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Review Article

Enterococcus: Review of its Pathogenesis, Antibiotic Resistance, and Treatment

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Abstract:

Enterococci are among the normal human microbiota inhabiting the gastrointestinal tract. However, they can translocate into blood causing infections. Many **Enterococci** species are identified, with **E. faecium** and **E. faecalis** being the most isolated species causing human infections. Both inherent and acquired anti-microbial resistance among **Enterococci** play a great role in the development and spread of MDR strains. This indicates a major challenge in treating enterococcal infections with empirical regimens. Also, the high level of resistance may be attributed to the excessive of these antibiotics for Gram positive bacterial infections in our locality. **Enterococci** are known to be biofilm producers. Biofilm production occurs in a dynamic process. Adherence of bacterial cells to specific surfaces, such as, medical devices, dead tissues, and catheters, is considered the first crucial step in biofilm production. Biofilm production aids to the virulence and pathogenicity of **Enterococci.** Biofilm production exhibits antibiotic resistance and failure of antibodies killing function. Our aim of this review is to demonstrate the characteristics of **Enterococcus** as regarding virulence factors, antibiotic Resistance, pathogenesis, and recent possible treatment.

Key words: Enterococci, Antibiotic resistance, biofilm production,

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Introduction:

Enterococci are among the normal human microbiota inhabiting the gastrointestinal tract. However, they can translocate into blood causing infections.(1) On the WHO's list of priority pathogens, **Enterococci** are frequently seen in hospital-acquired infections and are growing more important as a result of strains of the infection becoming resistant to several treatments.(2)

Enterococci are non-spore-forming bacteria. They can proliferate under a variety of diverse conditions. They are facultative anaerobe and are considered as lactic acid bacteria. (3) They can survive high salt concentrations; 6.5% Sodium Chloride (NaCl), in contrast to **Streptococci**. They can withstand extremes of temperature. They can grow at as low as 5°C, as high as 50°C. They can survive at 60°C for as long as 30 min. The optimum growth temperature ranges between 37 and 42.7°C. They can withstand pH extremes. The optimum pH for their growth is pH 7.5.(4)

Enterococci, belonging to the phylum **Firmicutes,** represents a natural element of the human microbiota. The human gut contains roughly 10^6 to 10^7 **Enterococcus** (up to 1% in the colon, 1% in the ileum,). Most are **E. faecalis** and **E. faecium** with **E. durans**, **E. hirae**, **E. avium**, **E. cecorum, E. casseliflavus**, and **E. gallinarum** to a lesser extent.(5)

Enterococci presence in the gastrointestinal tract is mainly limited to the ileum and the colon. Intestinal epithelial cells and Paneth cells secrete lectin Regenerating islet-derived protein III gamma (REGIIIγ) protein in response to heavy load of Gram-negative bacteria. Gram negative bacteria microorganism-associated molecular patterns (MAMPs), such as the lipopolysaccharide of the outer-membrane (within the intestinal lumen) and flagellin (within subepithelial tissues), are recognized by pattern recognition receptors (PRRs) such as Toll like receptors (TLR); TLR4 and TLR5, respectively.

Lectin REGIIIγ in turn blocks Gram positive bacteria intestinal colonization. However, massive antibiotic therapy can reduce the Gram-negative bacteria load , and thus the lectin REGIIIγ allowing massive colonization with Gram positive bacteria such as **Enterococci** especially the drug resistant strains such as VRE **(Fig. 1).**(6)

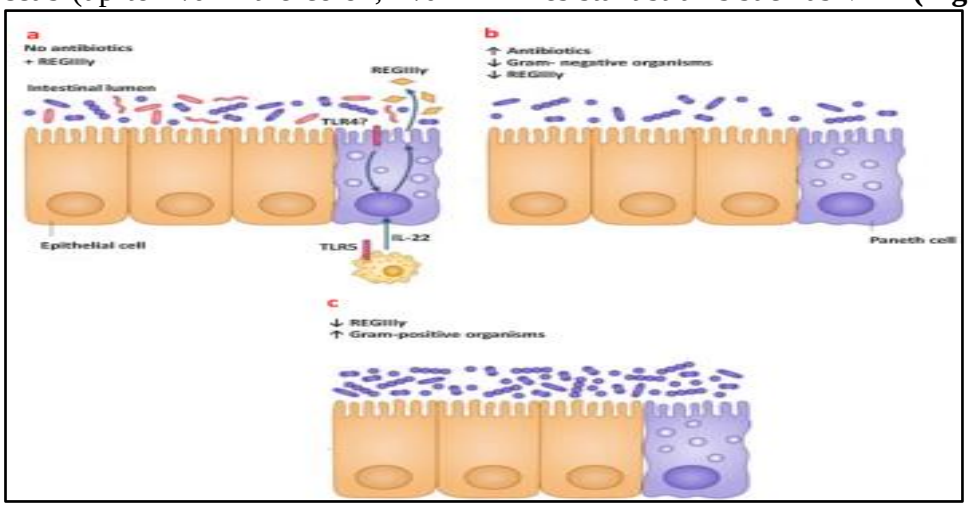

Figure (1): Effects of antibiotics on the gastrointestinal microbiota and VRE emergence.(7)

Enterococci are able to invade the extra intestinal regions, and reach blood, and lymphatics initiating human infections, so they can shift from commensals to pathogens.(8)

Virulence Factors:

Externally secreted factors:

1-Cytolysin:

Cytolysin is one of the secreted virulence factors that is expressed mainly by many ***E.*** **faecalis** strains. It's linked to human diseases, and increased patient morbidity and mortality. It's a hemolytic virulence factor and is also considered one of the bacteriocins as it exhibits lytic activity for both mammalian cells as human erythrocytes, polymorph nuclear leukocytes, retinal cells, and intestinal epithelial cells, and a widespread diversity of Gram-positive bacteria, including

lactobacillales, Clostridia, and **Staphylococci.**(9)

2-Gelatinase:

Gelatinase is an extracellular secreted bacterial Zinc-metallo-endopeptidase that breaks down many substances including collagen, gelatin, cas-

ein, and hemoglobin. [10] It also has a role in biofilm production, bacterial colonization, and persistence in some infection sites .[11] Gelatinase gives **E. faecalis** the ability to hydrolyze gelatin, hemoglobin, casein, collagen, and other peptides. It helps enterococcal evasion form innate immune response.[12]

3-Secreted antigen A:

Secreted antigen A is exclusively found in **E. faecium**. It has a role in cell growth, conceivably because of its interactions with the cell wall metabolism. It also binds to a range of extracellular matrix proteins and is associated with biofilm formation.[13]

4-Serine protease:

Serine protease is an extracellular secreted endopeptidase. It regulates bacterial autolysis and extracellular release of DNA. It also plays a role in biofilm production by **Enterococci**. It has been recognized to be related to enterococcal infections such as peritonitis, endophthalmitis, endocarditis, and orthopedic implant infections.[14]

5-Glycosyl hydrolase:

Glycosyl hydrolase is a hyaluronic acid degradative enzyme that breaks down hyaluronic acid increasing connective tissues permeability, thus, allowing spread of **Enterococci** through host tissues. It's encoded by a chromosomal gene called hyl. [15]

Cell-surface associated factors:

1-Enterococcal surface protein:

Enterococcal surface protein (Esp) is a surface protein with a high molecular weight. It's related to enterococcal endocarditis, bacteremia, and UTIs. It helps enterococcal colonization, evasion from host immune response, adherence to surfaces, and also biofilm formation. Enterococcal strains with Esp show higher antibiotic resistance to ampicillin and ciprofloxacin than those without Esp. [12]

2-Aggregation substances:

Aggregation substances are hair like glycoproteins that are located on the bacterial surfaces. Expression of aggregation substances leads to bacterial clumping and biofilm formation. Aggregation substances help **Enterococci** adherence to other bacterial cells, and to eukaryotic cells. They also reduce the superoxide function. [16]

Aggregation substances are a group of proteins encoded by plasmid on a gene called asa1 gene. Aggregation substances play a role in conjugation assisting transfer of plasmid carrying virulence traits, and also antibiotic resistance genes. Aggregation substances are also included in eukaryotic cell binding.[17]

1-Microbial Surface Components Recognizing Adhesive Matrix Molecules:

They are a subfamily of adhesins secreted by **Enterococci** and are attracted to specific proteins in human tissues.

1-Biofilm production

It has been reported that more than 80% of nosocomial infections caused by ***Enterococci*** are due to biofilm producing strains. The high prevalence is among urinary catheter associated infections, endocarditis, central venous catheter associated bacteremia. [18] Biofilm is defined as a population of mono or poly microbial aggregateions of cells that are irreversibly attached to each other, and to a living or non-living surfaces surrounded by a matrix of extracellular polymeric substances which are self-produced by the bacterial cells. [19]

The frequency of **E. faecalis** biofilm formation in UTIs isolates has been reported to be fifty-point four percent at Shenzhen Nanshan Hospital in China. [20]

Biofilms affect almost every aspect of human life, including energy consumption, equipment deterioration, infections, contaminated products, public health, and industrial issues. [21]

Biofilm associated infections is a great challenge to human health. It is considered a major threat of modern medicine due to difficulty of antibiotic penetration and thus eradication. [22]

In comparison to non-biofilm producing bacteria, **E. faecalis** biofilms are 1000 times more resistant to phagocytosis, antibodies, and antimicrobial agents. [23]

Antibiotic Resistance:

Enterococci infections treatment is a great challenge; owing to the high rate of antibiotics resistance of **Enterococci**. **Enterococci** are known with their capability of easily acquiring and sharing antimicrobial resistance. [24]

Intrinsic resistance is due to genetic determinants encoded within the core genome of all members

of a bacterial genus. A phenomenon called antibiotic tolerance, is defined as tolerance to antibiotic concentrations near their minimum inhibitory concentrations (MICs), is considered a form of intrinsic resistance. [25]

While, acquired resistance is due to acquisition of new genetic materials creating hybrid genomes among other **Enterococci**, and genes transfer across species through horizontal gene transfer, or through sporadic mutations to intrinsic genes. Horizontal gene transfer is defined as spread of genetic materials through plasmids and transposons. Acquired resistance is present only in some members of a bacterial genus. [26]

Glycopeptide resistance:

Glycopeptide antibiotics include vancomycin, teicoplanin, and newer derivatives. Vancomycin was considered an invincible antibiotic for a long time. It remained active against **E. faecium** and **E. faecalis** for almost three decades after its clinical introduction in the market. [27]

In the early 1980s, In USA, Ampicillin resistant **E. faecium** was observed. After that, in the 1990s, emergence of VRE was reported. While in Europe, the emergence of VRE was reported in 1986.[28]

Macrolides are used mainly to treat Gram-positive bacterial serious infections. The main mechanism of action of these antibiotics is interference with peptidoglycan layer synthesis. Peptidoglycan precursors ending in D-Ala-D-Ala peptide are incorporated by trans glycosylation and transpeptidation to strengthen the cell wall. Glycopeptide antibiotics bind with high affinity to the D-Ala-D-Ala peptide inhibiting peptidoglycan synthesis and thus impair the cell wall integrity.[29]

Enterococcal glycopeptide resistant strains exchange the D ala peptide ending for D-lac or D-Ser. These two substitutions have a much poorer affinity to glycopeptides compared with the D-ala (~1000-fold reduction for D-Ala-D-lac; ~7 fold for D-Ala-D-Ser). Therefore, the antibiotic can't bind onto peptidoglycan. [30]

Vancomycin resistance operon (Van) provides **Enterococcus** spp. resistance to glycopeptide antibiotics. This operon can be carried on mobile genetic elements. Certain glycopeptide resistance types are encoded as a part of certain enterococcal species genome. [31]

Aminoglycosides resistance:

Aminoglycosides antibiotics, such as gentamicin and streptomycin inhibit the bacterial protein synthesis. **Enterococci** are intrinsically resistant to Aminoglycosides antibiotics because their cell wall is naturally impermeable for aminoglycosides. [30]

Enterococci have intrinsic low-level resistance to Aminoglycosides with MICs ranging from 4 μg/mL to 256 μg/mL. While **Enterococci** growth at concentrations of 500 mg/L and 2000 mg/L of Gentamicin and Streptomycin, respectively, on brain heart infusion agar is defined as high level Aminoglycosides resistance (HLAR). HLAR is predominantly due to acquired genes encoding the aminoglycoside modifying enzymes (AMEs) such as aminoglycoside phosphoryl transferase (APH), aminoglycoside nucleotidyl transferase (ANT), and aminoglycoside acetyl transferase (AAC). The action of these enzymes reduces the synergistic activity of Gentamicin when combined with Ampicillin or Vancomycin. [32]

Aminoglycosides only are ineffective in enterococcal infections treatment, so they are mostly prescribed in combinations with cell wall synthesis inhibitors. [33]

The use of an antibiotic agent that inhibits the cell wall synthesis, such as β-lactam antibiotics, ampicillin, or vancomycin weakens the enterococcal cell wall allowing Aminoglycosides to enter the cells and exert their action. Here, the effect of these agents and Aminoglycosides together is higher than the sum of their effects individually; this is called (synergy). [30]

Erythromycin (Macrolides) resistance:

Macrolides are a group of antibiotics including, Erythromycin, Tylosin, and Azithromycin. Erythromycin is specially used as a therapeutic agent against enterococcal infections. Macrolides bind to the 50s subunits in the bacterial ribosomal 23S rRNA, thus, inhibiting bacterial protein synthesis.[34]

Erythromycin resistance gene; ermB is the most frequent erm gene in Erythromycin resistant **Enterococci** isolates. ermB gene encodes for the ribosomal RNA methylase that leads to Methylation of 23S rRNA hindering macrolides ribosomal binding. [35]

Daptomycin resistance:

Daptomycin causes alteration of the bacterial cell membrane function and characters. It binds with the bacterial cell membrane in presence of Ca^{+2} ions. When daptomycin interact with phosphateidylglycerol component of the bacterial cell membrane, it aggregates and enters through the cell membrane to the inside of the cell. Therefore, pores are formed on the cell membrane leaking ions depolarizing the cell membrane. (27)

Linezolid resistance:

Owing to its great pharmacodynamics and pharmacokinetic characters, Linezolid is widely overused in clinical practices in both developed and developing countries. (36)

Linezolid is mainly active against Gram positive bacteria such as **Staphylococci**, **Enterococci,** and **Streptococci**. Gram negative bacteria show intrinsic resistance to Linezolid. It's recommended in treatment of severe infections caused by these microorganisms such as nosocomial acquired pneumonia, bloodstream infections, skin and soft tissue infections especially those caused by MDR strains that showed therapeutic failure by other drugs. (37)

Linezolid is considered the last line of treatment in infections caused by Vancomycin-resistant **E. faecium** (VRE*fm*), Methicillin- resistant **Staphylococcus aureus** (MRSA) and Methicillin-resistant coagulase-negative **Staphylococci** (MRCNS) .(38)

Plasmid-born poxtA gene that leads to ribosomal protection was recently reported in **Enterococci.** (39)

Nowadays, one of the most challenging problems is the emergence of **Enterococci** resistant to Linezolid, which is the main antibiotic that can be used to treat Vancomycin resistant enterococcal infections. (40)

Enterococcal Infections:

Several retrospective studies have reported that the crude mortality rates for enterococcal bacteriemia are 13% to 68%. In the first wave of enterococcal infections it was reported that 90% of the overall cases was attributed to **E. faecalis**, while the remaining 10% was attributed to **E. faecium**. Currently, the remarkable resistance of **E. faecium** to several antibiotics including vancomycin, ampicillin, and aminoglycoside makes **E. faecium** one of the most common species isolated from enterococcal infections representing about 40% of all enterococcal infections. (41)

Enterococcal infections are commonly polymicrobial with poor prognosis and increased morbidity and mortality .(42)

Polymicrobial Enterococcal infections may be attributed to that **Enterococci** facilitate other pathogenic bacterial colonization and persistence. These polymicrobial enterococcal infections are difficult to be treated, and eradicated. Recently, researchers observed that biofilm formation, metabolic cross feeding; which is defined as "nutrients transfer among microorganisms", and virulence enhancement by **Enterococci** enhance the co-infection with **Staphylococcus aureus**, **E. coli**, and **Clostridioides difficile** .(43)

Urinary tract infections:

Enterococcal nosocomial UTIs are most commonly complicated UTIs, such as, perinephric abscess, pyelonephritis, and prostatitis. These complications are mainly related to urinary tract congenital anomalies, urinary catheters and prolonged antibiotic intake.

Moreover, high incidence of VRE colonizes the urinary tract, causing uncomplicated UTIs or asymptomatic bacteriuria, and is highly associated with increased patient morbidity and mortality. (44)

Endocarditis:

Enterococci are known to be the second most common cause of infective endocarditis. Vancomycin resistant **E. faecalis** endocarditis causes gastrointestinal or genitourinary manipulation, liver transplantation, damaged mitral valve infections, or aortic valve infections. Moreover, Vancomycin resistant **E. faecium** endocarditis is associated with tricuspid valve infections .(45)

E. faecalis also causes community acquired endocarditis with fever or a new murmur as the most prominent signs of infection.

Enterococcal blood stream infections:

Enterococcal blood stream infections are mainly explained by **Enterococci** translocation from the gut into the bloodstream. Other routes of infection include endocarditis, UTIs, intravenous lines, and other abscesses. Mortality due to enterococcal blood stream infections is related to the severity of disease (based on APACHE II scores), age of the patient, and use of third-generation Cephalosporins, or Metronidazole. (46)

Oral cavity infections:

E. faecalis is commonly isolated from root canals infections. It's considered one of the causative agents of endodontic failed treatments. (47)

As an opportunistic pathogen, **E. faecalis** causes a variety of infections in the oral cavity, including root canal infections, dental caries, primary endodontic infections, marginal, periodontitis, persistent/secondary infections, peri-implantitis, peri radicular abscesses, and oral mucosal lesions. (48)

Intra-abdominal and pelvic infections:

Several intra-abdominal and pelvic infections, including abscesses wounds or peritonitis were found to be caused by VRE. Mostly it is a part of polymicrobial infection associated with other Gram negative or anaerobic organisms. Often the strains causing these are the patients' own intestinal flora. Also, enterococcal monomicrobial peritonitis is identified specifically in patients with liver cirrhosis or chronic peritoneal dialysis. (49)

Skin and skin structure infections:

Mostly skin and skin structure infections are polymicrobial infections. **Enterococci** are considered part of these microbes. (50)

Enterococcal Infections Treatment:

Intra-abdominal enterococcal infections:

Empirical coverage of **Enterococci** is not recommended, unless, detected in cases with peritonitis, immunosuppressed patients, cases with severe sepsis, and in cases with persistent collections. (24)

Ampicillin can be used. For Ampicillin resistant **Enterococci**; Vancomycin or Teicoplanin can be used. While, for VRE, treatment options include Daptomycin and Linezolid. (51)

Infective endocarditis:

The main line of treatment is Ampicillin combined with Gentamycin or Vancomycin combined with Gentamycin.

Also, combinations of Ampicillin and Ceftriaxone are effective against (HLAR) ***Enterococci*** strains. (52) Daptomycin combinations with β lactams is also an effective treatment option. (51)

Enterococcal blood stream infections:

The most common **Enterococcus** species isolated from patients with enterococcal blood stream infections is **E. faecium**. Daptomycin and Linezolid are the first line treatment options for VRE, with device removal and metastatic infection identification. (53)

Enterococcal central nervous system infections:

For VR***E*****. faecium** CNS infections treatment, Linezolid is safe and effective, but, with considerations of drug monitoring with prolonged treatment period. Intrathecal or intraventricular administration of Daptomycin may be also effective. (54)

Enterococcal urinary tract infections:

Ampicillin, being highly concentrated in urine, is an effective therapeutic option for Enterococcal UTIs. For treatment of uncomplicated cases; Amoxicillin, Nitrofurantoin, or Fosfomycin are effecttive. If the isolated strains are not sensitive; Ampicillin, Vancomycin, Daptomycin, Fluoroquinolones, and Oxazolidinones can be used. For treatment of complicated cases; intravenous Ampicillin, Oxazolidinones, Fluoroquinolones, Vancomycin, or Daptomycin are recommended. For treatment of severe infections, Ampicillin combinations with Streptomycin or Gentamicin are recommended. (26)

Enterococcal Infections Prevention and Control:

Infection prevention and control strategies that help reduce the spread and occurrence of VRE:

- ✓ Vancomycin judicious usage.
- ✓ Antibiotic stewardship programs limiting Vancomycin and broad-spectrum antibiotics use play a crucial role in prevention of the spread and occurrence of VRE.
- ✓ Cleaning measures to decontaminate the environment, such as, routine Chlorhexidine bathing in ICU and the non-touch automated mobile ultraviolet units, also had a significant impact in preventing VRE spread and acquisition in hospitals. (55)
- ✓ Hospital staff education programs, focusing on the epidemiology of VRE and its implication on health.
- ✓ Defining the role of hospital microbiology labs for identification of **Enterococci** and detection of vancomycin resistance.
- ✓ Contact isolation of VRE positive patients .(56)
- ✓ Hand hygiene measures which showed a significant impact in preventing VRE spread. Hand hygiene is associated with a 47% reduction of VRE hospital acquisition.

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