2. REVIEW OF LITERATURE

The species of the genus *Enterococcus* were originally classified as enteric gram-positive coccii and later included in the genus *Streptococcus* (Murray, 1990). After the establishment of the Lancefield serological typing system, enterococci were classified as group D streptococci and were differentiated from the non-enterococcal group D streptococci by distinctive biochemical characteristics (Lancefield, 1933). Subsequently, it was recommended that the term "*Enterococcus*" should be used specifically for streptococci showing unique growth characteristics (Sherman, 1937). In 1984, Schleifer and Kilpper-Balz removed enterococci from the genus *Streptococcus* and placed in their own genus, *Enterococcus* based on genetic differences.

*Enterococcus* spp. are widely distributed in nature and the intestinal tracts of humans and animals is the natural habitat of these isolates. However, they are opportunistic human pathogens and globally, many studies have revealed the fact that enterococci tend to be one of the leading human infections. (Facklam and Collins, 1989; Murray, 1990 and Fridkin and Gayness, 1999). Resistance to environmental conditions such as heat or desiccation allow prolonged survival and results in the rapid spread of enterococci (Murray, 1990 and Patterson et al., 1995). Significantly, strains of enterococci have acquired resistance to essentially all antimicrobial agents over the past three decades (Edmond, 1996) including against the antibiotic of last resort, vancomycin. The increased emergence of resistant enterococci to a wide range of drugs poses problems, including the lack of available antimicrobial therapy for these organisms, and the possibility that the resistance genes present in enterococci can be transferred to other gram-positive bacteria (Handwerger et al., 1993 and Jordens et al., 1994). Because of these increasing difficulties in dealing with enterococcal infections, more effort is being devoted to understand the epidemiology enterococcal infections among persons (Dupont et al., 1998).
The following review focuses on the magnitude and nature of the problem posed by enterococci in general and gives a recapitulation of aspects which describe the scenario on enterococcal incidence, antibiotic susceptibility patterns, drug resistance among enterococci, and problems of vancomycin resistant enterococci, virulence factors supporting the infection and molecular approaches in the analysis of clinical Enterococcus spp. in particular.

2.1. Incidence of clinical Enterococcus spp.

Enterococci are a frequent cause of a wide variety of infections in humans (Evans and Chinn 1947; Duma et al., 1969 and Murray 1990). Hence, they are often isolated from different clinical specimens and reported widely both at national and global levels. A total of 120 isolates of enterococci were reported including 114 (95%) E. faecalis, and 6 (5%) E. faecium by Gulati et al. (1997). Agarwal et al. (1999) reported 150 enterococci of which 121 were identified to be E. faecalis and 21 E. faecium. Of 170 cases, as much as 23% was reported to be Enterococcus spp. (Anand et al., 2000). In 2003, Murdoch et al. reported 815 blood stream enterococcal isolates. Das et al. (2006) reported 8.1% of enterococci isolated from 1,680 clinically suspected cases of urinary tract infections and all were speciated to be E. faecalis. In a retrospective study, Mohanty et al. (2003) identified 30.17% of enterococcal isolates. Amorouche et al. (2004) isolated 133 bacterial isolates and confirmed 27 enterococci to the spp. level by conventional methods. Kolar et al. (2004) isolated 558 Enterococcus spp. from 5,283 clinical swab specimens. Karmarkar et al. (2004) isolated and reported 52 isolates of enterococci, 10 E. faecalis and 42 E. faecium, from 534 clinical specimens. A total of 374 clinical enterococci were isolated and reported by Willey et al. (1993) and further speciated and identified that a majority of them were E. faecium (n=234) and E. faecalis (n=112) from New York. Chen et al. (2000) studied a total of 156 clinical isolates of Enterococcus spp. Kirschner et al. (2001) isolated 18 enterococci and speciated based on
conventional identification methods as *5 E. faecium, 6 E. faecalis* and *3 E. hirae* and *4 E. durans*. Leavis *et al.* (2003) reported the enterococcal epidemiology characterized by a large community reservoir from Europe. Lee *et al.* (2003) reported 8.9% enterococcal isolates from 1, 22, 244 various bacterial pathogens of which 63.9% and 36.1% were noted to be *E. faecalis* and *E. faecium*. Similarly, a total of 984 isolates of enterococci were recorded by Richter *et al.* (2003) from North American medical centers during 1996-2000. Of 984 enterococcal isolates, 504 isolates were found to be *E. faecalis* and 480 as *E. faecium*. Although these two species are very common, causing various infections, Prakash *et al.* (2005) reported the emergence (46 isolates; 19%) of unusual species of enterococci causing infection in South India and brought their common occurrence from clinical specimens. Tyrrell *et al.* (2002) reported *E. gilvus* and *E. patterns* involved from human clinical specimens. Neely and Maley (2000) studied the long survival of *Enterococcus* on the commonly used hospital fabrics such as swab, suits, and lab coats and underscored the need for effective control procedures and careful disinfection methods to limit the spread of enterococci.

### 2.2. Antibiotypes of *Enterococcus* spp.

Microbial resistance to antibiotics is a world-wide health problem in human medicine (Lukasova and Sustackova, 2003). It is generally accepted that the main risk factor for the increase in the antibiotic resistance is extensive use of antibiotics. This has lead to the emergence and dissemination of resistance genes and resistant bacteria (Bogaard and Stobberingh, 2000a). According to World Health Organization (WHO) the resistance to antibiotics is an ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agents (Lochmann, 1994). In 1992, Levy reported the possible modes of antimicrobial resistance emergence in bacteria.

Enterococci have been known to be resistant to most antibiotics used in clinical practice. They are naturally resistant to cephalosporins, aminoglycosides and clindamycin
and may also be resistant to tetracyclines, erythromycin, penicillins and glycopeptides such as teicoplanin and vancomycin (Urbaskova, 1999). Enterococci are known to acquire antibiotic resistance with relative ease and to be able to spread these resistance genes to other species (Kuhn et al., 2000). *E. faecalis* has been reported to transfer plasmids harboring antibiotic-resistance traits to other species and other genera (Marcinek et al., 1998). Rice et al. (1995) showed that *E. faecium* conjugative transposons conferring resistance to vancomycin can be transferred to *Staphylococcus aureus*, streptococci and lactobacilli. They also brought out the fact that multidrug / vancomycin resistant enterococci can enter to human food chain as could be frequently isolated from humans, sewage, aquatic habitats, agricultural run-off and animal sources.

In 1990, Murray reported that complete or relative resistance to beta lactam antibiotics is a characteristic feature of the genus of *Enterococcus* spp. and stated that *E. faecium* is less susceptible than *E. faecalis*. In 1997, Murray showed that a concentration of ampicillin or penicillin between 1 and 8 µg/ml is required to inhibit *E. faecalis* whereas, the same is between 16 and 64 µg/ml or more for *E. faecium*. Burney et al. (1994) reported that enterococci resistant to ampicillin and vancomycin or aminoglycoside are a growing problem. Silverman et al. (1998) described 107 enterococci with gentamycin resistance occurred in 10 (14%) of *E. faecalis*. Ampicillin resistance in 2 (3%) of *E. faecalis* and 6 (23%) of *E. faecium* and no VRE was reported. They also described risk factors for enterococci with aminoglycoside or ampicillin resistance introduced prior to hospitalization and previous antibiotic therapy. Gulati et al. (1997) reported the antibiogram-resistogram of 120 isolates and showed that 100 (83.3%) isolates were resistant to ampicillin. In a retrospective study, Mohanty et al. (2003) identified 30.17% of enterococcal isolates with high level aminoglycoside resistance.

Moellering and Weinberg (1971) reported two groups of streptomycin resistance occur in enterococci, one, the moderate – level resistance with MIC of 62 to 500 µg/ml
and the second, high-level resistance with MIC of more than 2000 μg/ml. Murry et al. (1990) reported inherent low-level aminoglycoside resistance among the species of Enterococcus with MICs between 2 and 16 μg /ml. The high level aminoglycoside resistance (HLAR) among some strains of enterococci was reported by Sahm et al. (1991) and estimated MICs as more than 2000 μg/ml. Further, of the 52 isolates reported, 12 were resistant to vancomycin with an MIC of ≥4μg/ml but were sensitive to teicoplanin (Karmarkar et al., 2004).

Rice et al. (1995) investigated 248 isolates of enterococci for HLAR. The highest percentage of the resistance was reported for kanamycin, closely followed by tobramycin and to a lesser degree by streptomycin and gentamycin. They recorded multiple antibiotic resistance patterns in about 95% of HLAR isolates. The most frequently occurred multiple resistance pattern of their study was HLAR to both kanamycin and tobramycin, followed by multiple resistance to streptomycin, kanamycin and tobramycin. In their study, all isolates that exhibited gentamycin resistance were also resistant to kanamycin and tobramycin, but not to streptomycin. They concluded that these enterococci may contribute to the dissemination of HLAR among other strains.

Murray (1998) stated the likelihood that gentamycin resistance appeared and disseminated in staphylococci before it did in enterococci since this trait was well established in staphylococci by the 1970s but HLAR to gentamycin was not reported in enterococci until 1979. In 1971, Moellering et al. reported the lack of high-level gentamycin resistance among enterococci. Watanakunakorn (1989) reported the absence of high level gentamycin resistance among 126 enterococci from 1980 to 1984, with HLAR subsequently occurring in 1985. Phillips et al. (1990) from the UK reported no highly gentamycin resistant strains in 1969 to 1979 or 1980 to 1985 and appearance of strains in 1986. Zervos et al. (1986) stated that only one of 269 isolates of E. faecalis had high-level gentamycin resistance in 1981; and this figure gradually increased to 7.7% in 1984. Eliopoulos et al. (1988) indicated that high-level gentamycin resistance in E. faecium
appears to have occurred after its appearance in *E. faecalis*, with the first report occurring in 1988.

The problem of multidrug-resistant enterococci promises to be with us for the foreseeable future (Murray, 1998). The *Enterococcus* has likely emerged as a major nosocomial pathogen in part because of its resistance to multiple antibiotics, which allows it to survive and subsequently infect patients. With its propensity to acquire new traits, such as high-level gentamycin, penicillin, and vancomycin resistance, the *Enterococcus* spp. continues to create new therapeutic problems and dilemmas. Landmann and Quale (1997) also stated that the treatment of patients with enterococcal infection also been complicated by the convergence of strains posing high level resistance to aminoglycosides, penicillin and most recently glycopeptides. Allen et al. (2002) reported that as the incidence of infection caused by MDRE increases the importance of desiring additional synergistic drug combinations for this bacterium is magnified. They suggested a number of novel antimicrobial combinations that may be useful in managing enterococci. Aleboouyeh et al. (2005) reported two isolates of 149 with complete resistance to all tested antibiotic (total resistance) and others varied degrees of resistance pattern and also reported gentamycin and tetracycline resistance as common. Further, 10 isolates had multi drug resistance and MIC for the tetracycline resistant strains ≥16 μg/ml except in 2 where it was 64 μg/ml and MIC for gentamycin and erythromycin ≥1000 μg/ml and 32 μg/ml respectively and two vancomycin resistant enterococci had a MICs ≥64 μg/ml and ≥128 μg/ml.

Noskin et al. (1999) compared the activity of linezolid to those of other antimicrobial agents against 3,945 clinical isolates of enterococci. Linezolid had potent activity against all isolates tested and appeared to be a promising new antimicrobial agent for the treatment of gram positive bacterial infections. Abraham et al. (1998) reported the emergence of antimicrobial resistance infection in cancer patients. Enterococci with penicillin resistance was reported which accounted for 10.2% from the total urine
cultures (Jesudason et al., 1998). Gulati et al. (1997) isolated 100 isolates of enterococci revealing resistance to ampicillicin among 120 isolates (83.3%). Nelson et al. (2000) isolated and characterized glycopeptide resistant enterococci from hospitalized patients over a 30 months period. A totally 90% of 154 isolates were reported to be *E. faecium* with *vanB* genotype. All were susceptible to vancomycin, teicoplanin, ampicillin, gentamycin, streptomycin and ciprofloxacin. Auckland et al. (2002) reported from London the first 3 samples of linzolid resistant enterococci among which 2 were *E. faecalis* and 1 *E. faecium* with MIC ≥64 μg/l.

Lee et al. (2003) reported a total of 6,914 enterococci of which 2% and 87% of *E. facalis* and *E. faecium* respectively had resistance to ampicillin and 0.9% and 20% to vancomycin. And from a total of 3912 *E. facalis* and *E. faecium* isolates, 43% and 86% of ciprofloxacin, 82% and 35% of tetracycline and 84% and 96% of erythromycin respectively were resistant. Jureen et al. (2003) reported ciprofloxacin resistance as more common among the ampicillin resistant enterococci. High level aminoglycoside resistance was also found exclusively among ampicillin resistant rectal isolates. No resistance to linzolid or glycopeptides was reported and 90% of the isolates had an ampicillin MIC of ≥ 64 μg/l, ciprofloxacin MIC of ≥32 μg/l, vancomycin and teicoplanin MIC respectively 1.5 and 0.5 μg/l and gentamycin of 4 μg/l. Simonsen et al. (2003) reported that ampicillin resistance was not found in *E. faecalis* when compared to 48.8% in *E. faecium* isolates. They further reported that the lowest level of the hospital antimicrobial use will lead to the lowest emergence of resistance among *E. faecalis*. Donelli et al. (2004) reported a total of 19 clinical strains of enterococci isolated from occluded biliary stents. Most of their isolates had different patterns of antibiotic resistance. Percentage of isolates with resistance was high in the presence of chloramphenical, erythromycin and tetracycline and six strains were MDRE with no identified VRE.
The first known penicillinase-producing strains of Enterococcus spp. were isolated from a patient in Houston, Texas, in 1981. From then infrequent isolation of beta lactamase producing enterococci was reported, several other strains had been reported during 1986, 1988 (Murray, 1992). In 1989, Patterson et al. reported beta lactamase producing enterococci with high level gentamycin resistance. These isolates had also been reported from the United States, Lebanon, Canada, and Argentina (Rhinehart et al., 1990; Murray et al., 1991 and Wells et al., 1992). In 1998, Murray insisted upon the importance detecting beta-lactamase production by clinical enterococci. He indicated that the activity of the penicillinase in conferring resistance against antibiotics such as ampicillin and penicillin is reversed by the beta-lactamase inhibitors clavulanate, sulbactam, and tazobactam and such inhibitors have been shown to markedly enhance the therapeutic efficacy of ampicillin or penicillin.

2.3. Vancomycin resistant Enterococcus spp. (VRE)

Vancomycin is a cell wall active antibiotic that represents the lost line of defense against many multiply resistant gram positive pathogens. Enterococci are considered as vancomycin resistant if the MIC was ≥32 µg/ml or if the zone of inhibition by disc diffusion was 14mm (Fridkin et al., 2002). Vancomycin and teicoplanin are glycopeptide antibiotics had been in clinical use for more than 30 years without the emergence of marked resistance (Ingerman and Santoro, 1989). Because of their activity against methicillin-resistant staphylococci and other gram-positive bacteria, these drugs have been widely used for therapy and prophylaxis against infections due to these organisms (Gold and Moellering, 1996). In 1988, Uttley et al. reported the first isolate of vancomycin-resistant enterococci in England. Shortly after the first isolates of vancomycin-resistant enterococci (VRE) were reported in the UK and France (Leclercq et al., 1988), similar strains were reported from hospitals located in the Eastern Half of the United States (Friden et al., 1993). Purva et al. (1999) made the first report from India on
VRE from blood of a patient with non Hodgkins lymphoma. Anand et al. (2000) reported that 23% of Enterococcus spp. from 170 cases and all were found to have resistance to vancomycin. Subsequently, VRE have spread with unanticipated rapidity and are now encountered by hospitals in most states (Centers for Disease Control and Prevention, 1993; Boyce, 1995 and Jones et al., 1995).

VRE had also been reported from many other countries including Australia, Belgium, Canada, Denmark, Germany, Italy, Malaysia, The Netherlands, Spain and Sweden (Woodford et al., 1995). Particularly, from 1989 through 1993, the percentage of nosocomial enterococcal infections reported to the Centers of Disease Control and Prevention’s National Nosocomial Infections Surveillance System that were caused by vancomycin resistant enterococci (VRE) increased from 0.3 to 7.9% (Centers for Disease Control and Prevention, 1993).

Nicas et al. (1989) reported 180 clinical isolates of VR E. faecium from England and reported that resistance to vancomycin was transferable to other enterococci. Tomita et al. (2002) reported a total of 640 VREF were isolated during 1994-1999 from Michigan. Of the 640 strains, 611 and 29 were vanA and vanB VRE respectively as confirmed by PCR. Of which, 492 (77%) had a gentamycin MIC between 64 μg/ml to 1024 μg/ml. Willey et al. (1993) reported a total of 230 VRE from 374 clinical isolates of enterococcal species. Gordts et al. (1995) investigated the prevalence of fecal carriage of VRE and reported that 22 of 636 were VRE carriers. Of which 18 were identified to be highly resistant and found to be E. faecium, three E. gallinarum and one E. faecalis. Swenson et al. (1995) reported the use of E. faecalis (ATCC 51299) and E. faecalis (ATCC 29212) as controls for screening vancomycin high level aminoglycoside resistance in enterococci. Pegues et al. (1997) identified and reported 139 VRE from 169 patients. Kim et al. (1998) isolated a total of 287 strains of enterococci from nosocomical infections in Korea identified only one VRE.
Vancomycin resistant enterococci were isolated and analyzed from turkeys, turkey farmer, turkey slaughters and sub urban residents in the south of the Netherlands and proved the transmission of vancomycin resistance from animals to humans (Stobberingh et al., 1999). Singh-Naz et al. (1999) in their cohort study, the surveillance cultures of patients admitted to the hematology unit revealed a colonization rate of 24% and 30 VRE isolates were identified from 123 children. However, Austin et al. (1999) analyzed the VRE emergence in intensive care hospital settings and found that after treatment and infection control the prevalence was reduced from a predicted 79% to 36%. von Gottberg et al. (2000) reported 184 VRE isolated from rectal swabs. Yeh et al. (2002) reported four different species of vancomycin resistant isolates such as E. faecalis, E. faecium, E. gallinarum and E. casseliflavus from 27 isolates.

The prevalence of van genes (A, B, C) among clinical isolates of VRE at a large tertiary hospital in Sao Paulo, Brazil was reported by Caiafae Filho et al. (2003) and showed the presence of vanA type in 43 strain of E. faecalis and E. faecium. Sahm et al. (1997) applied a phenotypic approach to 378 surveillance cultures of enterococci among which VRE included 83 E. faecium, 33 E. gallinarum and 5 E. casseliflavus. Dahl et al. (1999) reported his findings on molecular analysis of 17 genomically unrelated clinical vanB type VRE from hospital in Germany, Norway, Sweden, UK and three subtypes from United States was in accordance with previous sub typing of the ligase gene sequence. They also reported no correlation between vanB subtype and levels of vancomycin resistance. All strains studied carried 9 structurally conserved vanB gene clusters as shown by long range PCR which led to the findings of 3 distinct vanB gene clusters. Aeschlimann et al. (1998) analyzed pharmodynamic activity of Quinupristin-Dalfopristin against VRE with differing minimum bactericidal concentrations. Bell et al. (1998) developed multiplex PCR assays for vanA, B, C1, C2, C3 isolates of enterococci with resistance to glycopeptides which had reemerged in Australia. They had found that PCR method was a rapid and accurate method for determining the genotype and
confirming the identification of glycopeptide resistant enterococci. Eliopoulps et al. (1998) characterized the VRE isolates from the United States and their susceptibilities in vitro to Dalfoprisitin-Quinspristin and identified 72% of the stain were vanA type, 28% were of vanB type.

Endtz et al. (1998) reported a collection of genetically unrelated VRE including 50 vanA, 15 vanB, 50 vanC1 and 30 vanC2 using eight detection methods. Friedkin et al. (1998) isolated and reported 43% of dominant VRE from Massachusetts general hospital from 99 patients of which 2.7% were from blood, each 19% from urine and wound infection specimens. Moretti et al. (2004) analyzed clonal dissemination of vanA type glycopeptide resistance among E. faecalis between hospitals of two cities located 100 km apart and all were highly resistant to vancomycin and teicoplanin. Particularly, three strains had minimum inhibitory concentration >256 μg/ml. All strains had the presence of transposon Tn1546 by PCR and were closely related. Clark et al. (1998) reported the nature of intrinsic low level resistance to vancomycin. Kolar et al. (2004) isolated 558 Enterococcus spp. from 5,283 swabs and 9 strains (1.6%) were identified as VRE of which, 2 were vanA, one vanB and 2 strains of E. gallinarum were vanC type. Satake et al. (1997) detected VRE from fecal samples using PCR. Ke et al. (1999) developed and reported PCR assay for rapid detection of enterococci which were capable of detecting all clinically important enterococci. Fridkin et al. (2001) analyzed the effect of vancomycin and third generation cephalosporin on vancomycin resistant enterococci in 126 US adult ICU. Schoutten et al. (2001) studied the relatedness of resistance in 18 clinical of European vanA VRE. Lee et al. (2001) isolated 25 isolates of E. faecium confirming vanB genotype recovered from single Korean hospital. Kawalec et al. (2001) reported VRE from Poland, which had vanB pheno type which emerged independently in two hospitals. Erika et al. (2001) characterized VREF in Tennessee and identified a total of 34 different strains types. Zhanel et al. (2001) studied the activity of nitrofurontoin and stated that this drug may be effective against UTI caused by VRE. They tested
nitrofurantoin against 300 isolates of *E. faecium*, *E. feacalis*, *E. galinarum*, none were resistant to nitrofurantoin and had a vancomycin MIC of >128 µg/ml.

The VRE were confirmed using multiplex PCR and *vanA* gene was detected in all the VRE isolates (100%) by Novais *et al.* (2004) and further reported that of 33 VRE isolates a total 94% resistance to erythromycin, 88% to ciprofloxacin and 82% to gentamycin and kanamycin. Karmarkar *et al.* (2004) reported 52 isolates of enterococci of which 12 were resistant to vancomycin with an MIC of ≥4µg/ml but were sensitive to teicoplanin. Collins *et al.* (2001) isolated 193 enterococci from different sources and a part representing VRE. Miyazaki *et al.* (2002) reported the vancomycin and teicoplanin MICs against vancomycin susceptible enterococci as 2 and ≥128 µg/l and 128µg/l and ≥0.25µg/l against vancomycin resistant enterococci respectively. They indicated that linzolid as a suitable therapeutic agent when compared to VRE and VSE bacterimia and vancomycin as suitable for vancomycin susceptible bacterimia. Palladino *et al.* (2003) described rapid detection of *vanA* and *vanB* genes directly from clinical specimens enrichment broths using real time multiplex PCR, performed directly with enrichment broth and found to be significantly more sensitive than enrichment broth culture. They stated that negative samples can be identified significantly earlier by PCR than by culture alone. Zhanel *et al.* (2003) reported a total of 616 (84.4%) of 697 VRE from 28 US medical centers. More than 75% (584) of all VRE isolates demonstrated a *vanA* genotype by PCR. They concluded that VRE urinary isolates were common in the US and more primarily of the *vanA* genotype and very susceptible to linzolid, nitrofurantoin and chloramphenicol where as, in Canada, VRE urinary isolates remain uncommon. Brown *et al.* (2003) reported 128 GRE isolates from 93 (43%) specimens with enterococci carrying *vanA* or *vanB* genes.

Patel *et al.* (2000) described that *Paessibonillns papillae*, a biopesticide has a vancomycin resistant gene cluster homologous to the enterococcal *vanA* vancomycin resistant gene cluster, also stated that the genes in *P. popilline* may have been a precursor
to or have had ancestral genes in common with vancomycin resistant genes in enterococci. The use of *P. papillae* biopesticidal population in agricultural practice may have an impact on botanical resistance in human pathogens. Drupe *et al.* (2003) reported that *E. faecium* were highly resistant than *E. faecalis* and a high percentage of resistant isolates were observed in the presence of penicillin, erythromycin and imipenem. A large number of isolates exhibited resistance to high levels of aminoglycosides. 10% (3) of them was VRE with MIC of >32µg/ml and glycopeptides were reported to be the most effective antimicrobials. Moellering *et al.* (1999) reported 396 VRE with *vanA* phenotype from 467 patients examined having a mean age of 53.4 ± 16.8 with 8 patients being <8 years of age. They also reported that majority were male patients (59.3%) and most of the isolates were MDRE (100% resistance to ampicillin) and all were susceptible to chloramphenicol. Padiglione *et al.* (2003) stated that the rates of the colonization with VRE, the polyclonal nature of the most isolates and the possible association with the use of broad spectrum antibiotics are consistent with either the endogenous emergence of VRE or the proliferation of the preciously detectable colonization with VRE among high risk patients managed under conditions in which the risk of nosocomial acquisition was minimized.

Jayaratne *et al.* (1999) recommended PCR method for the rapid detection of clinically significant genotypes of VRE. The diagnostic values of PCR compared to the phenotypic method were as following 94% sensitivity, 99.8% specificity, 98.8% positive predictive value and 99.3% negative value. Coombs *et al.* (1999) stated that genotyping such as PCR should be done on all the isolates whether *vanA* or *B* genes are present.

Bischoff *et al.* (1999) reported a total of 413 VR *E. faecium* isolates from urine (52%) wounds (16%) blood (11%) cathertip (6%) and other sites (15%). The number of VREF isolates progressively increased overtime with higher rates of isolation during winter. The results implied urgent need for stringent remedy with more effective infection control measures. All the isolates had high level vancomycin resistance with
MIC >128 µg/ml. They also identified high level resistance to the aminoglycosides such as to gentamycin the MIC was more than 1026 µg/ml. Palepou et al. (1998) reported 20 VRE strains with resistance to vancomycin and teicoplanin and had vanA genotype. Patel et al. (1997) identified 100 clinical isolates of enterococci with 63 vancomycin resistant isolates. Paule et al. (2003) used multiplex PCR assay using vanA and vanB primers which provided rapid results and was more sensitive than culture on selective media.

Kuriyama et al. (2003) reported over a 3 months period 134 isolates of VRE, 89 E. faecium and 45 E. faecalis. Multiplex PCR detected either vanA or B genotypes for all VRE isolates studied and vanA predominated (146 of 158 isolates; 92%). Montecavlo et al. (1996) determined whether enhanced infection control strategies reduce transmission of VRE in an endemic setting. Reported that during the use of enhanced infection control strategies incidence of VRE blood stream infection decreased significantly. Katz et al. (2002) reported cross contamination of clinical specimens with VR E. faecalis in Ontario, Canada. Balzereit-Scheurlein and stephen (2001) reported 4.9% as positive for VRE from healthy non hospitalized patients in Switzerland. All the isolated strains carried vanA resistance gene. Of the isolates, 47 were E. faecium and E. faecalis and 2 E. durans. Vancomycin and teicoplanin MICs were between 512 and 1024 µg/ml and 32 and 1024 µg/ml respectively. The isolates had resistance against 5 antibiotics and 2 were resistant to 6 antibiotics. Weinstein et al. (1996) stated that patients whose gastrointestinal traits are colonized with VRE may act as reservoirs for nosocomial transmission. Wendt et al. (1998) compared the abilities of 3 vancomycin resistant E. faecum and E. faecalis and 5 vancomycin sensitive enterococci (VSE) and found that they can survive under dry conditions up to 16 weeks. But found VRE has no advantages over VSE with respect to their ability to survive under dry condition.
2.4. Virulence factors of Enterococcus spp.

The Enterococcus species to act as pathogens must first adhere to host tissues. During the process of tissue invasion, enterococci encounter an environment vastly different than those at sites of colonization, with higher redox potentials, limited essential nutrients, phagocytic leukocytes, and other host defenses. Infecting enterococci likely express genes favouring growth under these alternate environmental conditions. As has become increasingly apparent in recent years, enterococci express factors that permit adherence to host cells and extracellular matrix, facilitate tissue invasion, effect immunomodulation, and cause toxin-mediated damage. This part of the review discusses enterococcal traits with the potential to contribute to virulence.

The first examination of enterococcal virulence was reported in 1899 (MacCallum and Hastings 1899), the same year the organism was discovered (Thiercelin 1899). The bacterium expressed cytolytic (or hemolytic) and protease (or gelatinase) activities and was isolated from a fatal case of endocarditis from the Osler service at The Johns Hopkins Hospital. However, the first systematic study of the properties of an enterococcal virulence factor was the study of cytolysin or hemolysin by Todd in 1934. He observed that although some strains of E. faecalis produced discernible zones of hemolysis on blood agar plates, "... this group failed to produce haemolytic filtrates when grown in Hewitt's broth". Although he called these strains "pseudo-hemolytic," cytolytic activity was detected by using a "horse flesh infusion" medium. But Jett et al. (1994) reported that modified by substitution of ground beef for less readily obtainable horse flesh, to be superior to commercially available media for production of cytolysin in liquid culture, although only modest cytolytic activity is produced. The basis for production in some media and not others is unclear and is the subject of ongoing studies on cytolysin expression. Cytolysin production by E. faecalis is recognized by the development of clearing around colonies on certain blood agar media. This E. faecalis phenotype, however, frequently has been overlooked in clinical microbiology laboratories because
sheep erythrocytes, the target cells commonly used in blood agar plates are refractory to cytolysin-mediated lysis. Erythrocytes from rabbits, humans, horses, and cows are readily lysed by the *E. faecalis* cytolysin and should be used instead to identify this phenotype (Basinger and Jackson, 1968 and Kobayashi 1940). The cytolysin genes are transmissible by plasmids and virulence trait can be transferred one to another (Dunny and Clewell 1975).

The other secreted products long associated with enterococci, but less well studied, are hyaluronidase (Rosan and Williams 1964) and protease (or gelatinase) (Andrewes and Horder, 1906). Further, bacterial adherence to host tissues is a crucial first step in the infection process (Baddour et al., 1990). Aggregation substance is a surface-bound protein encoded by pheromone-responsive plasmids of *E. faecalis* and expressed in response to pheromone induction. Aggregation substance converts the surface of the donor bacterium into one adherent to potential recipient cells, causing aggregation or clumping and facilitating transfer of plasmids (Dunny, 1990).

Further, the purification of a 28- to 32-kDa metalloproteinase from *E. faecalis* was first described 30 years ago (Bleiweis and Zimmerman, 1964). In 1989 Makinen et al. published a description of the substrate specificity of protease produced by *E. faecalis* OG1-10, a human oral isolate capable of hydrolyzing gelatin, collagen, casein, hemoglobin, and other small biologically active peptides. Su et al. (1991) subsequently reported the sequence of the protease gene, *gelE*. Coque et al., 1993 stated that protease production can be easily detected using semisolid media supplemented with 3% gelatin or 1.5% skim milk and analyzed 95 enterococcal isolates from patients with endocarditis and other nosocomial infections and reported 54% producing protease. In this same study, the production of protease by only 12 and 14% of enterococcal isolates from uninfected hospitalized patients and healthy volunteers, respectively was stated. A potential contribution of enterococcal protease to virulence was first suggested in 1975 by Gold et al. Kuhnen et al. (1988) reported protease-producing *E. faecalis* to be common
among enterococci (63.7%) isolated from surgical and neurosurgical intensive care units in Germany.

Gulhan et al. (2006) studied and reported the virulence factors of 146 _E. faecium_ and _E. faecalis_ strains isolated from fecal samples of dog, human and cats. Furumuru et al. (2006) reported 32 clinical isolates of _E. faecalis_ with increased virulence factors. Out of which 24 (7.5%) produced haemolysis with sheep erythrocytes and cell free heat stable hemolysis was detected in all isolates (100%) of _E. faecalis_ when grown in BHI-GA with 1% glucose and 0.03% L-arginine. Schlievert et al. (1998) investigated aggregation and binding substances that enhance pathogenecity in rabbit models of _E. faecalis_ endocarditis. Wells et al. (2000) analyzed and stated that by inducing the expression of _E. faecalis_ aggregation substance surface protein, bacterial internalization can be facilitated. In 2001, Waters et al. reported that pheromone inducible aggregation substance proteins of _E. faecalis_ are essential for high efficiency conjunction of the sex pheromone plasmids and also serve as virulence factors during host infections particularly for host cell adhesion. Drupe et al. (2003) isolated 47 clinical isolates between 1998 and 2001 and reported 15 _E. faecalis_ and 32 _E. faecium_ with virulence factors such as gelatinase, aggregation substance and extracellular surface proteins (esp) etc.

Baldassari et al. (2004) isolated 11 _E. faecalis_ causing endocarditis which were further screened for possible virulence factors using PCR and phenotypic characters. Donelli et al. (2004) demonstrated that slime production and pheromone response are both present in isolated enterococci suggesting that clinical strains with these features might have relative advantage in colonizing biliary stents. Lucilla et al. (2004) evaluated the contribution of extra cellular polysaccharide expressed by _E. faecalis_. Also stated that isolates expressing extracellular polysaccharides were able to survive for more hours then negative isolates. Alebouyeh et al. (2005) reported that the virulence factors frequency is more in _E. faecalis_ than in _E. faecium_ and identified enterococci with virulence factors.
such as haemolysin toxin, aggregation substance formation, gelatinase, Dnase production, haemagglutination in 144 *E. faecalis* and 35 *E. faecium*. Seno *et al.* (2005) investigated the potential relationship between biofilm formation and pathogenicity of 352 *E. faecalis* between 1991 and 2002. Of 352, 315 possessed aggregation substance (*asal*), 315 *esp* genes, 63 hemolysin positive and 167 gelatinase positive, 59 and 94 isolates respectively possessed both *asal* and *esp* genes. Importantly, *E. faecalis* isolates with both *asal* and *esp* genes formed biofilms at significantly higher rates than those with neither gene. They also stated that *E. faecalis* isolates that had accumulated virulence genes were apt to form persistent biofilms in the urinary front.

2.5. Molecular analysis of *Enterococcus* spp.

Epidemiological investigations involve the collection and analysis of both epidemiologic and microbiologic data. Several techniques have been used in medical microbiology for acquiring information on the spread of pathogenic bacteria with in the hospital environment and outside in the community. Among these, molecular typing has gained popularity and is frequently used to support and / or initiate epidemiological investigation. Accurate study of epidemic bacterial isolates is aided with the determination of genetic relatedness among isolates such that both differences and similarities are found (Antonyshyn *et al.*, 2000). In epidemiological studies molecular characterization of enterococci can provide additional information regarding the isolates of different origin and various geographic dispersion and the precise variation among isolates as to identify the molecular relatedness (Jensen *et al.*, 1998). Thal *et al.* (1998) stated that molecular evaluation would also be of helpful to determine whether clonal, plasmid, or transposon dissemination or a combination of mechanisms is responsible for some of the resistance that is being observed among *Enterococcus* spp.

The “genome scanning” techniques such as restriction endonuclease analysis (REA) and macro restriction analysis of genomic DNA followed by pulsed-field gel electrophoresis (PFGE) are preferred by the epidemiologists and referred to be the “gold
standard" for molecular typing. Murray et al. (1990) compared genomic DNAs of different enterococci isolates using restriction endonuclease with infrequent recognition sites. However, stated that epidemiologic correlation of enterococci has been limited by the lack of a sample and effective method for comparison. Woodford et al. (1993) studied the RFLP of rRNA genes (ribotype) and vanA genes to evaluate the cluster of the isolates. Savor et al. (1998) compared the ability of restriction endonucleases analysis (REA) with results previously determined by PFGE to distinguish strains at the subspecies level. Willems et al. (1999) reported the genetic diversity in Tn1546 combined with epidemiological data and suggested that the DNA polymerase among Tn1546 variants can successfully be exploited for the tracing of the routes of transmission of vancomycin resistant genes. Tsiodras et al. (2000) had reported differentiation of entrococcal species based on the domain V of 23S rRNA gene sequence in different enterococcal species and identified the fact that domain V is considered to be highly conserved; substantial differences were proved between several entrococcal species. Singh et al. (2001) reported that msrC gene is associated with resistance phenotype against erythromycin, azithromycin etc. Homan et al. (2002) elaborated multilocus sequence typing scheme for E. faecium. They described that VSEF isolates were genetically more diverse than VREF isolates. Nallapareddy et al. (2002) elaborated the utility of MLST and Pulsed Field Gel Electrophoresis (PFGE) for subspecies differentiation of E. faecalis. They also suggested that similar to PFGE the sequence based typing method (MLST) may be useful for differentiating isolates of E. faecalis to the subspecies level in addition to identifying outbreak isolates. Top et al. (2004) described the genetic relatedness of enterococci based on the multiple locus variable number tandem repeat (VNTR) analysis (MLVA) as a novel typing method. Similarly, SDS-PAGE has also been used for differentiation studies using proteins of whole cell and extra cellular purified from Enterococcus spp. Prakash et al. (2005) had applied SDS-PAGE for species differentiation and confirmation among enterococci.