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ENTEROCOCCI : TRANSITION FROM NORMAL FLORA TO A COMMON NOSOCOMIAL PATHOGEN

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ABSTRACT

Enterococcus species were considered as harmless commensals for many years. But today they have become one of the most common nosocomial pathogens. This review highlights the need for a greater understanding of this genus, including its ecology, virulence factors and epidemiology, to justify this dramatic change.

Key Words: Enterococci, Virulence factors, Antibiotic Resistance, Nosocomial Pathogen

INTRODUCTION

The name "Enterocoque" was first used by Thiercelin in 1899[1]. The name was proposed to emphasize the intestinal origin of this new Gram positive diplococcus. Same year Mac callum and Hastings reported a case of endocarditis caused by an organism which they called *Micrococcus zymogenes* but later studies suggested that this organism was actually a hemolytic enterococci[2]. Enterococci were originally classified as enteric Gram-positive cocci and later included in the genus Streptococcus[3]. The name *Streptococcus faecalis* (faecalis, relating to feces) was first coined in 1906 by Andrewes & Horder, who isolated this organism from a patient with endocarditis[3]. In the 1930s, with the establishment of the Lancefield serological typing system, enterococci were classified as group D streptococci and were separated from non-enterococcal Group D Streptococci mainly by biochemical characteristics[4]. Sherman (1937) further recommended that the term "Enterococcus" should be used specifically for Streptococci that grow at both 10^0C and 45^0C, at pH 9.6 and in 6.5% NaCl and survive if kept at 60^0C for 30 minutes. These organisms were also noted to hydrolyze esculin in the presence of bile[5]. In 1984, using DNA hybridization and 16S rRNA sequencing, it was established that the species *Streptococcus faecium* and *Streptococcus faecalis* were distinct from other Streptococci and to be designated as another genus: Enterococcus. Nine species were transferred from the Streptococcus groups and now Enterococcus includes 28 species[6].

E.faecalis is the predominant enterococcal species, accounting for 80-90% of all clinical isolates and *E.faecium* accounts for 5-15%. Other Enterococcal species such as *E.gallinarum, E.casseliflavus, E.durans, E.avium* and *E. raffinosus* are isolated less frequently[5].

The genus Enterococcus consists of Gram-positive organisms that are ovoid in shape and may appear on smear in short chains, in pairs, or as single cells. They are non-motile except *E.casseliflavus* and *E.gallinarum*. They are non-capsulated. Although some strains of *E.faecalis* may be capsulated. It grows readily on simple media. On MacConkey's agar, it forms small (0.5-1mm), usually magenta-colored colonies. Growth on Blood agar is usually non-hemolytic, but sometimes it may be alpha or beta hemolytic. It also grows on media with high salt content (eg.6.5% NaCl). Enterococcus species are facultative anaerobes. They grow at a range of temperatures from 5 to 50^0C. Both *E.faecalis* and *E.faecium* can withstand heating at 60^0C for 30 minutes. *E.faecalis* and *E.faecium* will grow in a wide range of pH (4.6-9.9), with the optimum being 7.5. Enterococcus species can also grow in presence of 40% bile salts. Enterococcus species do not have cytochrome enzymes and are thus catalase negative, although some strains do produce pseudocatalase[3]

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VIRULENCE FACTORS

Several virulence factors have been identified in *E.faecalis* and *E.faecium*. The surface protein, known as Aggregation substance (AS) is a pheromone inducible surface protein of *E.faecalis* which promotes mating aggregate formation during bacterial conjugation[8]. AsaI, AspI and Acs 10 are the best studied AS proteins. AS is also an important virulence factor in *E. faecalis*. Asa1 increases adherence to renal tubular cells and adherence to and survival in human macrophages. Asc10 increases internalization and intracellular survival in polymorphonuclear leukocytes (PMNs). Both Asc10 and Asa1 have been found to increase virulence of *E. faecalis* in a rabbit endocarditis model[9]. Aggregation substance production can be determined by adding 18 hour culture supernatant of the pheromone producing *E.faecalis* JH2-2 strain grown in TH (Todd Hewitt) Broth into enterococcal strain being tested and observing for clumping after incubation for 24 hours at 37^0C[10]. Another virulence determinant is the enterococcal surface protein(Esp) that is present in both *E.faecalis* and *E.faecium*[9]. Esp of *E.faecium* is involved in biofilm formation and contributes to the pathogenesis of experimental endocarditis, urinary tract infection and bacteremia[9]. Gelatinase, another virulence factor of *E. faecalis* , has an important role in biofilm formation . The production of gelatinase can be determined by using Todd-Hewitt agar plates containing 3% gelatin. After overnight incubation at 37^0C, colonies with opaque zones are considered positive for gelatinase[10]. Pili has also been implicated as a virulence factor of *E. faecalis.* Several surface proteins belonging to MSCRAMM (Microbial surface components recognizing adhesive matrix molecules) family play a role in pathogenesis of *E.faecalis* and *E.faecium* infections. The presence of ecbA (*E. faecium* collagen binding protein A) is associated with hospital-acquired *E. faecium* isolates[9].

LTA (Lipoteichoic acid) and Epa (Enterococcal polysaccharide antigen), present in the cell wall of *E.faecalis* are another important virulence factors. The disruption of different genes of the epa locus results in decreased biofilm formation, lower resistance to killing by PMNs (Polymorphonuclear Neutrophils) [9]. *E.faecalis* serotype C and D possesses a capsule locus (cps) which confer resistance to complement–mediated opsonophagocytosis of serotype C and D strains. Recent studies have demonstrated an important role of enterococcal membrane glycolipids in virulence[9]. Hemolysin (or cytolysin) is a cytolytic protein that can lyse human, horse and rabbit erythrocytes. Hemolysin producing strains of *E.faecalis* are associated with increased severity of infection in human[8]. Hemolysin production can be detected by inoculating Enterococci on Columbia agar supplemented with 5% (v/v) fresh human blood. A clear zone of ß-hemolysis around colonies after incubation at 37^0 C for 24 hours is taken as positive[10].

ENTEROCOCCI : AS NORMAL FLORA

Enterococci are found in the faeces of most healthy adults. Enterococci are less commonly found at other sites such as vagina and mouth. They have also been reported in dental plaques of healthy people[3]. The ability of Enterococcus species to survive a range of adverse environments allows multiple routes of cross-contamination of enterococci in causing human disease, including those from food, environmental and hospital sources[6].

PATHOGENECITY

The commonly encountered Enterococcal infections are Urinary Tract Infections which includes cystitis, pyelonephritis, prostatitis, perinephric abscesses and complications associated with bacteremia. Risk factors include frequent instrumentation, prior therapy with antibiotics that select for resistant organisms and structural abnormalities[3]. Enterococcal bacteremias are usually secondary to Urinary Tract Infections and Gastro-intestinal Infections[3]. Enteroccoal bacteremia are also associated with high mortality[2].Enterococci can also cause acute or sub-acute endocarditis, especially in middle aged men. Endocarditis occasionally occurs in children and rarely in infants. Common risk factors include Genito-urinary or Biliary tract Infections and underlying heart disease. The valves involved by enterococci are usually aortic and mitral[3].

In addition to causing neonatal sepsis, enterococci can also cause Central Nervous System (CNS) infections in older children and adults. Long term primary illness (eg. diabetes, malignancy, renal insufficiency), invasive procedures of the CNS (eg. shunt replacement), prior antibiotic therapy are reported to increase the risk[3]. Enterococci can also cause and contribute to abdominal and pelvic abscess. Enterococci have also been reported to cause acute salphingitis, endometritis and wound

and soft tissue infections. Enterococcal peritonitis associated with chronic ambulatory peritoneal dialysis have also been reported[3]. Enterococci has become one of the most common nosocomial pathogen, with patients having a high mortality rate of upto 61%[6]. Enterococci are now the second most common cause of Nosocomial Urinary tract and Wound infections and third most common cause of Nosocomial Bacteremias[11]. Enterococci is also a commonly isolated micro-organism in transplant recepients especially after liver and kidney transplantation. Blood-stream infections (mainly intravascular catheter related), intra-abdominal or biliary tract and wound infections are common infection reported in solid organ transplantation[12]. Enterococci also play a role in endodontic failure and are often isolated in root canal system[6]. Tomomi et al (2005) have also reported Enterococcal Endopthalmitis caused by *E.mundtii* in an elderly immuno-compromised patient[13]. Vincenzo et al (2008) have also reported a case of vaginal infection caused by *E.raffinosus* in an immuno-compromised patient[14].

RESISTANCE TO ANTIBIOTICS

Emergence of Enterococcus species as an important nosocomial pathogen can be attributed to their resistance to many antimicrobial agents and ease with which they attain and transfer resistant genes[15]. Antimicrobial resistance in enterococci is of two types: inherent / intrinsic and acquired resistance. Intrinsic resistance is species characteristic and is chromosomally mediated. Enterococci exhibits intrinsic resistance to penicillinase susceptible penicillin (low level), penicillinase resistant penicillins, cephalosporins, lincosamides, nalidixic acid, low level of aminoglycoside and low level of clindamycin. Co-trimoxazole combination is not effective in enterococcal infections as enterococci are able to incorporate preformed folic acid and thus can bypass the inhibition of folate synthesis produced by Co-trimoxazole. Acquired resistance on the other hand results from either mutation in DNA or acquisition of new DNA, and the examples include resistance to penicillin by ß lactamases, High Level Aminoglycoside Resistance (HLAR), vancomycin, chloramphenicol, erythromycin, high level of clindamycin, tetracycline and fluoroquinolone etc[15].

Enterococci are intrinsically resistant to most ß lactam antibiotics due to low affinity penicillin binding proteins[15]. Penicillin resistance is directly proportional to the amount of PBP5 (Penicillin Binding Protein 5) produced[5]. Most of the isolates of *E.faecalis* can be inhibited by concentration of penicillin achievable in the plasma (MIC 1 -8 µg/ml), but this is usually not the case with *E.faecium* (MIC 16-64 µg/ml). Higher level of resistance in *E.faecium* has been attributed to over production of low affinity PBP5, a protein that can take over the function of all PBPs. In addition,enterococci are "tolerant" to the activity of ß- lactams, that is , enterococci are inhibited but not killed by these agents. This property is an acquired characteristic[15]. Enterococci also acquire resistance to ß lactams through ß-lactamase enzyme, production of which is constitutive, plasmid mediated and inoculum dependent[5]. The resistance of beta-lactamase-producing strains is not detected by routine disk susceptibility testing because of an inoculum effect. When a low inoculum is used (like that used for disk testing), strains appear susceptible, but at a high inoculum (eg.10^7CFU/ml) strains appear resistant (MIC >500 µg/ml). An inoculum effect is due to the fact that low numbers of cells do not produce sufficient beta-lactamase to cause resistance[3]. *E.faecalis* strains producing ß-lactamase are not susceptible to anti-staphylococcal penicillins but are susceptible to ampicillin, amoxicillin and piperacillin combined with drugs that inhibit penicillinase such as clavulanic acid, sulbactam and tazobactam[15].

Low level resistance to aminoglycosides (MIC 8-256 µg/ml)[15] is also an inherent property of enterococci and is due to low uptake of these agents. If this is the only type of aminoglycoside resistance expressed, then the addition of an aminoglycoside to a cell-wall-active agent such as penicillin or vancomycin characteristically results in enhanced killing .This enhanced killing, called synergism, is defined for enterococci as a >2 log 10 increase in killing versus the effect of the cell-wall-active agent alone when the aminoglycoside is used in a subinhibitory concentration[16]. HLAR (High level aminoglycoside resistance) i.e., Streptomycin MICs >2000 µg/ml and Gentamicin MICs > 500 µg/ml is an acquired resistance[11]. HLAR occurs due to the presence of AME (Aminoglycoside modifying enzymes). The most frequently encountered enzyme include dual function 2′phosphotransferase and 6′acetyl transferase conferring HLR to all available aminoglycoside (kanamycin, gentamicin, amikacin, netilmicin , tobramycin) except streptomycin[15]. Hence, gentamicin resistance is a good predictor of

resistance to other aminoglycosides except streptomycin[5]. Another Aminoglycoside modifying enzyme, 3′ phosphotransferase codes for HLR to Kanamycin and Penicillin-Amikacin synergy without HLR to Gentamicin. 6′adenyl transferase another AME is resposible for HLR to streptomycin but does not inactivate other useful aminoglycosides. 30% of VRE (Vancomycin resistant enterococci) strains can produce multiple enzyme types and thus are highly resistant to all known aminoglycosides. AME are coded by plasmid and are transferable[15]. The composite transposon Tn5281 (IS256-related) has been shown to harbor the aac(6′)-Ie-aph(2′)determinant as part of conjugative enterococcal plasmids.Other transposons that have been linked to HLAR are Tn 924,Tn1547, Tn 5384, Tn 5385, Tn 5405,Tn 5482, Tn 5506[17]. Lall and Basak have reported that 60.5% of Enterococcus strains in their study were HLAR.[18]

Until recently, vancomycin was the only drug that could be used for the treatment of infections caused by multi-drug resistant enterococci. But vancomycin resistance also emerged . In 1988, Uttley et al were the first to report the isolation of Vancomycin resistant *E.faecalis* and *E.faecium* in England[19]. Shortly after this Vancomycin resistant enterococci (VRE) were reported from UK, France and worldwide. Under normal conditions of peptidoglycan synthesis in enterococci, two molecules of D-alanine are joined by a ligase enzyme to form D-Ala–D-Ala, which is then added to UDP-N-acetylmuramyltripeptide to form the UDP-N-acetylmuramyl-pentapeptide.The UDP-N-acetylmuramyl-pentapeptide, when incorporated into the nascent peptidoglycan (transglycosylation), permits the formation of cross-bridges (transpeptidation) and 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 crosslinking[5]. Vancomycin resistance involves modification of the vancomycin-binding target by synthesis of peptidoglycan precursors with peptide side chains that terminate in D-lactate (vanA, vanB and vanD) or D-serine (vanC, vanE, vanG, vanL and probably vanN) for which vancomycin has lower affinity than for the normal D-alanine side chain terminus. Vancomycin resistance in enterococci is an acquired resistance, except intrinsic low level resistance in *E.gallinarum* and *E.casseliflavus*. The van A and van B genotypes are the most commonly encountered forms of acquired glycopeptide resistance and have primarily been reported in *E.faecium* and *E.faecalis*[17].

Strains with the van A genotype characteristically display inducible, transposon-mediated, high level resistance to both vancomycin (MIC, 64-1000 μg/ml) and teicoplanin (MIC, 16- 512 μg/ml)[11]. Besides *E.faecalis* and *E.faecium*, van A genotype has also been reported in *E.avium, E.casseliflavus, E.durans, E.gallinarum, E.hirae, E.mundtii, E.raffinosus* and other bacteria such as *Staphylococcus aureus, Arcanobacterium haemolyticum*, etc[17]. Van A gene cluster is found on the transposon Tn 1546[5]. Enterococci with the van A phenotype are most worrisome because these strains are able to transfer van A resistance markers by a conjugative mechanism to other Gram-positive organisms, including *Staphylococcus aureus*[11].

Strains with the van B genotype have inducible resistance to various concentrations of vancomycin (MIC, 4-1000 μg/ml) but remains susceptible to teicoplanin (MIC, 0.5-1 μg/ml), although rare van B strains may also be resistant to the latter antibiotic[11]. Besides *E.faecalis* and *E.faecium*, van B genotype has also been reported in *E.casseliflavus, E.durans, E.gallinarum, E.hirae* and other bacteria such as *Staphylococcus epidermidis, Streptococcus, Clostridium* , etc. The vanB operon can be transferred between enterococci as part of large conjugative chromosomal elements or by conjugative plasmids. The vanB ligase gene has been divided into three subtypes, vanB1-3, based on nucleotide sequence differences. The vanB2 subtype as an integral part of Tn1549 / Tn5382-like conjugative transposons is the most widespread vanB type in clinical enterococci[17].

Strains expressing the van D genotype are constitutively resistant to both vancomycin (MIC, 64-128 μg/ml) and teicoplanin (MIC, 4-64 μg/ml). Resistance is not transferable to other enterococci[20]. Besides *E.faecalis* and *E.faecium*, van D genotype has also been reported in *E.avium, E.gallinarum, E.raffinosus* and Non-enterococcal faecal flora[17].

Rare *E.faecalis* strains expressing the van E genotype express inducible, low level resistance to vancomycin (MIC, 16 μg/ml), yet remain susceptible to teicoplanin (MIC, 0.5 μg/ml)[19].

The van G phenotype is associated with low level resistance to vancomycin (MIC, 16 μg/ml), but susceptibility to teicoplanin (MIC, 0.5 μg/ml)[19].

Conjugative transfer is possible in van G phenotype but not in van E phenotype[17].

Isolates that have the van C genotype display intrinsic, constitutive, low level resistance to vancomycin (MIC, 2-32 µg/ml) and are susceptible to teicoplanin (MIC, 0.5-1 µg/ml). The van C gene cluster is not transferred by conjugation to other organisms and is chromosomal in origin[11].

An interesting enterococcal phenomenon that has developed in some strains of van A and van B type VRE is that of vancomycin dependence[5]. Vancomycin dependence refers to strains of enterococci that grow only in the presence of vancomycin.[11]. Recently, VDE has been reported from India by T. Banerjee et al[21].

Enterococci also exhibit intrinsic, low level resistance to clindamycin and lincomycin (MIC, 12.5-100 µg/ml). High level resistance (HLR) to clindamycin is an acquired characteristic, which occurs as a part of the macrolide-lincosamide-streptogramin B resistance phenotype[3]. The gene involved is known as ermB, which is carried by transposon Tn917[17].

Erythromycin resistance in enterococci is an acquired characteristic. Erythromycin resistance occurs as part of the macrolide lincosamide-streptogramin B resistance phenotype. The mechanism involves methylation of an adenosine residue in the 23S rRNA. Different erythromycin resistance determinants exist, but an especially common one (ermB) is carried by Tn917[3].

Various genes for acquired tetracycline resistance in enterococci have been identified, including tetL, tetM, tetN, tetO, tetS. These genes confer resistance by two different mechanisms; tetL mediates active efflux of tetracycline from cells, while tetM and tetN mediate resistance by a mechanism that protects the ribosomes from inhibition by tetracycline. tet M is carried by the conjugative transposon, Tn916[17], and tet L is contained in plasmid pAMα1[3].

Chloramphenicol resistance in enterococci is an acquired characteristic, mediated by chloramphenicol acetyltransferase.

Linezolid is an oxazolidinone antibiotic that has been approved for management of complicated and uncomplicated skin and soft-tissue infections, community and hospital acquired pneumonia and drug-resistant Gram-positive infections including infections with vancomycin resistant enterococci[22]. Although linezolid resistance in enterococci is uncommon, it has been reported [23,24,25]. Linezolid resistance in enterococci is an acquired characteristic, which affects the binding affinity between the target and the drug. It is not transferable and spreads clonally[17].

LABORATORY DIAGNOSIS

In clinical laboratories, enterococci have been presumptively identified for years by their appearance on smear and culture plus the demonstration of their ability to hydrolyze esculin in the presence of bile and to grow in the presence of 6.5 % NaCl[3]. For rapid identification of enterococci, few screening procedures are available. One of them is Sodium-chloride-Esculin hydrolysis test[26] and another method available for the rapid identification of enterococci is PYR (L-pyrrolidonyl-ß-naphthylamide) test, which gives results within 10 minutes[27].

The speciation of enterococcus is done by Carbohydrate fermentation tests (mannitol, sucrose, arabinose, raffinose, lactose, melezitose), Motility test, Arginine dihydrolase test, Yellow pigment detection test and Pyruvate fermentation test[11].

Commercially available systems available for the identification of enterococcus include the API 20S and the API Rapid ID32 STREP systems (bioMerieux) etc. Identification of unusual species by a commercial system should be confirmed by a reference method before being reported[28].

Molecular procedures proposed for the identification of enterococcus species include: analysis of WCP (whole-cell protein) profiles by sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) as WCP is species specific [28]; vibrational spectroscopic analysis; proton magnetic resonance spectroscopic analysis; randomly amplified polymorphic DNA (RAPD) analysis; sequencing analysis of the 16s RNA gene; fragment-length polymorphism analysis of amplified 16S rDNA etc.

EPIDEMIOLOGY

The epidemiology of Enterococci with VRE (Vancomycin resistant enterococci) varies widely in different geographical areas[29]. In Europe, VRE have been isolated from sewage and animal sources such as frozen poultry, pork, feces or intestines of horses, dogs, chickens, and pigs. The use of glycopeptide-

containing animal feeds (avoparcin) in some regions of Europe have been blamed for development of resistance in man.

Risk factors for VRE infection among hospitalized individuals include longer duration of hospitalization (≥ 7 days), the need for intrahospital transfer to another ward, the need for surgical reexploration following liver transplantation and a prolonged stay in Surgical ICU (SICU) post-operatively[30]. Other risk factors include previous antimicrobial therapy, exposure to contaminated medical equipment such as electronic thermometers[31], proximity to a previously known VRE patient, and exposure to a nurse who was assigned on the same shift to another known VRE patient[32]. Parenteral vancomycin use, duration of vancomycin use (>7 days) and receipt of third-generation cephalosporins are risk factors for colonization or infection with VRE[33]. Oral vancomycin use may also be a risk factor for VRE colonization. The high prevalence of skin colonization might explain the importance of VRE as a cause of catheter-related sepsis and bacteriaemia.

Presently, hospitalized patients with gastrointestinal carriage of VRE appear to be the major reservoir of the organism. Examples of items that may be contaminated include patient gowns and linen, beds, bedside rails, floors, door knobs, wash basins, glucose meters, blood pressure cuffs, electronic thermometers, electocardiogram monitors, electrocardiograph wires, intravenous fluid pumps, and commod[34,35,36] .

Transmission of VRE by health care workers (HCWs) whose hands become transiently contaminated with the organism while caring for affected patients is probably the most common mode of nosocomial transmission[37].

To minimise nosocomial transmission of VRE, CDC Hospital Infection Control Practices Advisory Committee (HICPAC) has recommended a multidisciplinary approach. It includes prudent use of Vancomycin, education of hospital staffs, effective use of Microbiology laboratory, and implementation of infection control measures[38] C.difficile colitis is another risk factor for colonization or infection with VRE in hospitalized patients[39].

Once VRE have been detected in a patient, enterococci recovered from all body sites should be tested for susceptibility to Vancomycin. Infection control personnel must be immediately notified about the presumptive identification of VRE, so that the patient can be placed on appropriate isolation precautions promptly.

Isolation precautions include : Placement of VRE-infected or colonized patients in single rooms or in the same room as other patients with VRE, use of clean non-sterile gloves when entering the room of a VRE-infected or -colonized patient and use of a clean nonsterile gown when entering the room of a VRE-infected or colonized patient if substantial contact with the patient or environmental surfaces in the patient's room is anticipated or if the patient is incontinent or has diarrhea, an ileostomy, a colostomy, or wound drainage not contained by a dressing. Gloves and gowns should be removed before leaving the patient's room and hands should be immediately washed with an antiseptic soap[5]. In addition to these isolation precautions, the use of noncritical items such as stethoscopes, sphygmomanometers, or rectal thermometers should be dedicated to a single patient or cohort of patients infected or colonized with VRE[40]. Patients infected and/or colonized with VRE can be removed from isolation precautions when VRE-negative cultures on at least three consecutive occasions, 1week or more apart, for all cultures from multiple body sites (including stool, rectal swab, perineal area, axilla or umbilicus, and wound, Foley's catheter, and /or colostomy sites if present) are obtained[41].

HICPAC also recommends hand and rectal swab cultures from hospital personnel. If found VRE-positive, he should be removed from the care of VRE-negative patients until the carrier state has been eradicated. Some hospitals may perform focused environmental cultures before and after cleaning rooms, housing patients with VRE.

Various drugs have been tried for eradication of gastrointestinal colonization of VRE e.g. Novobiocin, Doxycycline, Tetracycline and bacitracin[5]. No regimen has been found to be uniformly effective in eradicating VRE from the gastrointestinal tract[42].

TREATMENT

Serious enterococcal infections (e.g., bacteremia and endocarditis) require treatment with a bactericidal combination of antibiotics that should include penicillin (e.g., ampicillin or penicillin G) to which the Enterococcus isolate is susceptible and an aminoglycoside (e.g., gentamicin or streptomycin) to which the Enterococcus isolate does not exhibit

high-level resistance. Vancomycin in combination with an aminoglycoside has demonstrated synergistic activity against enterococci both in vitro and in vivo[43] and it is recommended as the drug of choice in patients with serious penicillin allergy or in the treatment of ampicillin- and penicillin-resistant strains of bacteria. However, enterococci are becoming increasingly resistant to traditional antibiotic therapy. In addition to high-level aminoglycoside resistance and ampicillin resistance, rapid spread of vancomycin resistance has resulted in limited therapeutic alternatives. Treatment of infections due to VRE, especially *E. faecium* is extremely problematic, because these organisms are resistant to multiple antibiotics. Penicillin or ampicillin with or without a synergizing aminoglycoside would be a reasonable choice in the non-allergic patient infected with vancomycin-resistant *E.faecalis*. Almost all *E.faecalis* strains are at least moderately susceptible to ampicillin. Therefore, if vancomycin resistance emerged predominantly in *E.faecalis*, the treatment of most of these infections could be relatively easy. Unfortunately, vancomycin resistance has preferentially appeared in *E.faecium*, which is inherently more resistant to penicillin and ampicillin[5]. Teicoplanin is another glycopeptide that is active in vitro against most VanB-type enterococci, but resistance has been reported[5]. The oxazolidinones inhibit enterococcal translocation at the initiation of protein synthesis and in vitro selection of resistant mutants does not occur readily[44].

Hence, not only the detection of Enterococci especially VRE and HLAR producing Enterococci is important for hospitalized patient or patient with medical device. The health care workers (HCWs) have to follow the prevention and control guidelines in management of patients colonized or infected with Enterococci.

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