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Study of enterococcus physiology and their characteristics- A short review

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Abstract *The genus Enterococcus is composed of 38 species, the most important of which are Enterococcus faecalis and Enterococcus faecium—both human intestinal colonizers. Hospitals within the United States and around the world commonly isolate these bacteria because they cause of bacteraemia, urinary tract infections (UTIs), endocarditis, wound infections, meningitis, intra abdominal and pelvic infections, and nosocomial and iatrogenic infections. Given the ubiquity of enterococci within the human population, it is important for laboratories to be able to distinguish these agents within hospitalized patients from other bacterial genera and also differentiate different species within the Enterococcus genus as well as different strains within each species. Unfortunately, the enterococci are emerging as serious pathogens in both the developed world, where surveillance needs to be improved and speciation procedures are inadequate or cumbersome, and in developing nations, which lack the trained hospital personnel or funding to sufficiently identify enterococci to the genus or species level. This review explores the Enterococcus genus and highlights some of the concerns for national and international clinical microbiology laboratories.*

Keywords: *enterococci, Staphylococcus, antibiotic resistance, bacteriology, microbiology*

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I. Enterococcus genus

The human intestine is consists of its own normal microbial flora. Among these normal flora some bacteria are resident flora & some are transient . Enterococci, as resident flora of intestinal tract colonise in other organs mucosal and also skin surfaces, can cause disease[1]. Thiercelin first named the intestinal bacteria as "enterococcus" which is a gram-positive diplococcus organism [2]. This same microbes when isolated from a patient is named as Streptococcus faecalis by Andrewes and Horder [2]. Streptococcus faecium was a fecal organism later described by Orla-Jensen to have similar characteristics to Streptococcus faecalis [2]. According to Rebecca Lancefield enterococci were classified as Group D streptococci because their Group D cell wall C carbohydrate antigen is made up of glycerol teichoic acid [3,4], Pyogenes ,viridans, lactic and enterococcus are the four distinct groups of Streptococci in combination with S. faecalis and S. faecium form the enterococci [5]. For years enterococci were classified in Streptococcus genus until Kalina proposed that they are placed in their own genus [5]. There is a significant difference between the streptococci and enterococci were observed when DNA-DNA and DNA-rRNA hybridization were observed [1,4]. From humans and animals total 38 species within the Enterococcus genus were observed [5]. The most important species are the potential human pathogens Enterococcus faecalis and Enterococcus faecium, though Enterococcus gallinarum and Enterococcus casseliflavus have also been studied because they are inherently vancomycin-resistant and colonize the intestinal tract [6,5].

Enterococcus physiology

In a variety of hostile conditions Enterococci proliferate which are robust non-spore forming bacteria. Enterococci are facultative anaerobic organisms that can proliferate in both oxygen rich and oxygen deficient environments i.e both in oxidation and fermentation [7]. Gram positive streptococci can't survive in 6.5% NaCl solution but enterococci can survive and grow in high salt concentrations [8,9,10]. They can survive in wide temperature variation like 5^{o}C, 50^{o}C and in 60^{o}C for 30 min [9,10]. They proliferate optimally at 37–42.7°Cdepending on the growth media [9,10]. Enterococci can be able to survive in a high pH range as 4.8and 9.6 [9,10]. In 40% bile salt concentrations enterococci can survive but Streptococcus pneumoniae would destroy [8,10,9]. The ability of enterococci to withstand broad pH ranges is likely due to their membrane durability and impermeability to acid and alkali, while their resistance to temperature is attributed to membrane lipids and fatty

acids [8]. The extreme conditions by which enterococci can survive allow them to colonize a wide range of niches, which could have relevance for their clinical importance.

Pathogenesis

Pathogens produced virulence factors that are traits or molecules. Virulence factors help these pathogens with colonization, immune evasion and immunosuppression of their hosts. Consequently these virulence factors are often responsible for causing disease. Enterococci like some other bacteria do not produce potent toxins. They possess virulence factors in the form of aggregation substance, cytolysin, gelatinase, enterococcal surface protein (Esp) and antibiotic resistance genes [11]. In the extracellular environment they can release superoxide ion [12].Many of the genes for the enterococci virulence factors are found on conjugative plasmids or encoded within transposons are easily transferable[13,14,15,16]. Enterococci can exchange these genetic determinants with bacteria of a different genus and among themselves [17,18].Among the all Enterococci species, three species could lead the exchange of genetic determinants [19]. The first conjugative system involves the sex pheromone plasmids that are unique to the enterococci, have a narrow host range, and can be transferred at high frequencies in laboratory and in vivo environments[20,21]. Plasmids act as a second conjugative system are readily transferrable among the enterococci,staphylococci, streptococci, and other bacterial species [22].Transposons found in Gram-negative and Gram-positive bacteria and the last conjugative system involves the transposons [23,24].Though conjugative system is another method of genetic material exchange, bacteriophage used by enterococci to allow for the spread of virulence factors or antibiotic resistance [25,26]. 91 E. faecalis and 79 E.faecium a total of 170

Enterococcus species were isolated and screened for putative enterococcal virulence genes. Enterococcal virulence genes (gelE, asa1, esp, cylA and hyl) was screened and isolated from California and Puerto Rico. PCR primers and multiplex method developed used by Vankerckhoven et al. [27]. Eighty-seven (80.6%) E.faecalis isolates from PR beach water harboured one or more of the following genes: gel E (98.1%), asa1 (44.4%), esp (11.1%) and cyl A (3.3%). 26.3% E.faecalis isolated from septage contained gel E (21%), asa 1(5.3%), cyl A (5.3%) and hyl (5.3%). Eighteen E.faecalis isolated from clinical and non-clinical specimens contained gel E (17.4-100%), asa1 (50-100%), esp (33.3-40%) and cyl A (19-60%) [28].

A haemolytic protein cytolysin can be produced by the enterococci. The gene encoding the cytolysinis found in an operon i.e carried on a plasmid and into the bacterial chromosome [29,30,31]. Enterococci that possessing cytolysin have selective advantage for growth and survival for gram positive bacteria but not for gram negative bacteria [32]. On a blood agar plates when β-hemolytic reactionis observed it indicates the presence of cytolysin containing strains. The cytolysin is able to lyse red blood cells from either human or horse blood, but sheep blood is somewhat less susceptible to lysis[33]. Cytolysin can present in many bacteremic strains of enterococci and it lyse macrophages and neutrophils to gain additional nutrients and escape immune clearance [34]. Finally, virtually all bacteremic strains of E. faecalis produce extracellular superoxide, but the precise purpose for this chemical is still unknown, though there is some suggestion that it may have a role in the lysis of red blood cells . Gel E present in E. faecalis strains produce gelatinase [35]. Gelatinase is a protease that hydrolyzes gelatin, collagen, casein, and hemoglobin and appears to be responsible for enhanced virulence for endocarditis caused by E. faecalis in animal models [35,36].

Clinical disease

Enterococci are found in communities and hospital that are responsible for various clinical diseases. These diseases normally affect the elderly, the immunocompromised, patients with serious underlying illness, patients treated with broad spectrum antibiotics, and patients with additional bacterial infections. Enterococci are the cause of urinary tract infections (UTIs), bacteremia, endocarditis, meningitis, wound infections and intra abdominal and pelvic infections [37,38]. Enterococci causes nosocomial infections in hospitals and iatrogenic infection [39,38]. According to the National Nosocomial Infections Surveillance (NNIS)system conducted by the Centers for Disease Control and Prevention (CDC), the enterococci are the fourth leading cause of hospital-acquired infections and the third leading cause of bacteremia [40]. Normally, the routes for enterococcal infections are (1) endogenous i.e. spread of the patient's own microbial flora into other body sites as a result of over antibiotic usage or improper patient usage of antibiotics (opportunistic infections), (2) spread of antibiotic resistant bacterial strains in a hospital setting (nosocomial and iatrogenic infections), and (3) wound infections (largely attributed to surgery, decubitus ulcers and burn wounds). Death associated with enterococci is normally due to endocarditis [41].Among all the species the most two important pathogens are , E. faecalis and E. faecium. E. faecalis causes 80%-90% infection and E. faecium(20%) of the infection [42]. Clinical isolates include E. casseliflavus and E. gallinarum [43].Most UTIs infection caused by enterococcal species in patients using urinary catheters [37,44]. In Cedar Sinai in Los Angeles, California, USA most UTI cases are caused by E. faecalis not by E.coli. The second most common enterococcal infection, a polymicrobial infection, arises from intra abdominal and pelvic infections in which the enterococci are usually associated with other organisms [37]. They may cause wound infections [37].Bacteremia is the third most common infection [45,46,38].

Patients undergoing enterococcal bacteremia have an increased likelihood of developing endocarditis [45,46,38]. In neonates meningitis caused by enterococcal bacteremia [1].In some literature it is reported that dental infections are caused by enterococci [47,48]. If the dental infection progresses to a bacteremia, like it can withStreptococcus mutans and Eikenella corrodens, then it could cause endocarditis. Again, enterococcal endocarditis is an infection that can lead to significant morbidity and mortality and can occur from sources of infection involving the genitourinary tract, the gastrointestinal tract, and wound infections and, as stated above, bacteremia [45,46,41].The mortality of endocarditis is 20%-40% associated with antibiotic treatment and 17%-100% without appropriate antibiotic treatment [41]. Endocarditis is a common cause of morbidity and mortality as enterococci is a commonly antibiotic resistant.

Antibiotic resistance to antimicrobial agents

Enterococcal infections are treated by the synergistic effects of an aminoglycoside and by cell wall active antibiotic ampicillin or vancomycin..Enterococci can resist antibiotics and causes nosocomial and iatrogenic infections [49,50]. In hospital these organisms provide an evolutionary pressure for selective advantage [49]. Enterococci can transfer resistance genes through exchanging plasmids via conjugation and resistance genes on transposons or via bacteriophages [51,52,53,54,55,56,57].Mutations causes high-level resistance to various antibiotics. Enterococci reside in the gastrointestinal tract and they come in contact with both Gram-negative and Gram-positive organisms and can exchange resistance genes with other bacterial genera [58,59].Enterococci have resistant to plethora of antimicrobial agents, resistance to aminoglycosides via aminoglycoside modifying enzymes, tetracyclines via genes such as tetM and tetN, chloramphenicol via chloramphenicol acetyltransferase,and the glycopeptides via enzymes that modify the vancomycin target or remove the susceptible target [60]. Penicillin, ampicillin and related drugs by transferable plasmids of enterococci [61,62].When we discuss about vancomycin resistance, six gene clusters have been associated with both phenotype VanA to VanG [63]. Plasmid borne or chromosomal borne diseases can be formed by constitutive or inducible cluster [63]. Within the VanA cluster, genes VanS and VanR are involved in a two-component system that can alter the enterococcal cell wall composition from the peptidoglycan precursor (D-Ala-D-Ala) to D-Ala-D-lactate (D-Lac).Vancomycin has reduced affinity for D-Lac i.e the basis for the vancomycin resistance [64,63,65].Glycopeptide resistance genes can be transferred to Methicillin-resistant Staphylococcus aureus [66,67,68]. VanA is highly resistant to vancomycin and teicoplanin and VanB shows variable resistance to vancomycin only. The most common forms of acquired glycopeptide resistance phenotypes are transferable via plasmids or transposons [69].VanA phenotype is found in 60% and VanB found in 40% of VRE [70]. The genes conferring the VanA phenotype and transferred from E.faecium to MRSA [67].

Sugar Metabolism and Identification of the genus

In clinical laboratories enterococci which act as a disease causing agents isolated from hospital patients and identified to the genus and species level. Classical culture and biochemical techniques were used by clinical microbiologists to identify the enterococci to the genus level. Enterococci, being fastidious organisms,grow readily on nonselective media such as blood agar and chocolate agar, but "enriched sheep blood agar" enhances their growth [71,72]. After 24 h of incubation on blood agar, depending on the ability to partially or fully destroy red blood cells which appear as non-hemolytic, or may present as α-hemolytic, or, rarely, β-hemolytic [71].The microscopic structure of the enterococci in the gram stain is gram positive, ovoid shape and arranged in pairs and short chains [71,72]. As enterococcus and streptococcus both have similar morphologies, cannot be separated by normal microscopic examination [71]. Like the streptococci, enterococci are catalase-negative [73].Enterococci are different from streptococci by their ability to survive and grow in high salt concentrations(6.5% NaCl), proliferate within a broad temperature range (5–50°C) detected by heat sensitivity test , withstand extremes of pH(4.8 to 9.6), and resist bile salts [73,74,71,72]. Group D antigen present in the cell wall of enterococci reacts with Lancefield group D antiserum, but several streptococcal species are also known to react positively [76]. Enterococci are resistant to optochin that do not dissolve in bile and produce L-pyrrolidonylarylamidase(PYR) [73,71,72]. The enterococci also reveal positive reactions in the β-glucosidase and leucinearylamidase tests [75]. The ability to utilize hexose and pentose carbohydrates is a key component of metabolism for a majority of the eubacteria. The two members of the genus Enterococcus are utilized over 30 more species. At least 13 sugars are metabolized by all Enterococcus species. Enterococcus researchers help in the genomic sequencing of additional carbohydrates that have the potential to be metabolized by this genus. Carbohydrate sources include not only a diverse array of monomers but also many naturally abundant carbohydrate polymers. The capsular polysaccharide of Enterococcus faecalis and its relationship to other polysaccharides in the cell wall.

The challenge for clinical microbiology

In both animals and plants ubiquitous organisms enterococci can colonize .They grow in the intestinal tract of humans and other animals, are flourishing in hospitals and communities, and are even present in the waters and sands of the world's beaches, such as the coastline ofSouthern California, where sewage contamination and river runoff have increased their numbers [77].In developing countries for nosocomial infections lack a quality healthcare infrastructure. Different strains of Enterococcus species will be differentiated in the diagnostic laboratories. With the help of classical culture and basic biochemical methods enterococcus can be differentiated from other bacterial genera [78,79].A costly, time consuming procedures are nucleic acid or protein molecular techniques required to differentiate species within the genus [80,81].In developing countries hospital acquired infections have become a leading cause of death. The cost of molecular tests for developing nations is cited as the primary reason for their absence [82,83]. In the developing world HIV/AIDS and tuberculosis has overwhelmed many resources. In most of the literature symptoms of these two diseases have been published. When one observes the situation for bacterial induced diseases, one finds many alarming trends: (1) antimicrobial resistant bacteria, such as enterococci, are on the rise in both hospitals and communities within the developing world, (2) developing world hospitals have less than basic clinical microbiology facilities, (3) developing world hospitals lack the trained staff to perform molecular testing and even basic classical culture and analysis (such as the Gram stain procedure), and (4)developing world hospitals lack adequate infection control programs and surveillance [82,83]. This investment is cost effective in the identification of the enterococci in the species and genus level.The results from classical culture tests aid the clinical diagonostic scientist help the clinical microbiologist to treat enterococcus infection in an inexpensive manner.The biomedical research community must be realistic in its expectations ofwhat technologies and procedures for identification and speciation of the enterococci can feasibly be adopted in developing world hospitals, especially given that most of the budget in these hospitals is earmarked for HIV/AIDS,malaria, and tuberculosis. Identification of Enterococcus species is necessary to select antibiotics to treat patients and cure patients infection. To acquire such data antibiogram diagnostic testing is necessary. Unfortunately, wealthy donor nations and world health governing bodies have not recognized basic clinical microbiology as a priority.In these developing nations there is a lackof quality clinical microbiologists. To train their clinical staff these nations acquire assistance from wealthy donor nations. In United States and other nations clinical microbiology is also dwindling due to a variety of factors. Consequently, basicsurveillance of the enterococci and other disease causingbacteria is presently inadequate and could lead to the inabilityof hospitals to properly diagnose their patients and treat themeffectively with the correct antibiotics in such a way as toprevent the further emergence of antibiotic-resistant pathogens [84,85].In developing world governments focused oncurbing HIV, malaria and tuberculosis. Nevertheless, the enterococci and other bacteria must also be addressed. Quality clinical microbiology laboratories must be established to train the staffs .There is also necessary to treat hospital infections caused by enterococci. Rapid development, cost effective, high-throughput and technically feasible diagnostic methods used to identify and speciate the enterococci. These methods mustbe successful, but simple and cost effective enough to be adopted by developing world hospitals, which presently do not have access to the more expensive and complicated molecular technologies with respect to bacteria. Finally,extensive surveillance needs to be continually carried outwith the enterococci, and, indeed, all bacterial genera tomonitor the emergence of antibiotic resistance and theprevalence of the enterococci in hospitals and communities,and so curb the future threat of the enterococci in nationseverywhere.

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