7.6. Hyaluronidase

Hyaluronidase acts on hyaluronic acids and is a degradative enzyme which is identified in infections with tissue damage by facilitating the invasion of enterococci through host tissue (Hynes et al., 2000). Hyaluronidase is a major virulence factor of Enterococcus faecalis. Hyl gene is important in the pathogenesis of this bacterium (Wu et al., 2007). Hyaluronidase production is responsible for tissue destruction and has a role in caries formation (Rosan and Williams, 1964)

2.7.7. Extracellular polysaccharides

An operon encoding synthesis of capsular polysaccharide is found in Enterococci. This type is most commonly expressed by clinical isolates of E. faecalis (Hancock and Gilmore, 2002). Antibodies may be useful for prevention of enterococcal infections.(Huebner et al., 2000).
2.7.8. Extracellular superoxide

Extracellular superoxide production has a role in bacterial translocation across the epithelium. And it contributes to chromosomal instability associated with intestinal polyps and colorectal cancer. This anion production was found to enhance the in vivo survival of *E. faecalis* in mixed infection (Huycke and Gilmore, 1997).

2.7.9. Lipoteichic acid (LTA)

They are present on the cell surface of gram positive including enterococci and have a role in plasmid transfer. Through its lipidic moiety, the LTA molecule has been found to bind to a variety of eukaryotic cells, including platelets, erythrocytes, lymphocytes, PMN leukocytes and epithelial cells (Beachey, 1981).

2.7.10. Gelatinase

Gelatinase is an extracellular zinc-containing metalloproteinase from *E. faecalis* which was first purified and described by Bleiweis and Zimmerman, (1964). It can hydrolyze gelatine, collagen, fibrinogen, casein, hemoglobin, insulin etc. Collagen hydrolysis by them play a role in the pathogenesis of periapical inflammation. Lethality of gelatinase producing strains was described in a study by Singh *et al.*, (1998). Gelatin liquefying strain induced caries in rats and nonproteolytic strains did not produce caries (Gold *et al.*, 1975). A locus called fsr, positively regulates expression of gelatinase and serine protease in *E. faecalis* OGIRF (Qin *et al.*, 2001).

2.7.11. BIOFILM

Biofilm is a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each
other and are embedded in a matrix of extracellular polymeric substances, proteins, polysaccharides and nucleic acids. Biofilm formation is a complex developmental process involving attachment and immobilization on a surface, cell-to-cell interaction, microcolony formation, formation of a confluent biofilm, and development of a three-dimensional biofilm structure (O'Toole et al., 2000). Problems associated with biofilm formation can be varying based on site of production. Biofilm production in the surfaces results in reduced heat exchange, increased frictional resistance in pipes, deterioration of water quality, with in municipal drinking water systems, plugging, and petroleum souring. Biofilms is also formed on specific devices like prosthetic heart valves, central venous catheters, urinary catheters, contact lenses, intrauterine devices, and dental unit water lines. Biofilm has recently been suggested to be an important factor in the pathogenesis of enterococcal infections (Di Rosa et al., 2006; Mohamed et al., 2004). Bacteria in biofilms are resistant to phagocytosis; making biofilms extremely difficult to eradicate from living hosts (Lewis, 2001). More than 60% of all microbial infections are caused by biofilms (Lewis, 2001). Common biofilm associated infections are urinary tract infections caused by catheters, middle-ear infections, common dental plaque formation, and gingivitis (Costerton et al., 1999). And all of these biofilm mediated infections, are hard to treat or frequently relapsing.

However, the exact processes by which biofilm-associated organisms elicit disease in the human host are only poorly understood. Suggested mechanisms include the following: firstly the detachment of cells or cell aggregates from indwelling medical device biofilms, resulting in bloodstream or urinary tract infections, secondly by the production of endotoxins, thirdly by the resistance to the host immune system, and then by
Chapter 2

providing a niche for the generation of resistant organisms. When the inherent resistance of biofilm to industrial biocides was first discovered, this property was attributed to a limitation in mass transfer conferred by the matrix material (Donlan and Costerton, 2002).

Resistance may be due to delayed penetration of the antimicrobial agent through the biofilm matrix (Mah and O’Toole, 2001). The matrix presents a diffusional barrier for these molecules by influencing either the rate of transport of the molecule to the biofilm interior or the reaction of the antimicrobial material with the matrix material. Suci et al., (1994) have reported the altered growth rate of biofilm organisms. Biofilm-associated cells grow significantly more slowly than planktonic cells and, as a result, take up antimicrobial agents more slowly (Donlan and Costerton, 2002).

Factors affecting biofilm formation

Nutrient contents of the growth medium, such as glucose, serum, availability of iron and CO₂, NaCl, pH, and temperature influence biofilm production among different bacteria. Carbohydrate metabolism regulates biofilm production among various Gram-positive bacteria, including _E. faecalis_ (Pillai et al., 2004). _Fsr_ is a quorum-sensing locus associated with virulence controls biofilm development through the production of gelatinase. It has been suggested that a glucose-dependent transcriptional regulator may directly or indirectly control _fsr_ (Pillai et al., 2004). TSB medium with 1 % glucose supplementation can enhance the biofilm production in _E. faecalis_ compared to TSB without glucose (Baldassarri et al., 2001). Another study found a reduction in biofilm production by _E. faecalis_ as the glucose concentration increased from 0 to 0.2 % in the culture medium (Kristich et al., 2004).
Recently it was also shown that alkaline pH increase biofilm formation and these effects were dependent on the cation-responsive regulatory protein NhaR (Goller et al., 2006). Changes in the osmotic strength also affect biofilm formation in *E. faecalis*. A study showed that biofilm production was abolished by exposure to a medium with high osmolarity (2–3 % sodium chloride).

Biofilm-producing *E. faecalis* isolates survive better in macrophages than the non-biofilm producers (Baldassarri et al., 2004). Such isolates expressing extracellular polysaccharide were found to survive within rat peritoneal macrophages (>24 h) for a longer period of time than the polysaccharide-negative strains (Baldassarri et al., 2004).

There are a number of factors described to be involved in biofilm formation. They were revealed as a result of the availability of genomic and proteomic approaches. Multiple genes are reported to be associated with biofilm formation such as fsr, gelE, EPA (enterococcal polysaccharide antigen), atn (autolysin) (Mohammed et al., 2004). Products of these genes are associated with the formation of capsular polysaccharide or exopolysaccharide.

The association of gelatinase and biofilm production has been reported in enterococcal isolates collected from Italy (Baldassarri et al., 2004; Di Rosa et al., 2006). Another study has shown that gelatinase was not required for biofilm production in *E. faecalis* and *E. faecium* (Di Rosa et al., 2006). These results suggest that fsr has an effect independent of gelatinase on biofilm formation in *E. faecalis*, and that this effect is in the same direction as that of agr of staphylococci.
Effective strategies to prevent or control biofilms on medical devices must take into consideration the unique and tenacious nature of biofilms. Intervention strategies prevent initial device contamination, minimize initial microbial cell attachment to the device, penetrate the biofilm matrix and kill the biofilm associated cells. Again by mechanical cleaning and by using oxidative biocides; the former removes biofilms, and the latter kills the sessile cells.

Several classes of chemical compounds have shown promise in combating biofilms when used in conjunction with traditional antimicrobials. The vast majority of these compounds exert their anti-biofilm properties through disruption of "quorum sensing," a common means of intercellular communication in bacterial communities that allows coordinated expression of virulence factors and facilitates formation of the complex architecture of mature bacterial biofilms. New techniques for biofilm disruption are ultrasound, hydrolysis of extracellular polymeric substance etc.

2.8. ENVIRONMENTAL PARAMETERS AFFECTING GROWTH

The enterococci are able to survive a range of adverse environmental conditions. Enterococcus species (including VRE) survive for months on dry surfaces (Neeley and Maley, 2000).

2.8.1. Influence of pH on enterococci

Enterococci grow over a broad pH range. The optimum pH for their growth is 7.5 (Van den Berghe et al., 2006). However their ability to grow in a medium at pH 9.6 is used for the identification of enterococci as this ability is a general characteristic of enterococci. Viability of enterococci at pH 3 (Galaz et al., 2004) and at a high alkaline pH of 10 (Hartke et al., 1997) has been noticed.
2.8.2. Sodium chloride tolerance

The ability of NaCl resistance can be used in the presumptive identification of Enterococcus. NaCl treatment was found to be effective in the induction of general stress proteins in some enterococci (Ahmad et al., 2002). NaCl resistance is due to its cation homeostasis.

2.8.3. Influence of temperature

Enterococcus grow at a wide range of temperature from 5°C-50°C (Fisher and Phillips, 2009). Enterococcus survives heating at 60°C for 30 min and this property helps to distinguish Enterococcus from other closely related genera. Enterococcus are highly thermo resistant bacteria as mentioned in a previous study (Perez et al., 1982).

2.8.4 Interaction with other bacteria

Enterococcus produce a range of bacteriocins like A, B, I, L, Ej97, CRL35, N15, AS-48, 012, AT06, KS and P which are active against many bacteria like Listeria, Clostridia, Staphylococci, Bacillus, Acinetobacter, Klebsiella and Pseudomonas (Campos and Rodriguez, 2006). Because of its antagonistic effect the genus Enterococcus is also used as probiotics. Probiotics are practical solution against gastrointestinal infections (Havenaar et al., 1992). E. faecium SF68 has been used to treat diarrhea and it is considered as an alternative to antibiotic treatment (Marteau et al., 2001).

2.9. DISINFECTANT RESISTANCE

Large quantities of disinfectants are used in a range of purposes in hospitals and in modern society. The skin disinfection can be done externally before the surgical procedures. More recently it has become a trend to incorporate biocide into a variety of materials to reduce cross infection like
chopping boards, soaps and creams. Common agents for disinfection purpose include alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, hydrogen peroxide, iodophors, peracetic acid, phenolics, and quaternary ammonium compounds.

Different factors affecting the disinfectant activity include the intrinsic property of the organism, the chemical and physical environment. The greater, the concentration of the disinfectant the greater is the efficacy and the shorter the time necessary to achieve microbial kill. Temperature, pH, relative humidity and water hardness also influence the disinfection (CDC Guidelines, 2008). Organic matter from patient sources, such as faecal matter, body secretions and blood can interfere with the antimicrobial activity (Oliveira et al., 2010).

Glutaraldehyde is active against gram positive and gram negative organism (Gorman et al., 1980). Glutaraldehyde was effective in many of the studies. Nevertheless intrinsic resistance was reported (Fraise, 2002).

Hypochlorites are the most widely used chlorine disinfectant and are available as liquid (e.g., sodium hypochlorite) or solid (e.g., calcium hypochlorite. A 1:10–1:100 dilution of 5.25%–6.15% sodium hypochlorites mainly household bleach has been recommended for decontaminating blood spills (Weber et al., 1999). Numerous studies support that NaOCl was effective against Enterococcus (Abdulla et al., 2005). But there are studies showing NaOCl were not effective by exvivo or invivo (o’Hara et al., 1993). VRE are known to tolerate sodium hypochlorite indicating the potential of the organism to survive in the environment. (Kearns et al., 1995; Bradley and Fraise, 1996).
Tincture or aqueous solutions or alcoholic solution have been used for more than hundreds of years as antiseptics. Povidone iodine was found to be effective against enterococci (Block et al., 2000).

QAC are the most useful antiseptic and disinfectant (McDonnell and Russell, 1999). They are used in a variety of clinical purpose like preoperative disinfection and disinfection of noncritical surface and also used for hard surface cleaning. It is known that some quaternary ammonium compounds (QACs) are subjected to resistance mediated by the qacA efflux pump. Many of the researchers are in agreement with its efficacy, qacZ gene was detected in enterococci (Braga et al., 2010).

The phenolics are used for disinfection of ward floors, in discarding jars in laboratories and disinfection of bedpans. 5% Lysol (a saponified cresol) is the most commonly used household disinfectant.

Although the mechanism of resistance vary from agent to agent they involve one or more of the following methods like alteration of the drug target in the bacterial cell, enzymatic modification or destruction of the drug itself, or limitation of drug accumulation as a result of drug exclusion or active drug efflux. Plasmids are also involved in the resistance. Biocide resistance will result in inadequate decontamination of medical devices, causing a risk of cross infection. Some investigations have implied that there is disinfectant cross-resistance exists with antibiotics (McDonnell and Russell, 1999).

2.10. HEAVY METAL RESISTANCE IN ENTEROCOCCI

Enterococci have shown an increasing resistance to heavy metals. There are a number of interactions between microorganisms and heavy metals exist in nature. Some of the implications are useful such as cleaning
up the metal contaminated sites, it helps the bacteria to survive in the contaminated environments and it plays an important role in the biogeochemical cycling of toxic heavy metal.

Zinc is another essential trace element. Fard et al., (2011) has reported zinc resistance in Enterococcus. Bacterial cells accumulate zinc by a fast, unspecific uptake mechanism (Nies, 1999). General efflux mechanisms are present. A chromosomal gene, zntA, was found to be responsible for the transport of zinc and other cations across cell membranes (Beard et al., 1997).

Bacterial resistances to cadmium have been identified in enterococci (Laplace et al., 1996). Two resistant determinants were found on penicillinase-plasmid pI258, called cadA (also called cadCA operon) and cadB. Though cadmium resistance in enterococci was absent in a study (Nakipoğlu et al., 2009).

The use of metallic silver as an antimicrobial agent has long been recognized (Klasen, 2000). Silver-coated dressings are used extensively for wound management, chronic leg ulcers (Karlsmark et al., 2003), diabetic wounds (Hilton et al., 2004) and traumatic injuries.

Mercury resistant bacteria could resist 10ppm (mg/l) of mercury (HgCl2). Mercury resistance associated with plasmids was detected in a study (Zscheck and Murray, 1990)

In order to stay alive in the wild conditions, bacteria develop different mechanisms to confer resistance to the heavy metal. These mechanisms include the efflux of metal ions outside the cell, accumulation and complex formation of the metal ions inside the cell, and reduction of the heavy metal ions to a less toxic state mainly by enzymatic detoxification (Nies, 1999).
The metal tolerance mechanisms may contribute to antibiotic resistance. The genes encoding heavy metals are located together with antibiotic resistance genes or alternatively because bacteria can have unspecific mechanism of resistance common to different substances including heavy metals and antibiotics (Dalsgarrd and Guardbassi, 2002) & (Calomiris et al., 1984). Products such as disinfectants, sterilants, and heavy metals used in industry and in household products and antibiotics, create a selective pressure in the environment that leads to the mutations in microorganisms that will allow them better to survive and multiply (Baquero et al., 1998).

2.11. ANTIBIOTIC RESISTANCE IN ENTEROCOCCI

Enterococci have an amazing ability to survive in an environment with antibiotics. Enterococci are intrinsically resistant to beta-lactams particularly cephalosporins and penicillinase-resistant penicillins, lincosamides, nalidixic acid, low concentrations of aminoglycosides, low concentrations of fluoroquinolones, clindamycin, trimethoprim-sulfamethoxazole and nitrofurantoin (Cetinkaya et al., 2000). They can also acquire resistance to high concentrations of ß-lactams, aminoglycosides, glycopeptides (vancomycin, teicoplanin), tetracycline, erythromycin, high concentrations of fluoroquinolones, rifampin, chloramphenicol and fusidic acid.

2.11.1. Beta-lactams

The beta lactam antibiotics act by inhibiting the cross linking stage of cell wall synthesis by binding to enzymes known as penicillin binding protein (PBP) (Fontana et al., 1983) . The enterococcal beta-lactamase hydrolyzes penicillin, ampicillin, and piperacillin and other ureidopenicillins. But there is little or no inactivation of penicillinase- resistant semisynthetic penicillins, cephalosporins, or imipenem. ß-lactamase producing enterococci
have been reported and can be detected by chromogenic cephalosporin method using nitrocefin (Murray and Mederski-Samoraj, 1983). The activity of the penicillinase is reversed by the beta-lactamase inhibitors clavulinate, sulbactam, and tazobactam.

Other mechanisms of resistance to β-lactams can be due to low affinity of the PBP (Williamson et al., 1985). Most enterococci are tolerant to cell wall active agents like penicillin or glycopeptides. If these agents are given alone often fail to cure serious infections like endocarditis and meningitis which requires bactericidal therapy. And this is achieved by synergistic effect of penicillin/ampicillin plus aminoglycoside. *Enterococcus* rapidly develops tolerance to penicillins following exposure to a few doses of penicillin (Marothi et al., 2005).

Resistance to cephalosporins may be due to poor permeability of the drug into bacteria, lack of penicillin-binding proteins, or degradation by β-lactamases. None of the currently available agents are active against the enterococcus. Enterococcal superinfections occur in patients receiving cephalosporins or it alongwith aminoglycosides (Dancer, 2001).

Tolerance property is reported in enterococci in which the enterococci are killed only by many fold concentration higher than the MIC. As most enterococci are tolerant to cell wall active agents, penicillin or glycopeptide, alone often fail to cure serious infections like endocarditis and meningitis which require bactericidal therapy and this is achieved by synergistic effect of penicillin/ampicillin plus aminoglycoside and is the standard treatment for serious infection. MBCs of penicillin, ampicillin and other penicillins in broth macrodilution systems are typically more than
100µg/ml. This is an acquired property. Enterococci rapidly develop tolerance to penicillins following exposure to a few doses of penicillin.

**2.11.2. Clindamycin**

The lincosamide, clindamycin inhibit bacterial protein synthesis by blocking the peptidyltransferase activity of the 50S ribosomal subunit. It is inactive against *Staphylococci and Enterococci*. Resistance to clindamycin and lincomycin are the intrinsic property of the genus. Specific lincosamide resistance is encoded *lnu (B)* in enterococci (Leclercq, 2002). High level resistance to these agents are also described by some authors.

**2.11.3. Aminoglycosides**

Antibiotics included in this group include streptomycin, gentamicin, tobramycin, amikacin; netilimycin and spectinomycin. Low level resistance due to the low uptake is also found as an inherent property of enterococci. But when grown in the presence of cell wall inhibitors such as penicillin or vancomycin the uptake can be enhanced. (HLGR) have been reported in enterococci (Keddy *et al.*, 1996).

Different mechanisms have been described like ribosomal resistance and enzymatic modification by adenyltransferase is coded by plasmid (Eliopoulos *et al.*, 1984). Gentamicin resistance is predominantly due to the result of the presence of the inactivating enzymes 2”'-phosphotransferase - 6’-acetyltransferase conferring resistance to gentamicin, tobramycin, netilmicin, amikacin, and kanamycin (Chow, 2000). No single enzyme can inactivate all available aminoglycosides. 6’adenyl transferase produces HLR to streptomycin but does not inactivate other aminoglycosides (Murray, 1990).
2.11.4. Trimethoprim/sulphamethoxazole (TMP/SMX)

Trimethoprim-sulfmethoxazole resistance in enterococci is controversial. Acquired trimethoprim resistance can be due to chromosomal mutations and expression of an acquired dihydrofolate reductase (DHFR), encoded by \textit{dhrF} have also been found among enterococci (Woodford, 2005).

2.11.5. Fluoroquinolones

Endogenous efflux pumps seem to be widespread among wild-type strains of enterococci and might be the explanation for the intrinsic low-level resistance of most enterococci to the fluoroquinolones (Robicsek et al., 2006). A variant of the plasmid-mediated aminoglycoside 6-Nacetyltransferase, which can acetylate and inactivate fluoroquinolones has been described by Shin et al., (2009). This plasmid-mediated quinolone resistance determinant is named QnrA (Rodriguez-Martinez et al., 2008).

2.11.6. Chloramphenicol

The first demonstration of transferable resistance among enterococci was made in chloramphenicol resistance, from one \textit{E. faecalis} to another (Raycroft and Zimmerman, 1964). Chloramphenicol resistance could be mediated by chloramphenicol acetyltransferase (Brunton et al., 1984)

2.11.7. Erythromycin.

Erythromycin resistance mediated by plasmids and transposons are also commonly found in enterococci (Franz et al., 2001). Erythromycin resistance occurs as a part of the macrolide-streptogramin B resistance phenotypes. The same determinant confers resistance to clindamycin.
Another erythromycin resistant determinant namely ermB carried on Tn917 in enterococci (LeBlanc et al., 1986).

2.11.8. Tetracycline

Resistance to tetracycline is distributed widely in enterococci. Several different genes tetL, tetM tetN and tetO have been found to be involved in tetracycline resistance (Fraser, 2012). Resistance can also be through active efflux of tetracycline from cells or by protecting the ribosome from inhibition by tetracycline (Burdett, 1986). Tetracyclines can be used to treat vancomycin resistant strains which are susceptible to tetracycline.

2.11.9. Bacitracin

Bacitracin is used in hospitals, agriculture or in animal feed in different areas. Enterococci are known to be bacitracin resistant. Recent works showed the spread of high levels of bacitracin resistance (Manson et al., 2004)

2.11.10. Linezolid

Linezolid is a member of oxazolidone which is chemically unrelated to currently available agents (Woodford, 2005). It binds to the 23S ribosomal subunit and inhibits the formation of the 70S ribosomal initiation complex. (Lin et al., 1999). Linezolid has been reported as sensitive to all gram positive cocci including enterococci. Resistance against this drug has started to emerge. A mutation in the domain of 23rRNA is responsible for linezolid resistance in enterococci (Prystowsky et al., 2001).
2.11.11. Tigecycline

Some in vitro models suggest that synergistic action of tigecycline can be attained when combined with vancomycin, gentamicin, doxycycline or rifampin. It can be used for certain strains of *E. faecalis* and *E. faecium* compared to tigecycline alone. Recently, successful therapy of endocarditis with the combination of tigecycline plus daptomycin has been documented (Babinchak *et al*., 2005).

2.11.12. Quinupristin-dalfopristin

It is available intravenously for the treatment of *E. faecium* infections. Quinupristin-dalfopristin resistance may be the result of several mechanisms like modification of enzyme, active efflux and target modification (Hershberger *et al*., 2004).

2.11.13. Daptomycin

Resistance to *E. faecalis* and *E. faecium* to daptomycin, a newer cyclic lipopeptide antibiotic has been reported (Montero *et al*., 2008). In vitro synergism with rifampin, fosfomycin and gentamicin has been described against *E. faecalis*. However, resistance in VRE isolates has been reported (Rahim *et al*., 2003; Tsiodras *et al*., 2001).

2.11.14. Glycopeptide resistance

2.11.14.1. Teicoplanin

It is an inhibitor of cell wall synthesis and bacteriostatic against enterococci. Teicoplanin is widely used in Europe and is effective against *VanB* type of VRE. (Cetinkaya *et al*., 2000). However, in those strains expressing *VanB* phenotype, the development of resistance to teicoplanin has been noted.
2.11.14.2. Vancomycin

As most gram-positive bacteria are susceptible to glycopeptides, they have become agents of choice and the last resort for treating infections with multiple antibiotic resistant organisms.

Vancomycin a glycopeptides, though itself is not bactericidal for enterococcus, addition of a synergistic aminoglycoside produce bactericidal effect. Under normal conditions transpeptidation contributes to the strength of the peptidoglycan layer. Vancomycin binds with high affinity to the D-Ala–D-Ala termini of the pentapeptide precursor units, blocking their addition to the growing peptidoglycan chain and preventing subsequent cross linking (Wu et al., 1995; Yamaguchi et al., 1994)

There are 6 six different phenotypes of glycopeptides resistance in enterococci termed vanA, vanB, vanC, vanD; vanE and vanG are known to date. In vanA resistance the acyl-D-Ala-D-Ala ter minus of peptidoglycan precursors to which glycopeptides bind has been replaced by acyl-D-Ala-D-lactate with the loss of a crucial hydrogen bond in the binding site (Bugg et al., 1991). VanA and vanB are carried on a transposon Tn1546 and Tn1547 respectively or in closely related transferrable elements (Centinkya et al., 2000).

The phenotypes with high resistance to vancomycin (MIC≥256mg/L) and teicoplanin (MIC≥128mg/L were termed as vanA phenotypes. This type of resistance is inducible and transferrable on plasmids or on transposons. These can be transferred on to other bacterial species like S.aureus (Yamaguchi et al., 1994). Bacteria resistant to vancomycin and sensitive to teicoplanin consistent with the vanB phenotypes also may possess vanA gene. It can be due to the mutation in the vanS regulatory gene and result in
impaired resistance to teicoplanin (Hashimoto et al., 2000). Earlier studies also revealed that deletion in vanZ also could result in a loss of teicoplanin resistance (van den Bogaard et al., 1997).


Although it is identified in *E. faecium* and *E. faecalis*, the genetic determinants of the VanA phenotype have now appeared in *E. gallinarum* and other enterococcal species (Dutka-Malen et al., 1994).

VanB phenotypes are predominantly found in *E. faecalis* and in *E. faecium*, but have also been observed in rare isolates of other enterococcal species, including *E. casseliflavus* (Haenni et al., 2009). The MIC of vanB resistance vary among the isolates but teicoplanin sensitivity remains typical. VanB glycopeptide resistance in enterococci is mediated by an abnormal ligase (VanB) that is structurally related to VanA ligase. VanB protein also favors the production of the pentadepsipeptide terminating in D-Ala–D-Ser or D-Ala–D-Lac. VanB cluster is often located on the host chromosome or on plasmids and are transferable (Quintiliani & Courvalin, 1994).

Low-level resistance to vancomycin is characteristic of *E. gallinarum*, *E. casseliflavus*, and *E. flavescens* (Clark et al., 1998). Resistance may be inducible or constitutive and is not transferable and is related to the presence of species-specific genes vanC-1 and vanC-2, respectively (Sahm et al., 1995).

The VanD and VanE are the most recently described phenotypes. They are characterized by low to moderate resistance to vancomycin and low-level resistance to teicoplanin. The genes encoding are located on the
chromosome and transfer of these to other enterococci has so far not been demonstrated. VanD was first described in a New York Hospital in 1991 (Perichon et al., 1997).

The vanE vancomycin resistance gene has recently been described in *E. faecalis* BM4405, which is resistant to low levels of vancomycin (MIC, 16 mg/ml) and susceptible to teicoplanin (MIC, 0.5 mg/ml) (Fines et al., 1999). This new resistance phenotype has similarities to the intrinsic VanC type of resistance.

There are variants of vanA and vanB enterococci require glycopeptides for growth. These dependent strains had defective D-ala-D-ala ligase and were unable to synthesize normal peptidoglycan. A likely explanation for the phenomenon of vancomycin dependence is that these enterococci turn off their normal production of D-Ala–D-Ala and then can grow only if a substitute dipeptide like structure is made. Reversion to vancomycin independence has been observed; it probably occurs by either a mutation that leads to constitutive production of D-Ala–D-Lac or one that restores the synthesis of D-Ala–D-Ala. Some described them as superbug (Quintiliani and Courvalin, 1996).

**2.12. ENTEROCOCCAL RESISTANCE GENES**

Gene transfer in bacteria is responsible for the evolution of pathogenic potential and metabolic diversity. The transferable resistance genes arise from acquisition of “foreign” DNA by conjugation, transduction, or transformation.
2.12.1. Plasmids

Several plasmids have been reported in *Enterococcus* species and often mediate resistance to antimicrobials and particular heavy metals, contribute to enhanced virulence (Garcia-Migura et al., 2007). The conjugative plasmids in enterococci are two major groups; pheromone responsive plasmids and broad host range plasmids; the pheromones are produced by a recipient. The best-studied pheromone-induced plasmid transfer systems in Enterococci are pAD1, pCF10, pPD1 and pAM322 (Grohmann et al., 2003). Plasmids that carry sex pheromone genes also harbor genes encoding hemolysins, bacteriocins, and antibiotic resistance (Clewell and Dunny, 2002). Dissemination of vancomycin resistance genes via pheromone-responsive plasmids among enterococci is known to occur (Heaton et al., 1996).

Pheromone-independent plasmids generally encode resistance over a broad host range (Clewell, 1981). Broad host range plasmids are plasmids that are able to transfer between enterococci and other gram positive organisms including streptococci and staphylococci (Poyart-Salmeron et al., 1990). Plasmids pIP501 and pAMB1 are two well-characterized examples in this group (Grohmann et al., 2003). These plasmids carry multiple antimicrobial-resistance genes, including genes that confer resistance to macrolides, lincosamides, and the streptogramin B (MLS) group. Specifically, Inc18-like vanA plasmids were identified in VRE isolates from Michigan (Flannagan et al., 2003).

2.12.2. Transposons

Composite transposons have been reported in enterococci. The most widespread is the Tn5281 which hold resistance to high levels of
aminoglycosides (Bjørkeng, 2010) and Tn5385 holding resistance to erythromycin, aminoglycosides, mercuric chloride, streptomycin and penicillin (Bjørkeng, 2010).

Unit transposons encode often an enzyme which is engaged in the excision and integration of the genetic element. An example of a unit transposon is Tn1546 encoding the VanA phenotype in Enterococcus (Sletvold, 2010).

Conjugative transposons were also discovered in E. faecalis (Grohmann et al., 2003). A conjugative transposons identified was 18 kb Tn916, Tn917, Tn1546. Tn917 carry macrolide-lincosamide streptogramin resistance (Shaw and Clewell, 1985).

2.13. TREATMENT OF VRE INFECTIONS

All strains of glycopeptide-resistant E. faecalis have remained moderately susceptible to penicillin and ampicillin and these agents represent the drugs of choice for treating serious infections due to vancomycin-resistant E. faecalis. Linezolid, an oxazolidinone antibiotic, is available orally and intravenously and is used to treat infections including VRE. Nitrofurantoin is effective in the treatment of enterococcal urinary tract infections, including many caused by vancomycin-resistant enterococci (VRE) strains.

Telavancin is a novel lipoglycopeptide that is rapidly bactericidal against many Enterococcus species (Entenza and Moreillon, 2009). Oritavancin is a new parenteral semisynthetic glycopeptide that is active against all gram-positive pathogens, including VRE. Its advantages include in vitro activity against bacteria embedded in biofilms (Poulakou and Giamarellou, 2008). Dalbavancin has activity against non-VRE enterococci
and VRE are more susceptible to this drug than vancomycin (Aktaş et al., 2012). Because of long half-life weekly administration is possible (Candiani et al., 1999).

Quinupristin/dalfopristin (Synercid) has been used to treat a number of patients infected with multiresistant VREF. Tigecycline also can be used to treat vancomycin-sensitive E faecalis infections. Teicoplanin has been used in patients infected with enterococci exhibiting the VanB phenotype.

A number of experimental drugs are currently being evaluated for activity against VRE. These include the everninomycin Zeracin (Schering), and the glycopeptide LY333328 (Eli Lilly & Company) (Nicolaou et al., 2000). It is currently too early to tell whether any of these agents will have great utility in the management of infections due to VRE.

2.14. ENTEROCOCCAL INFECTIONS

Infections commonly caused by enterococci include urinary tract infections, endocarditis, bacteremia, catheter-related infections, wound infections, and intra-abdominal and pelvic infections. Most of infecting strains originate from the patient's intestinal flora.

2.14.1. Endocarditis:

Enterococci cause 5-15% of bacterial endocarditis, and E.faecalis is responsible for majority of these infections (McDonald et al., 2005). Other species associated with infections are E.faecium, E.aves, E.casseliflavus, E.durans, E.gallinarum and E.rafinosus. Enterococcal endocarditis occurs in older patients and rarely in infants (Fraser, 2010). Risk facors for enterococcal endocarditis include genitourinary and biliary portal, UTI, abortion and instrumentation. Infection also occurs in drug addicts. Most
cases of enterococcal endocarditis are left-sided. The aortic valve was involved more often than the mitral valve (Cabell et al., 2003).

2.14.2. Enterococcal bacteremia

It is much more common than enterococcal endocarditis. Various studies have shown that patients with hematological malignancies, a history of transplantation or severe burns have been more likely to experience bacteremia. The source of bacteremia is the urinary tract where it occurs by urinary and intravascular catheter. Other sources are intra abdominal, biliary, pelvic, wounds, peripartum infection, burn and intravascular catheters etc (Murray, 2000).

2.14.3. Urinary tract infection

Enterococci are found as the leading cause of UTI (Shepard and Gilmore, 2002). A rise in enterococcal infections is seen in case of instrumentation, after antibiotic therapy, in structural abnormality and recurrent UTI. Cystitis and pyelonephritis are common urinary tract infections caused by enterococci followed by enterococcal prostatitis and perinephric abscess (Fraser, 2012) Several studies proved a potential role for Agg in adhesion to renal epithelial cells and Esp in colonization and persistence of E. faecalis at the urinary tract (Shankar et al., 2001).

2.14.4. Central nervous system infections

In addition to causing neonatal meningitis, Enterococcus can also cause central nervous system infections in elderly, children and adults. Most cases seem to be related to an underlying disorder like long term primary illness, invasive procedure of central nervous system and prior antibiotic therapy (Iaria et al., 2005). Enterococci have also been reported as a cause of central nervous system shunt infections (Lai et al., 1997).
2.14.5. Intra abdominal and pelvic infections

*Enterococci* cause intra abdominal and pelvic sepsis and abscess. Spontaneous peritonitis by them is seen in patient with cirrhosis and nephrotic syndrome. Enterococci also cause acute salpingitis, peripartum infections and endometritis (Gibbs *et al.*, 1977).

2.14.6. Skin and soft tissue infections

*E. faecalis* accounts for up to 5% of isolates from skin and soft tissue infections (Luginbuhl *et al.*, 1987). *Enterococcus* generally cause infections only in previously damaged tissues and are not apparently responsible for primary cellulitis. It causes wound infections after abdominal surgery (Murray, 2000).

2.14.7. Biomaterial associated infections

Many nosocomial enterococcal infections are associated with medical devices such as intravascular or urinary catheters, bile drains and prosthetic heart valves (Sandoe *et al.*, 2003).

2.14.8. Nosocomial infections

Enterococci are most often associated with hospital-associated UTIs, wound infections, bacteremia, pneumonia and endocarditis (Murray, 2000). Enterococci are currently the third leading cause of all Hospital acquired infections (HAI), (Hoffmann and Moellering, 1987) and the second leading cause of HAI UTI in humans. In 2001, the SENTRY Antimicrobial Surveillance Program identified enterococci as the fifth most prevalent cause of HAIs in ICU patients (Low *et al.*, 2001).

Enterococci were ranked as the leading cause of bloodstream infections in North America; Enterococci had risen to the second leading
cause of Blood stream infections (BSI) by 2006-2007, causing 6% of reported catheter related BSI (Reigadas et al., 2012). Meningitis may be secondary to bacteremic spread or may complicate neurosurgical procedures or penetrating wounds into the cerebrospinal fluid.

In 1989, VRE was first reported in New York City; subsequently, VRE has spread rapidly throughout the United States. In recent years the percentage of nosocomial VRE isolates in ICU patients are increased rapidly.

VRE are being isolated in Indian hospital laboratories that look for them. Tertiary care centers see varying numbers of VRE. Thus one centre in north India, 38% of blood culture isolated enterococci from Intensive Care Unit patients were vancomycin resistant (Wattal et al., 2008). Two other studies examined 52 and 685 enterococcal isolates (Karmarkar et al., 2004; Ghoshal et al., 2006). In the former study 12 isolates (53%) of VRE of the van B type were seen, in the latter study from north India a low level of resistance(1.4%) was reported.

2.14.9. Community acquired infections

Enterococci are important cause of community acquired infections. These infections are endogenous. Little is known concerning the epidemiology or the prevalence of enterococci in the community settings. Community-acquired infections with VRE, however, have rarely been described (Coque et al. 1996), in community acquired infection; enterococci are commonly associated with urinary tract and wound infections, most often caused by E. faecalis. In infected wounds, such as diabetic foot wounds, E. faecalis is regularly isolated as part of a polymicrobial flora and the clinical significance of enterococci in such cultures are often ambiguous.
2.14.10. Food borne infection

Strictly speaking, *Enterococcus* is not a food borne pathogen. But because of the resistance of enterococci to pasteurization temperatures (McAuley *et al.*, 2012), and their adaptability to different substrates and growth conditions implies that they can be found either in food products manufactured from milk or meat and in heat-treated food products. They can contaminate finished products during food processing. Both *E. faecalis* and *E. faecium* have been implicated in spoilage of cured meat products, such as canned hams and chub-packed luncheon meats (Franz *et al.*, 2002)

2.15. EPIDEMIOLOGY OF ENTEROCOCCAL INFECTIONS

Epidemiological studies have provided evidence for the spread of enterococci through different modes form the following reservoirs or sources.

2.15.1. Environmental reservoirs

Several epidemiological studies provided evidence for epidemic spread of enterococci in a hospital setting and nosocomial acquisition of enterococci (Zervos *et al.*, 1987). In USA studies have shown that VRE spread by the nosocomial transmission (Coque *et al.*, 1996).

2.15.2. Human beings

The ecological niche of enterococci is the GI tract. Faecal colonization has been found to precede infection. Among the enterococci colonising the intestinal tract the commonest are *E. faecium* and *E. faecalis*. *E. casseliflavus*, *E. durans*, *E. gallinarum* & *E. hire* were also distributed among the microflora. Gelsomino *et al.*, (2003) have reported that *E. faecium* was the dominant enterococcal species of fecal flora.

Colonized patients may provide a reservoir of VRE which may cross colonize and cause infection in other patients. VRE colonization typically persists months, but has been documented up to 1 year following discontinuation of antibiotics (Livornese *et al.*, 1992; Boyce 1994; Lai *et al.*, 1997). Colonized patients contaminate themselves at another body site and each other (Lai *et al.*, 1997).

Vancomycin use is associated with VRE colonization and infection. Third-generation cephalosporins, aminoglycosides, aztreonam, ciprofloxacin, imipenem, clindamycin, and metronidazole have been associated with VRE colonization. Increased duration of exposure to individuals colonized with VRE and close proximity to other colonized patients increase the likelihood of VRE exposure.

2.15.3. Drinking water sources

*Enterococcus* is the inhabitant of humans and animals intestinal tract and is used as indicators of faecal contamination of drinking water. The presence of enterococci in water poses risk to consumers in terms of virulence factors and the antibiotic resistance of enterococcal isolates. The prevalence of antibiotic resistant *Enterococcus* in water can spread the bacterial resistant genes to other bacteria via mechanisms such as
conjugation, in which the plasmid and conjugative transposon can be exchanged, as previously mentioned.

Budnick et al., (1996) have isolated *E. casseliflavus* or *E. gallinarum*, *E. faecium*, *E. casseliflavus*, *E. gallinarum* and *E. duran* (Budnick, et al., 1996). In another study the most prevalent species in drinking water sample sources was *E. faecium* followed by *E. faecalis* (Grammenou et al., 2006).

In order to prevent the exposure to waterborne pathogens, disinfection of water is required. Commonly used chemical agents for disinfection of water are chlorine compounds like chlorinated lime, oxidants like ozone, potassium permanganate and halogens. Household water can be disinfected by chlorination, distillation, UV devices and ozonation. However microbes differ greatly in their sensitivity of disinfection. *Enterococcus* are also described to be more resistant to disinfection than *E.coli* (Tree et al., 2003).

Microbes found in water pose no risk for healthy individuals. They can be opportunistic pathogens capable of causing serious and life threatening infections in severely immune compromised individuals. Several outbreaks of waterborne nosocomial infections are reported in each year (Anesssiea and Costa, 2001). Polluted water can contaminate environmental surfaces, medical equipments, endoscopes, respiratory equipments and ultimately lead to patient exposure.

### 2.15.4. Animal reservoirs

VRE were most common in the faecal samples from broilers. In broiler farmers a significantly higher prevalence and degree of VRE was observed than in the other human populations (van den boggard et al.,
1997). The use of avoparcin, a glycopeptides in animal feed supplement might select VRE and there are studies documenting an association between avoparcin-containing feeds and VRE recovery from animals (McDonald et al., 1997). The genetic similarity of the strains isolated from animals and workers indicates that VRE can be transmitted from animals to humans (van den boggard et al., 1997; Jensen et al., 1998). One study found high carriage rates of VRE among meat eating elderly persons in Holland but absence of VRE among elderly vegetarians (Van den Braak et al., 1997).

2.16. EPIDEMIOLOGICAL TYPING METHODS

The role of pathogen typing is to determine whether epidemiologically related isolates are genetically related. The analysis of nosocomial pathogens in an outbreak has relied on a comparison of phenotypic characteristics. Traditional methods such as bacteriocin typing, phage typing, serotyping or biotyping are moderately useful for Enterococcus (Backeljau et al., 1996 & Barbut, et al., 1993).

2.16.1. Phenotyping Methods

Biotyping is based on properties such as biochemical reactions, morphology, and environmental tolerances. Little attempt seems to have been made to use biotyping for epidemiological purposes although Facklam, (1972) has successfully typed them. The API-20 Strep Kit (API System SA, La Balme-les- Grottes, France) was also used for biotyping strains (Colman and Ball, 1984). The PhenePlateTM RF (PhP-RF) system is a recently developed phenotypic method, (Kuhnen et al., 1987) for typing it.

Antibiograms are often used by clinicians to assess local susceptibility rates, as an aid in selecting empiric antibiotic therapy, and in
monitoring resistance trends over time within an institution. Similar anti-
obigrams of all isolates suggests clonal dissemination in outbreak (Kaufman et al., 1998).

No systematic study on serotyping of Enterococcus faecalis has been reported since 1964 when M.E. Sharpe conducted serotyping of group D streptococcus in U.K. Enterococcal serotyping was described by Sharpe and Shattock, (1952). Based on capsular polysaccharides, five different serotypes types have been described for Enterococcus faecalis.

Several reports exist on bacteriophage typing of enterococci (Hoch and Herman, 1971; Caprioli et al., 1975). Pooled phages can be used for rapid group and species identification. Natkin, (1967) showed that phage typing of enterococcal isolates from the oral cavity could be useful. Smyth et al., (1986) suggested that the epidemiology of infections due to enterococci might be tackled by such a tripartite typing (biotype/phage type/serotype) approach (Smyth et al., 1986).

Differentiation and typing of enterococci based on enterocin has been successfully performed in some studies (Kuhnen et al., 1988).

2.16.2. Genotyping methods

Different genotypic methods are used for genotyping studies of epidemiologically related isolates, collected during an outbreak of nosocomial disease.

Molecular methods like Pulsed-field gel electrophoresis (PFGE) is currently considered to be the “gold standard” for subtyping enterococci and has been used extensively for molecular epidemiological characterization of VRE outbreaks (Lina et al., 1993; Noskin,1997). PFGE
is highly discriminatory and has excellent reproducibility (Tenover et al., 1997).

The genes coding for rRNA are highly conserved. The unique riboprints help to determine the identity of bacterial isolates. 16S rRNA gene sequences of Enterococcus spp. could be used as molecular markers for microbial source tracking to differentiate between Enterococcus spp. isolated from human and animal feces (Kim et al., 2010).

Plasmid DNA analysis was the first tool to be applied for epidemiological analysis of enterococci (Flannagan et al., 2003). Plasmid restriction is also commonly used for the analysis of staphylococci and enterococci (Bonilla et al., 1997).

A novel fingerprinting method, amplified fragment length polymorphism (AFLP) analysis, has recently been applied to enterococci for fingerprinting enterococci from nosocomial outbreaks and in epidemiological studies (Antonishyn et al., 2000).

MLST could be used for global epidemiology and tracking the worldwide inter hospital spread of virulent clones and now been applied to enterococci (Willems et al., 2009).

Fragments resulting from RAPD PCR typing is randomly amplified and resolved in common agarose gel. This method can be used empirically without the knowledge of the complete genomic sequence. Several researchers have successfully used RAPD for enterococci (Van den Braak et al., 2000 & Quednau et al., 1998). Compared to PFGE and ribotyping, this method demonstrated reproducible results with a high degree of discriminatory power.
In an effort to control the nosocomial transmission of VRE, Hospital Infection Control Practices Advisory Committee (HICPAC) published recommendations (2006) to detect vancomycin resistance promptly and accurately to minimize nosocomial transmission of VRE.