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Short Review

A Short Review on *Enterococcus faecalis*

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Abstract. The gastrointestinal tract gets colonized during the first two years of life and after this period, gut microbiota either stabilize or fluctuate throughout life. *Enterococcus faecalis* is one of the species found in gut and is responsible for various infections and colorectal cancer. Bacterium *E. faecalis* can easily be diagnosed in the laboratory, as it is gram-positive, present in pairs and short chains. Antimicrobial therapy can be used to treat enterococcal infections but its excessive use has led to the emergence of vancomycin-resistant *Enterococcus*. Enterococcal infections are usually nosocomial and can be reduced by taking preventive measures. The purpose of this study is to review all the aspects of *E. faecalis*, to create awareness to control Enterococcal infections and to promote the discovery of new drugs against the resistant *E. faecalis*.

Keywords: *Enterococcus faecalis*, nosocomial infection, enterococcus, enterococcal infections, anaerobes, gram-positive bacteria

Introduction

The collection of bacteria, archaea and eukaryotes colonizing the gastro-intestinal (GI) tract is called gut microbiota and has co-evolved with the host over thousands of years to form an intricate and mutually beneficial relationship also known as symbiotic relationship (Thursby and Juge, 2017). The GI tract gets colonized during the first two years of life and after this period, gut microbiota either stabilize or fluctuate throughout life (Dethlefsen *et al.*, 2006).

The human colonic microbiota is a large and complex microbial community. This can be gauged from the fact that in total, over 1000 bacterial species have been identified of which many remain uncultured, with about 160 species being found in the gut of any individual. The gene set of the gut microbiota (the gut microbiome) is estimated to be about 3 million genes-150 times larger than that of the human genome (Rowland *et al.*, 2018)

Several nutrients that are not digested by the human body, such as cellulose, resistant starch and some of the endogenous products like mucins, etc. which are some of the good sources of energy for microbiota. In 1955, a term (Prebiotics) was coined by Glenn Gibson and Marcel Roberfroid for such nutrients. According to these two researchers, "Prebiotic" is described as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health. However, in 2008, the 6th meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) defined "Dietary Prebiotics" as a selectively fermented ingredient that results in specific changes in the composition or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health (Davani-Davari *et al.*, 2019; Flint *et al.*, 2007).

There are several types of prebiotics, but the most essential ones are Fructans (Insulin and Fructo-oligosaccharide or oligofructose) (FOS), Galacto-oligosaccharides (GOS), Starch and Glucose derived Oligosaccharides and other oligosaccharides (originated from pectin a polysaccharide) (Davani-Davari *et al*., 2019).

Prebiotics help bacteria in the growth and formation of biofilm in the intestine (Flint *et al*., 2007). Biofilms are

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bacterial growths attached to a surface and encased in a matrix that may be composed of carbohydrates, DNA, or protein (Tendolkar *et al*., 2004). In biofilms, the bacteria are lodged in the matrix, especially extracellular. The extracellular matrix protects bacteria from the human immune system and also from antibacterial/ antimicrobial treatments. It also gives structural integrity, enhances the absorption of nutrients and helps the extracellular enzymes to stay close to the cells (Taglialegna *et al*., 2020).

The intestinal microbiota is heterogeneous, meaning it is composed of several different species of bacteria (Sekirov *et al*., 2010). These bacterial species are not only present in the lumen but some are also present in the mucus layer and epithelial crypts of the intestine. Like, the species of *Bacteroides*, *Bifidobacterium, Streptococcus* are the members of *Enterobacteriaceae, Enterococcus, Clostridium, Lactobacillus* and *Ruminococcus* which present in the lumen of intestine. Whereas, the species of *Enterococcus* are observed in both the mucus layer and epithelial crypts of the small intestine (Dieterich *et al*., 2018; Swidsinski *et al*., 2005).

Normal microbiota consists of many different species of bacteria. Usually, the majority are harmless, nonetheless, if some move from the intestine to any other part of the body, these can cause several adverse ailments, ranging from different kinds of infections to cancers. One of these is the genus *Enterococcus*, the spread of which can cause different infections ranging from periodontitis to urinary tract infection (Vu and Carvalho, 2011; de W. Blackburn and McClure, 2009).

Enterococci are gram-positive bacteria and facultative anaerobes that resemble streptococci (Malani *et al*., 2002). *Enterococcus* species can not only be isolated from the GI tract but also the oropharynx, genital tract of females and skin. Till now, fifty-eight different species of the *Enterococcus* genus which have been discovered (Teixeira *et al*., 2019). The *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum* and *Enterococcus casseliflavus* are of human concern (de Blackburn and McClure, 2009; Reid *et al.*, 2001; De Baere *et al.*, 2000; Hsueh *et al.*, 2000). However, *Enterococcus gallinarum* and *Enterococcus casseliflavus* have also been studied because they are inherently vancomycin-resistant and part of the GI tract (Murray *et al*., 2020; Reid *et al*., 2001).

The bacterium *Enterococcus faecalis* was previously considered as the D group of *Streptococcus* species. However, in 1984, this bacterium (*E. faecalis)* gained its genus i.e., *Enterococcus*, *Streptococcus faecalis* and *Streptococcus faecium* were the first species to be transferred to the new genus as *Enterococcus faecalis* (the type species) and *Enterococcus faecium*, respectively. Subsequently, other earlier *Streptococcal* species and sub species were transferred and received new denominations as members of the genus *Enterococcus*. The nucleic acid hybridization of this bacterium was considered as the basis for the generation of a new genus (Ryan *et al*., 2022; Fiore *et al*., 2019). Further, the continuous use of molecular approaches has allowed major developments in the classification of enterococci, resulting in the recognition of 58 *Enterococcal* species (Said *et al*., 2022; Teixeira *et al*., 2019).

The enterococci are commensal micro-organisms that act as opportunistic agents causing a variety of infections in humans such as urinary tract infection, endocarditis, periodontitis, bacteremia and lastly wound infection. Many of these infections have been suggested to arise from translocation of the Enterococcal cells from their major site of colonization in the GI tract. Moreover, this bacterium is also involved in the incidence of colorectal cancer. Unhygienic practices are the root cause of enterococcal infections. However, the prevalence of these infections and colorectal cancer can be reduced by adopting preventive measures and spreading awareness. Government should also play its role by creating awareness programs and campaigns related to hygiene and healthy living habits (Teixeira *et al*., 2019).

This article explains all the aspects including epidemiology, clinical manifestation, diagnosis and treatment of *Enterococcus faecalis*. It focuses on virulence factors, drug resistance and pathogenicity of the bacterium. The current study is also designed to create awareness to control the prevalence of Enterococcal infections and to promote the discovery of new drugs against *E. faecalis.*

Epidemiology. Several intrinsic characteristics of the Enterococci allow them to grow and survive under harsh conditions and persist almost everywhere, colonizing several ecological niches. The prevalence of different *Enterococcus* species appears to vary according to the host and is also influenced by age, diet and other factors that may be related to changes in physiological conditions, such as underlying diseases or prior

antimicrobial therapy (Teixeira *et al*., 2019; Vos *et al*., 2011; Devriese *et al*., 2006).

As previously mentioned, *Enterococcus* species usually do not cause life-threatening situations like other gram-positive bacteria, still they are a part of most infections. The reason behind this is the translocation of the Enterococcal cells from their major site of colonization in the GI tract to other body organs (Teixeira *et al*., 2019).

Almost everyone is susceptible to Enterococcal infections but the elderly patients with serious underlying diseases and other severely ill immune-compromised patients who have been hospitalized for prolonged periods, have been treated with invasive devices and have received broad-spectrum antimicrobial therapy are at higher risk to acquire enterococcal infections (Arias and Murray, 2012).

Further, due to the excessive use of antibiotics, resistance is an emerging problem. *Enterococcus* species are a major complication, especially in patients who receive broad-spectrum antibiotics because of antibiotic resistance (Fridkin *et al*., 2001; Vergis *et al*., 2001). Nowadays, with the excessive use of antibiotics, *Enterococcus faecalis* has become vancomycin-resistant, and is known as vancomycin-resistant *Enterococcus* (VRE) (Byers *et al*., 2001; Falk *et al*., 2000). The very first case of vancomycin-resistant Enterococci was reported in 1988 (Toc *et al*., 2022). Additionally, resistance to vancomycin is an independent cause of mortality in patients who have bacteremia due to enterococci (DiazGranados *et al*., 2005).

It is suggested that the ratio of infected subjects with asymptomatic vancomycin-resistant Enterococci and GI tract colonization is higher than those who are clinically recognized with infection, i.e. 10:1 (Byers *et al*., 2001). Therefore, many undiagnosed patients are a source of transmission of vancomycin-resistant *Enterococcus*. Furthermore, there is a need for proper diagnosis and screening of such patients.

Admittance to CCU, disease severity, encountering the patients having vancomycin-resistant enterococci infection, length of stay at the hospital and lastly, the use of antibiotics such as vancomycin, cephalosporins, quinolones and some agents having anti-anaerobic activity are some of the risk factors associated with vancomycin-resistant *Enterococcus* (VRE) infection (Stiefel *et al*., 2004; Padiglione *et al*., 2003).

Pathogenesis and clinical significance. The pathogenesis (Fig. 1) of enterococcal species related infections and diseases is largely governed by their transmission and virulence factors. The two points are discussed in detail in the following sections.

Transmission. As stated earlier, *Enterococcus* species are the causative agents for several infections, especially nosocomial infections including urinary tract infection, endocarditis, periodontitis, bacteremia and wound infection. These infections can occur due to the spread of the bacteria from the intestine to other body parts. The route of transmission can be unhygienic practices of health care workers, use of infective catheters, sharing of personal items, etc. Further, colonized patients are at higher risk of developing Enterococcal infections. Moreover, they are also a source of transmission of bacteria to other people (Hughes, 2008).

Virulence factors. Enterococci are usually found to be less virulent than other related infections, especially in humans. Virulence factors of *Enterococcus faecalis* such as cytolysin "hemolysin", aggregation substance (AS) (aggregation substance proteins), gelatinase (GelE), serine protease (SprE), Enterococcal surface protein (Esp), Ace protein and enterococcal polysaccharide antigen have a pathogenic role. Besides these, extracellular superoxide is also thought to be a virulence factor (Higuita and Huycke, 2014; Vu and Carvalho, 2011; Chen and Zervos, 2009; Gilmore *et al*., 2002; Vergis *et al*., 2002).

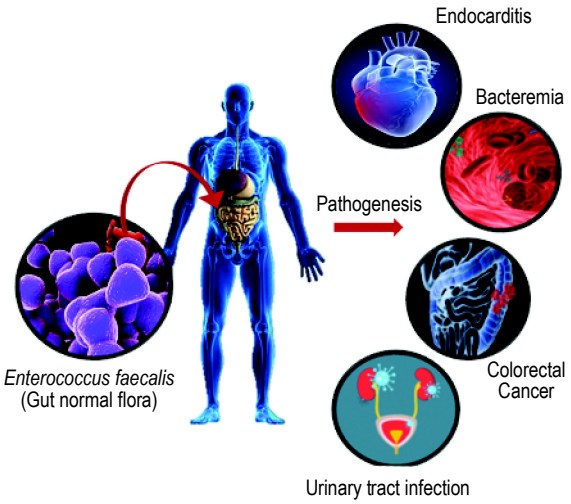

Fig. 1. Pathogenesis of *Enterococcus faecalis*.

Several studies reviewed the mechanisms behind these virulence factors (Table 1), which are summarized as follows:

- The bacterium *Enterococcus faecalis* produces a two-peptide cytolysin (a hemolytic protein) named bacteriocin. It helps in the colonization of bacterial species by inhibiting the growth of other gram-positive bacteria (Van Tyne *et al*., 2013).
- Aggregation substances. The mostly hair-like structures that are embedded in the primarily into the "old" parts of the cell wall or sometimes in cell membrane. These structures facilitate the attachment of bacterium (*E. faecalis*) to the host cell and enables cell-to-cell interaction between donor and recipient strains for conjugation. The sequencing of structural gene for the aggregation substance revealed the presence of conserved amino acid motif that enables the binding of *E. faecalis* to eukaryotic cells through integrins (a class of receptors) (Vu and Carvalho, 2011; Sartingen *et al*., 2000).
- Gelatinase and serine. These are two proteases that are involved in the virulence of the bacterium *Enterococcus faecalis*. These two, especially serine, are involved in bacterial autolysis and the formation of biofilm (Nešuta *et al*., 2017). The enzyme gelatinase is involved in the hydrolysis of gelatin and several other substances such as collagen, hemoglobin and casein.

Table 1. Virulence factors of *Enterococcus faecalis*

Virulence factors	Pathogenesis	References
Cytolysin	Colonization of enterococcal species and inhibition of other bacterial growth	(Van Tyne *et al.*, 2013)
Aggregation substances	Mediate the attachment to the host cell, cell to cell interaction	(Vu and Carvalho, 2011; Sartingen *et al.*, 2000)
Geletinase and serine	Causes autolysis and formation of biofilm	(Nešuta *et al.*, 2017; Vu and Carvalho, 2011)
Enterococcus surface protein	Ashesion, colonization, evasion of immune response, biofilm formation	(Ceci *et al.*, 2015; Tendolkar *et al.*, 2004)
Ace protein	Mediate the attachment to the host cell, cell to cell interaction	(Madsen *et al.,* 2017)
EPA	Biofilm formation, enterocyte translocation, antiphagocytosis and resistance of transduction by bacteriophages	(Vu and Carvalho, 2011; Sava *et al.*, 2010)
Superoxide	Hemolysis	(Vu and Carvalho, 2011)

Further, a study revealed that it is responsible for the increase in the severity of endocarditis in animals (Vu and Carvalho, 2011).

- Enterococcal surface protein. A gene product is a cell-wall-associated surface protein, which is linked to adhesion, colonization, evasion of immune response and biofilm formation and is believed to contribute to antimicrobial resistance (Ceci *et al*., 2015; Tendolkar *et al*., 2004).
- Ace protein. This is also known as Adhesion to collagen of *Enterococcus faecalis*, works almost in a similar way like aggregation substances, as this protein facilitates the adhesion of the bacteria to the cell wall (Madsen *et al*., 2017).
- All Enterococci produce a surface polysaccharide called EPA (enterococcal polysaccharide antigen) whose synthesis is encoded by the *Epa* locus. *Epa* locus also plays a role in the formation of biofilm like Esp. It is also involved in the enterocyte translocation, resistance to killing by PMNs (polymorphonuclear leukocytes) and resistance to infection by phages (Vu and Carvalho, 2011; Sava *et al*., 2010).
- Superoxide is produced by the bacterium that helps in the red blood cells lysis (Vu and Carvalho, 2011).

These virulence factors are generally involved in the adherence to the extracellular surfaces and biofilm formation as well as in the important processes in initiating colonization of bacteria and infections in the host (Sava *et al*., 2010). An investigation revealed that there are several factors involved in the determination of the virulence of *Enterococcus* species (Fisher and Phillips, 2009). These factors are mentioned as follow:

- Proficiency of the bacteria to colonize the GI tract, since the GI tract is usually the home for different bacterial species.
- The capability of the bacteria to stick to or attach with a variety of proteins present in the extracellular matrix, such as lactoferrin, thrombospondin and vitronectin.
- The ability of the bacteria to attach to the epithelium of the urinary tract and the oral cavity, as well as to the human embryonic kidney cells.

Generally, it is thought that Enterococcal infections are endogenous, mediated by the translocation of the bacteria via intestinal epithelial cells. After which they cause infection through lymph nodes, thus spreading to other cells of the body (Fisher and Phillips, 2009).

Enterococcus faecalis and *faecium* are usually the most frequent Enterococcus species isolated from the human

clinical specimens. However, the other less frequent enterococcal species causing enterococcal infections are *E. casseliflavus*, *E. gallinarum*, *E. raffinosus*, *E. avium*, *E. caccae*, *E. canintestini*, *E. cecorum*, *E. dispar*, *E. durans*, *E. gilvus*, *E. hawaiiensis*, *E. hirae*, *E. italicus*, *E. malodoratus*, *E. mundtii*, *E. pallens*, *E. pseudoavium* and *E. thailandicus* (Teixeira *et al*., 2019).

Clinical manifestations of *Enterococcus faecalis*. The genus *Enterococcus* is a diverse group of gram-positive bacteria that colonize the human gut. Their lifestyle ranges from being symbionts to nosocomial pathogens. This genus plays various roles in the food industry as they are used in the synthesis of probiotic products and in food production. Two species of this genus *Enterococcus faecalis* and *faecium* are of human concern because they play a major role in the colonization of the GI tract. Translocation of these two from the intestine can cause infection (Higuita and Huycke, 2014; Palmer *et al*., 2012; Neish, 2009; Aarestrup *et al*., 2002; Tannock and Cook, 2002).

Besides being a part of normal microflora, *Enterococcus faecalis* is also responsible for different infections such as urinary tract infection, endocarditis, periodontitis, bacteremia and lastly wound infection that are usually nosocomial, i.e., hospital acquired. It is also involved in the occurrence of colorectal cancer.

Fever, chills, fatigue, headaches, abdominal pain, pain or burning sensation, while urination, nausea, vomiting, sometimes diarrhea, chest pain, while breathing, neck stiffness, swollen and bleeding gums are some of the indicators of *Enterococcal* infections. Fortunately, these ailments caused by *Enterococcus faecalis* are curable and can be prevented by adopting several preventive measures. These preventive strategies/measures are discussed later in this paper. Following section explains the diseases caused by *E. faecalis* and their prevalence which are summarized in Table 2.

Enterococcal Infections. The *Enterococcus* genus is involved in the causation of a variety of infections in the human body and the term coined for these infections is "Enterococcal infections". The most common Enterococcal infections are urinary tract infections, bacteremia and endocarditis. Endocarditis, the most severe infection caused by *Enterococcus faecalis*, contributes upto 3 percent of the total enterococci bacteremia (Vergis *et al*., 2002). Enterococcal species are also found in several other infections, such as intra abdominal infections, pelvic infections, wounds and infections of the soft tissues. However, the above mentioned infections are usually part of the mixed infection caused by both aerobic and anaerobic bacteria. Fewer common infections caused by *Enterococcal* species are meningitis, osteomyelitis and septic arthritis.

Enterococcal Infection. *Urinary Tract Infection*. Urinary tract infections (UTIs) are considered as the most abundant bacterial infections commonly encountered in hospital environments. According to an estimate, UTIs are responsible for approximately 40% of the hospital acquired infections (Hughes, 2008). *Enterococcus faecalis (E. faecalis)* has been recognized as the third-most important uropathogen responsible for intermittent and chronic UTIs among intensive care unit (ICU) patients (Shankar *et al*., 2001). Additionally, as per the Centers for Disease Control and Prevention (CDC) national healthcare safety network, it is reported that Enterococci are the 2nd most common reason for urinary tract infection associated with the catheter, i.e., catheter-associated urinary tract infection (CAUTI) (Garsin *et al*., 2014; Hidron *et al*., 2008).

Enterococcal Infection. *Bacteremia*. Bacteremia is the presence of bacteria in the bloodstream. It can occur spontaneously, during certain tissue infections, with the use of indwelling genitourinary or IV catheters or after dental, gastrointestinal, genitourinary, wound care or other procedures. However, Enterococcal bacteremia usually arises from the urinary tract or an intra-abdominal focus of infection and is frequently associated with metastatic abscesses in multiple organs and high

Table 2. Prevalence of *Enterococcus faecalis* diseases

Diseases	Prevalence	References
Urinary tract infection	Approximately, 40% of the hospital-acquired infections of UTI have been reported caused by *E. faecalis*.	(Hughes, 2008)
Bacteremia	Hospital-acquired bacteremia due to *Enterococcus faecalis* (80.8%) is the most prevalent type of infection in comparison to community-acquired (6.7%) and the health care-associated one (12.3%).	(Jafari *et al.*, 2022)
Endocarditis	It is estimated that about 20% of the cases of native valve bacterial endocarditis and about 6 to 7% of prosthetic valve endocarditis cases are due to *E. faecalis*.	(Teixeira *et al.*, 2019)
Colorectal cancer	*E. faecalis* is responsible for 63-81% of cases, and malignancy is the most common comorbidity.	(Khan *et al.*, 2018)

mortality rates (Teixeira *et al*., 2019). Hospital-acquired bacteremia due to *Enterococcus faecalis* (80.8%) is the most prevalent type of infection in comparison to community acquired (6.7%) and the health care associated one (12.3%) (Jafari *et al*., 2022). Bacteremia may cause metastatic infections, including endocarditis, especially in patients with valvular heart abnormalities.

Enterococcal Infection. *Endocarditis*. Enterococci are the third most common cause of endocarditis which is an Enterococcal infection, is caused by *Enterococcus faecalis* and *Enterococcus faecium* (Murdoch *et al*., 2009). Endocarditis is the deadliest of all infections, whereby, the bacteria are involved in the biofilm formation at the place of damage, on the valves of the heart (Garsin *et al*., 2014). Furthermore, Enterococci have also been considered as an important cause of infective endocarditis and are estimated to account for about 20% of the cases of native valve bacterial endocarditis and about 6 to 7% of prosthetic valve endocarditis cases. Endocarditis remains one of the most difficult *Enterococcal* infections to treat because of limitations in the use of bactericidal antimicrobial therapy for Enterococcal infections, especially when caused by vancomycin-resistant enterococci (VRE) (Teixeira *et al*., 2019).

Colorectal cancer. *Enterococcus faecalis*, a member of phylum *Firmicutes* member, is every so often used as a probiotic product (Gong *et al*., 2017; Fisher and Phillips, 2009). However, sometimes, it can also act as a pathogenic microbe. According to some researchers, it plays a role in the development of colorectal cancer, since it can damage epithelial cell DNA of the colon (Huycke *et al*., 2002). As per recent studies, on average, it causes more than one hundred thousand cases every year (Vu and Carvalho, 2011). *E. faecalis* is the most common among enterococci responsible for 63-81% of cases and malignancy is the most common comorbidity (Khan *et al*., 2018).

Laboratory diagnosis of *Enterococcus faecalis.* Several tests and abilities of enterococci can be used for the detection of *Enterococcus faecalis,* which are hereinafter laid down.

Gram staining. The *Enterococcus* species can easily be identified by using gram staining procedure/ techniques (gram staining), as they appear to be gram-positive cocci present in pairs and short chains (Chen and Zervos, 2009), whereby, the gram-positive cells stain purple due to the retention of the crystal violet from the thick peptidoglycan in their cell wall (Wanger *et al*., 2017).

Pyrrolidonyl-beta-naphthylamide test. *Enterococcus* species can hydrolyze pyrrolidonyl-beta-naphthylamide. Pyrrolidonyl-beta-naphthylamide is a molecule used to detect the presence of pyrrolidonyl peptidase (a bacterial enzyme) (Chen and Zervos, 2009). Pyrrolidonyl aminopeptidase hydrolyzesthe l-pyroglutamic acid-β-naphthylamide to produce β-naphthylamine, which combines with *N*,*N*-dimethyl aminocinnamaldehyde reagent to form red colour which confirms the presence of *E. faecalis* (Compton *et al*., 2017).

Leucine aminopeptidase test. A class of enzymes like Leucine aminopeptidases or Leucyl aminopeptidases (LAP) usually hydrolyze the leucine (an amino acid) residues. These are usually secreted by some of the species of bacteria. LAP hydrolyzes leucine residues at the N-terminal. Enterococci are Leucine aminopeptidase positive and this feature can also be used for their detection from other catalase-negative, gram-positive cocci (Teixeira and Merquior, 2013; Huycke, 2002).

Bile-esculin test. The bile-esculin test can be used to differentiate *Enterococcus* species from non-*Enterococcus* species. *Enterococcus faecalis* are bile tolerant and can hydrolyze the esculin (a hydroxy-coumarin, i.e., a coumarin glycoside) to esculetin in the presence of 40% bile. Bile-esculin test was first described in 1926 and since then is has been used widely for the detection of *Enterococcus faecalis.* Also, this test is known to have a sensitivity of 100% and a specificity of 97% for identifying Enterococci (Goldman and Schafer, 2019; Yilema *et al*., 2017; Leber, 2016).

Catalase test. Catalase is an enzyme that plays a role in oxidative stress defense by degrading hydrogen peroxide to molecular oxygen and water. The bacterium *Enterococcus faecalis* is an anaerobe and does not produce catalase, i.e., catalase negative. Therefore, this feature can also be used for its detection (Baureder and Hederstedt, 2012; Facklam *et al*., 2002).

As previously mentioned, the bacterium *Enterococcus faecalis* generally lack the ability to synthesize catalase. However, under certain conditions such as the presence of heme in the growth medium, this bacterium can synthesize two heme proteins; catalase (KatA) and cytochrome *bd* (CydAB) (Baureder and Hederstedt, 2012).

Broth. Furthermore, this bacterium is capable of growing in the broth with 6.5% sodium chloride (for selective

isolation) as well as at 10 °C and 45 °C which help differentiate the *Enterococcus faecalis* from *Streptococcus* species (Goldman and Schafer, 2019).

PCR. Conventional identification methods are based on culturing techniques that require 2 to 3 days to obtain the results. PCR has provided a means for the culture independent detection of enterococci in a variety of clinical specimens and is capable of yielding results in just a few hours. PCR can help detect *Enterococcus faecalis* by aiming at the Tuf gene. The Tuf gene encodes the elongation factor EF-Tu that is involved in the peptide chain formation. It is a ubiquitous and evolutionarily conserved part of the core genome and is more discriminative than the 16S rRNA gene for identifying strains belonging to the genera *Staphylococcus*, *Streptococcus* and especially *Enterococcus* (Li *et al*., 2012).

Agars. TSA or trypticase soy agar, 5% blood agar, bile-esculin agar, chocolate agar and nutrient agar can be used for culturing *Enterococcus faecalis*. Furthermore, Enterococcal species can be found as smooth, creamy white colonies on agars. Additionally, *Enterococcus faecalis* are found to have a hemolytic property on blood agars containing sheep blood. Whereas, they are found to be β-hemolytic on the rabbit, horse, or human blood agar (Teixeira *et al*., 2019; Vos *et al*., 2011).

Susceptibility to antibiotics. Susceptibility of the genus *Enterococcus* to antibiotics means that the bacterium cannot grow if the specific antibiotic is present. In other words, the antibiotic is effective against that bacterium.

Cephalosporins, macrolides and clindamycin are some of the antibiotics that are frequently used for treating gram-positive bacterial infections. Unfortunately, Enterococci are usually resistant to such antibiotics. Several antibiotics, like glycopeptide, Penicillin, carbapenems, aminoglycosides, tetracyclines, quinolones, chloramphenicol and rifampin, *etc*. can be used for the treatment of Enterococcal infections. The most effective ones are penicillin and glycopeptide. However, *Ampicillin* is more effective than *Vancomycin* (Said *et al*., 2022; Riccardi *et al*., 2021; Xu *et al*., 2019; Enna and Bylund, 2008).

Antibiotics such as aminoglycosides have a lower ability to penetrate the bacterial cell wall. However, the synergistic effect of *Penicillin* with *Streptomycin* (an Aminoglycoside antibiotic) can enhance the bactericidal activity by damaging the cell wall and promoting the entry of the antibiotic into the bacteria (Xu *et al*., 2019). Additionally, Enterococci are tolerant to the bactericidal activity of the cell-wall active agents, such as β-lactam antibiotics and *Vancomycin*. *Enterococcal* tolerance to these antibiotics can be affected by combining the cell-wall active agents with an aminoglycoside to acquire synergistic bactericidal activity. Studies have shown that a higher concentration of aminoglycoside enters those cells that are also treated with agents that inhibit cell wall synthesis, therefore, suggesting that the cell wall active agents promote the uptake of the aminoglycoside like *Streptomycin*. Accordingly to treat infections caused by Enterococci, combination therapy with a cell wall-active agent and a synergistic aminoglycoside should be considered. Nevertheless, in the recent years, resistance to aminoglycosides and decreased susceptibility to β-lactam antibiotics and *Vancomycin*, makes their synergistic function less efficient. Therefore, the widespread resistance of Enterococci has a significant impact on the selection and use of synergistic antibiotics for the treatment of Enterococcal infections (Zavaryani *et al*., 2020).

Antibiotic resistance. Unlike susceptibility to antibiotics, antibiotic resistance means the bacteria can grow even if the antibiotic is present. An example of antibiotic resistance in the case of genus *Enterococcus* is the resistance to *Vancomycin* by Enterococci.

Vancomycin-resistant *Enterococcus*, or *Vancomycin*-resistant Enterococci, are bacterial strains of the genus *Enterococcus* that are resistant to the antibiotic *Vancomycin*. Since the first reports of *Vancomycin*-resistant Enterococci (VRE) in the 1980s, epidemiological studies have demonstrated serious health and economic impacts of VRE-associated infections and persistent colonization in human medicine (Toc *et al*., 2022; Ahmed and Baptiste, 2018).

Treatment. *Antimicrobial/antibiotic therapy*. Antimicrobial therapy can be used to treat Enterococcal (especially *Enterococcus faecalis*) infections. Infections, such as urinary tract infections, soft tissue infections, intra-abdominal infections, periodontitis and septic arthritis can be cured by using single antibiotic therapy (Riccardi *et al*., 2021; Teixeira *et al*., 2019). But in some cases, combinatorial antimicrobial therapy is needed, such as in bacteremia excluding Endocarditis and infective Endocarditis (an infection of the Endocardial surfaces of the heart, primarily of 1 or more heart valves, the mural Endocardium, or a septal defect). However, some studies proposed alternate theories (Riccardi *et al*., 2021; Chen and Zervos, 2009).

Ampicillin is the preferred antibiotic to treat *Enterococcus faecalis* infections. Besides this, other antibiotic options include *Daptomycin*, *Gentamicin*, Linezolid, Nitrofurantoin, *Streptomycin*, *Tigecycline* and *Vancomycin*.

Furthermore, for managing VRE infections, linezolid or *Daptomycin* are the options for adequate treatment (Riccardi *et al*., 2021; Chen and Zervos, 2009).

The period of antibiotic therapy can be determined by the below-mentioned two factors: (i) The response of the patient to antibiotic therapy and (ii) The site of infection. Antibiotic therapy, either oral or intravenous for urinary tract infection, is required only for a few days. Whereas, for bacteremia excluding endocarditis, antibiotic therapy is needed for at least 10 to 14 days. Lastly, in the case of Endocarditis, antibiotic therapy could last for at least six weeks. Moreover, without adjunctive therapy, antibiotic therapy may last longer than the usual duration (Riccardi *et al*., 2021; Chen and Zervos, 2009).

Combination therapy. As previously mentioned, for serious *Enterococcus faecalis* infections, such as bacteremia excluding Endocarditis and infective Endocarditis (IE), bactericidal agents, often as combination therapy, which are preferred. β-Lactam antibiotics lack bactericidal activity against Enterococci when used as monotherapy, making treatment of systemic infections, particularly challenging. Although *E. faecalis* is often susceptible to ampicillin, treatment failure of 60% and lack of bactericidal activity of cell wall-active agents (i.e., *Penicillin* G, *Ampicillin*, *Vancomycin*) prompted efforts to identify combination therapies that would yield a bactericidal effect in severe infections. Originally, *Penicillin* or *Ampicillin* was combined with *Gentamicin* or *Streptomycin* to facilitate intracellular uptake of aminoglycosides. The recognition of *in vitro* bactericidal synergism between β-lactams and aminoglycosides was supported by observational clinical data and led to improvements in IE cure rates up to 75%. However, rising high-level aminoglycoside resistance (HLAR), which may range up to 63%, prompted the need for an alternative therapy. Subsequently, dual β-lactam combination therapy emerged as a viable, safe treatment option for severe infections with *E. faecalis* (Beganovic *et al*., 2018). Usually, the combination therapy of antibiotics may include ampicillin or vancomycin plus gentamicin or streptomycin.

Adjunctive therapy. Although infections associated with *Vancomycin* resistant *Enterococcus faecalis* and *Vancomycin* resistant *Enterococcus faecium* are not virulent in comparison to others, still these infections are an emerging problem. However, these infections can be treated with antibiotic therapies but there are other ways to treat them, as well. Such treatments/ therapies are known as adjunctive therapies. An adjuvant therapy, also known as adjunct therapy and adjuvant care, is a therapy that is given in addition to the primary or initial therapy to maximize its effectiveness. Such as urinary tract infection catheter-associated is sometimes curable by just removing the catheter. Another example of this includes the drainage of abscesses related to Enterococcal infections along with antibiotic therapy can help in treating the infection (Linden *et al*., 2001).

Vaccine therapy. A lot of research is undergoing to develop a vaccine for Enterococcal infection (especially *Enterococcus faecalis*), however, there is no vaccine available in the market, yet. According to a study, the EbpA vaccine can be used for *Enterococcus faecalis* infection, especially urinary tract infection. EbpA is a minor subunit of Ebp or Endocarditis-and biofilm-associated pilus which is an extracellular fiber. This Ebp has three subunits (EbpC, EbpB and EbpA) and is involved in the formation of biofilm, followed by disease pathogenesis (Flores-Mireles *et al*., 2014; Chen and Zervos, 2009).

The mechanism of this vaccine involves the antibodies produced due to the EbpA vaccine that block the *Enterococcus faecalis* binding with fibrinogen. Thus, resulting in the inhibition of fibrinogen-dependent biofilm formation on the catheter.

Prevention. Taking preventive measures such as practicing good hygiene, especially in a hospital setting, can help prevent the spread of *E. faecalis* infection. Patients with the colonization of Enterococci are the main source of *Vancomycin* resistant *Enterococcus*. Such patients can cause the transmission more specifically indirect transmission of this bacteria (Kampmeier *et al*., 2020). Enterococci can outlive adverse conditions. This allows enterococci to cause diseases in humans via multiple routes of cross-contamination, including those from food, environmental and hospital sources (Van den Berghe *et al*., 2006). Therefore, preventive measures developed by HICPAC, i.e., the hospital infection control practices advisory committee must be taken to reduce its prevalence (Sartingen *et al*., 2000). Some of them are as follows.

Enterococcal infections can be prevented by taking hygienic measures, safe handling as well as preparation of safe, healthy and nutritious food and getting the vaccination done at a proper time. Prevalence can also be reduced by spreading awareness regarding the reduction of nosocomial infections. Likewise, by arranging some awareness programs and training sessions regarding hygienic measures for the staff of the health care system (Koch *et al*., 2004). Other preventive measures include: thorough washing of hands after the use of the bathroom, before eating or drinking anything, avoid sharing items such as utensils, toothbrushes or towels, ensure that healthcare professionals wash their hands before encountering IV lines, catheters and dressings and wash their hands or wear clean gloves, in-time identification of vancomycin-resistant *Enterococcus*, frequent screening of infective patients, avoid excessive and unwanted use of antibiotics and always sanitize the doorknobs, remote controls, telephonesor anyother commonly shared items (Koch *et al*., 2004).

Conclusion

Conclusively, *Enterococcus faecalis* is usually harmless, but its transmission from the GI tract to other body parts can pose severe health hazards. Several virulence factors are associated with the pathogenesis of *E. faecalis* infections that are mainly involved in the adherence to the extracellular surfaces, biofilm formation, processes in initiating colonization of bacteria and infections in the host. *E. faecalis* poses a health hazard through antibiotic resistance. There is an urgent need to identify and develop easy, cost-effective methods and potential drugs to reduce the risk posed by antibiotic-resistant *E. faecalis*. Additionally, extensive surveillance and monitoring are required to control the emergence of antibiotic resistance and the increasing incidence of *E. faecalis* in hospitals and as well as in communities.

Conflict of Interest. The authors declare that they have no conflict of interest.

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