German Journal of Veterinary Research
eISSN:2703-1322

Review article

Role of *Enterococcus* in spreading antimicrobial resistance genes and its public health significance

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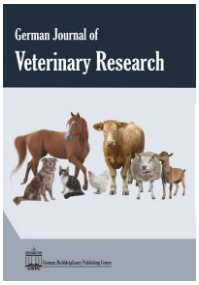

Article History:
Received: 20-Dec-2024
Accepted: 8-Feb-2025
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Abstract

Enterococci are ubiquitous Gram-positive, non-spore-forming, catalase-negative, chemo-organotrophic facultative anaerobic bacteria recognized as a significant public health concern due to their role in disseminating antimicrobial resistance (AMR) genes and disease-causing ability. Often found in various environments, such as the intestines of humans and other monogastric animals, green plants, silage, milk, and soil, it also regularly coexists alongside insects, birds, and other wildlife. Apart from coagulase-negative *Staphylococcus* and *Staphylococcus aureus*, it is the second most frequent cause of nosocomial bacteremia. It is a common bacterium that is naturally resistant to various antibiotics and can also develop resistance through point mutations and gene transfer. Thus, this review aims to explore the possible way Enterococci resist antibiotics through intrinsic mechanisms like altered penicillin-binding proteins and efflux pumps and acquired mechanisms such as horizontal gene transfer of resistance genes, mutations, and biofilm formation, enabling their survival and clinical persistence and also provides an overview of Enterococci general characteristics and its health implications in both human and animals. This review highlights the pressing need for intense monitoring, strict infection control protocols, and the creation of innovative therapeutic approaches to lessen *Enterococcus*'s impact on public health by combining data on its disease burden, epidemiology, and resistance mechanisms. A multidisciplinary strategy that integrates clinical care, microbiology, and public health initiatives is needed to address these issues.

Keywords: Enterococci, Antimicrobial resistance, Public health, Enterococcal diseases, Biofilms

Citation: Fahim, N. A. I., Masud, R. I., Salam, S., Hasan, M. A. E., Rahman, A. M. T., Punom, S. A., and Rahman, M. T., 2025. Role of *Enterococcus* in spreading antimicrobial resistance genes and its public health significance. Ger. J. Vet. Res. 5 (1): 95–12. https://doi.org/10.51585/gjvr.2025.1.0123

Introduction

Enterococci are common facultative anaerobic Gram-positive bacteria that can hydrolyze bile-esculin and L-pyrrolidonyl-B-naphthylamide (PYR) and grow at high pH and 6.5% NaCl concentrations. Previously thought to be members of Lancefield group D *Streptococcus*. DNA homology studies have revealed that they are a separate genus that has been isolated from soil, surface waters, and seawater in conjunction with plants in fermented food products, as a component of the gut microbiota of both vertebrates and invertebrates and as agents of human disease that have several adverse health effects on all bodily systems, including the digestive, respiratory, urogenital, and cardiovascular systems. It commonly causes inflammatory bowel disease (including Crohn's disease and ulcerative colitis), diarrhea, peritonitis, and colorectal cancer (CRC) (Karlinger et al., 2000). It emerged as a leading hospital-associated pathogen in the late 1970s and 1980s (Jett et al., 1994). Approximately

66,000 infections are caused by Enterococci annually in the United States, with an approximate 33% overall mortality rate. It is the second most frequent cause of nosocomial bacteremia, after coagulase-negative *Staphylococcus* and *Staphylococcus aureus* (Jett et al., 1994). The gastrointestinal tract (GIT) is frequently densely colonized before enterococcal bacteremia, from which Enterococci can move into the circulation. Mucositis, *Clostridium difficile* infection, and neutropenia are risk factors for enterococcal bacteremia, which has also been linked to GIT barrier disturbance and a lack of mucosal immunity. Enterococcal bacteria are responsible for over 10% of catheter-associated urinary tract infections (UTIs). Over one-third of all instances of enterococcal endocarditis are acquired in a hospital setting (Alfageme et al., 1993; Murdoch et al., 2009).

Antimicrobial resistance (AMR) is considered a global problem. It is predicted that if the AMR phenomenon is not managed, millions of people will die in the following decades. According to World Health Organization (WHO) estimations, In 2019, bacterial AMR directly caused 1.27 million deaths worldwide and contributed to 4.95 million fatalities. Besides causing mortality and suffering, AMR has substantial financial repercussions. The World Bank forecasts that by 2050, AMR could lead to US$ 1 trillion in higher healthcare expenses and US$ 1 trillion to US$ 3.4 trillion in GDP losses annually by 2030 (Jonas et al., 2017). According to the UK Government's commissioned Review on AMR, by 2050, AMR might claim 10 million lives yearly (de Kraker et al., 2016).

Since *Enterococcus* mainly inhibits the gut, it is essential for disseminating AMR genes throughout the species. It is a common bacterium inherently resistant to a wide range of antimicrobials. It can also develop resistance through point mutations and gene transfer. One common practice that increases AMR among bacterial species, including Enterococci, is using antibiotics in animal production. Consequently, this provides advantageous circumstances for developing and selecting resistant strains (Iweriebor et al., 2016). Numerous studies have revealed that antibiotics are widely used in animal husbandry, with most farmers utilizing them for prophylactic or therapeutic purposes and, to a lesser extent, for growth promotion (VE and C, 2016). Antibiotic-resistant bacterial strains may develop and proliferate through food or other environmental pathways due to human and animal pathogens being exposed to sub-inhibitory antibiotic dosages due to the persistence of antibiotic residues in animal products. Although Enterococci carry a great significance in spreading AMR genes and causing a wide range of diseases, limited articles have been published focusing on Enterococci. Through a comprehensive review of published research, we aimed to determine the mechanisms behind *Enterococci*'s resistance to antibiotics, both acquired (resulting from random mutations in intrinsic genes or from absorbing new genetic material) and intrinsic (found throughout the species' genome). Along with the AMR concept, this review offers an in-depth analysis of Enterococci and their effects on the health of humans and animals.

Enterococcus

The Enterococci are facultative anaerobes that are chemo-organotrophic, non-spore-forming, catalase-negative, and Gram-positive. It is primarily non-motile and poses homofermentative metabolism. They create lactic acid as the predominant outcome of carbohydrate fermentation and have less G+C content, between 34.29 and 44.75% (Torres et al., 2018).

In 1899, Leon Thiercelin, a French microbiologist found a group of diplococci in the intestinal content and named it 'entérocoque' (Švec et al., 2014). In 1906, Enterococci were renamed *Streptococcus faecalis* by Andrewes and Horder as they were able to form short chains like *Streptococcus*. *Streptococcus faecalis* was previously described by Orla-Jensen in 1919 as *Streptococcus glycerinaceus* with another species named *Streptococcus faecium*. These species were differentiated from the other streptococcal divisions by demonstrating promising D group antigen growth findings, growing at 45°C and 10°C in 6.5% NaCl and pH 9.6 (Devriese et al., 1993). In 1991, based on the small subunit rRNA reverse transcriptase sequencing, the *Enterococcus* genus was grouped into several species groups. At that time, 11 species were placed into 3 species groups (Williams et al., 1991). Only 30 *Enterococcus* (*E.*) species were identified between 1992 and 2012; among these, *E. sanguinicola, E. gilvus, E. pallens,* and *E. canintestini* were implicated in human infections

(Carvalho et al., 2008; Tan et al., 2010; Tyrrell et al., 2002). 43 species were recognized in 2014. Currently, more than 73 known *Enterococcus* species are organized in 'species groups'. More than 80% of the isolates are *E. faecalis* and *E. faecium*, making them the most common isolated species (Choi et al., 2024). Furthermore, these two species rank third and fourth globally in terms of nosocomial pathogen prevalence. The core gene nucleotide sequences of 37 Enterococci from different species were analyzed in a recent genomic analysis. Where the majority of the bird isolates belonged to the *E. casseliflavus* branch, while the majority of strains from humans and other mammals were grouped into the *E. faecium, E. faecalis, E. dispar,* and *E. pallens* branches (Torres et al., 2018).

They are ovoid cocci arranged in single pairs, short chains, or groups. It is discovered that only *E. gallinarum* and *E. casseliflavus* are mobile. Some species exhibit yellow pigmentation. Some bacteria can have pseudo-catalase activity. Many species can grow between 10°C to 45°C , but the optimum temperature is 37°C. Enterococci are inherently resistant to several antibiotics, particularly those that target cell wall synthesis. In addition to that, they also present inherited, acquired resistance determinants, which are encoded on various conjugative plasmids, transposons, and bacteriophages, which improve their resistance profile. Enterococci have a high acceptance of salinity levels and may withstand 6.5% NaCl, 40% bile salts, acidic and/or basic conditions (pH up to 9.6), and can last 30 minutes at 60°C. Almost all strains can ferment mannose, fructose, lactose, and galactose and utilize them as the primary energy source (Zhong et al., 2017). By adhering to host tissue, colonizing, boosting bacterial movement, and regulating the host immune system, *Enterococci*'s virulence factors have great potential for improving disease output.

The genomes of Enterococci are between 2.7 to 3.5 Mb, which is comparatively tiny. The average length is around 3.20 Mb, and the average G+C content is close to 37.99% (Zhong et al., 2017). This genus is well recognized for its diverse adaptive ability and resilience and is often found in diverse environmental and healthcare settings. Their genomic structure is characterized by a core genome, a large part involved in carbohydrate, protein, DNA, and RNA metabolism, and a pan-genome, including a dispensable genome and a core genome. Both provide valuable information on genetic diversity and Enterococcal evolution and the reasons behind their adaptability. Their core genome has at least 82 genes related to carbohydrate metabolism, which is almost 13.6% of the core genes (Zhong et al., 2017). The *Enterococcus* PAI was first discovered in the genome of a multidrug-resistant *E. faecalis* strain [MMH594] that was the source of a nosocomial infection outbreak in the 1980s (Huycke et al., 1991). Some genes were responsible for transposases, transcriptional regulators, and proteins associated with virulence (Hacker and Kaper, 2000). Virulence factors such as hemolysin, gelatinase, and aggregation substances aid Enterococci resistance. Enterococci open pan-genome indicates continuous gene exchange within and between species, with novel functions likely generated during evolution. This resistance has led to increased attention in nosocomial super-infections (Medini et al., 2005).

Role of *Enterococcus* in transmitting antimicrobial resistance genes

Antimicrobial resistance is one of the major concerns worldwide. The most common multidrug-resistant (MDR) pathogenic bacteria are *E. coli, Klebsiella pneumonia*, methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant Enterococci (VRE). These organisms are usually found in environments and cause disease in humans. However, among these organisms, *Enterococcus* is one of the significant MDR organisms mainly linked to human catheter-related diseases, posing a significant health risk. This is due to the ability of biofilm formation and resistance to multiple antibiotics, which also transfer to other organisms and foster developing resistance to antibiotics. As *Enterococcus* can usually be found in the guts of animals and humans, it can quickly transfer to environments and help spread resistance genes. Generally, they are innately resistant to a broad spectrum of routinely used antibiotics, including cephalosporins, aminoglycosides, macrolides, and trimethoprim-sulfamethoxazole (Khalil et al., 2023). However, stress, biofilm community, and environmental factors can also contribute to developing and disseminating antibiotic resistance.

Factors associated with resistance development in *Enterococcus*

Intrinsic factors

Peptidoglycan, a primary cell wall component for *Enterococcus*, shapes the outer structure and helps them to be protected from antibiotics and other drugs. Because of this cell wall, antibiotics have low permeability, which limits their ability to enter the bacterial cell and lessens their effectiveness. Efflux pump systems that actively extrude antibiotics from the bacterial cell are also expressed by Enterococci, lowering intracellular antibiotic concentrations. These efflux pumps facilitate resistance to various antimicrobial drugs, such as fluoroquinolones, macrolides, and β-lactams (Mirzaii et al., 2023). The structural relationship between lsa and ATP-binding cassette (ABC)-efflux pumps raises the possibility of drug efflux as a mechanism and resistance inherently to quinupristin, dalfopristin, and clindamycin (Singh et al., 2002).

Additionally, the main components of the cell wall are glycan strands alternating β-1,4-linked N-acetyl glucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residues cross-linked by short peptides made of l- and d-amino acids (Mesnage and Foster, 2013). Glycan strand polymerization and cross-linking between glycan chains are catalyzed by a class of enzymes known as penicillin-binding proteins (PBPs), which are involved in manufacturing and remodeling peptidoglycan (Sauvage et al., 2008). In *Enterococcus*, at least 5 PBPs were found, of which PBPs 4 was for *E. faecalis* and PBPs5 for *E. faecium* (Hollenbeck and Rice, 2012). These PBPs encode low binding affinity for ampicillin and cephalosporins (Signoretto et al., 1994) and develop resistance. Another inherent characteristic of Enterococci is their resistance to cephalosporins. It is partially controlled by a two-component signaling pathway called CroRS and a system with competing kinase and phosphatase activity (IreK and IreP) that regulates resistance expression while maintaining fitness. Some Enterococci exhibit different enzymes in response to antibiotics that are responsible for antibiotic resistance. Because the 6′-acetyltransferase enzyme AAC (6′)-Ii is present, Enterococci frequently have an innate resistance to most aminoglycosides. Almost all of the therapeutically available aminoglycosides, such as gentamicin, tobramycin, amikacin, kanamycin, and netilmicin, are ineffective against Enterococci that carry *aac*(60)-*Ie*-*aph*(200)-*Ia*, but not streptomycin (Ferretti et al., 1986). Specific *Enterococcus* species (e.g., *E. gallinarum* and *E. casseliflavus*) are intrinsically resistant to vancomycin by producing modified peptidoglycan precursors that reduce antibiotic binding. Meanwhile, *van*C1 and *van*C2 show intrinsic resistance to vancomycin, while *van*A, *van*B, *van*D, and *van*E acquire resistance through horizontal gene transfer (Gold, 2001). However, low-level penicillin, aminoglycoside, clindamycin, nalidixic, and cephalosporin are the most common antibiotics intrinsically resistant to *Enterococcus*.

Acquired resistance

Enterococci frequently evolve antibiotic resistance via exchanging resistance-encoding genes on pheromone-responsive plasmids, conjugative transposons, and other broad-host-range plasmids (Rice et al., 1995). Pheromone systems that are only used for plasmid transfer and integrated DNA elements, known as conjugative transposons, excise themselves to create a circular, covalently closed intermediate that can either transfer by conjugation to a recipient and integrate into their genome (intercellular transposition) or reintegrate-in the same cell (intracellular transposition) (Salyers et al., 1995). This process generally helps acquire resistant genes from other organisms or environments. There is acquired resistance to tetracyclines, linezolid, macrolides, glycopeptides (vancomycin), clindamycin, erythromycin, and chloramphenicol. For example, the Tn3-family transposon Tn1546 mobilizes *van*A, while the Tn5382/1549 or related conjugative transposons are the most common carriers of *van*B (Hollenbeck and Rice, 2012).

Introducing foreign resistance genes through horizontal gene transfer or mutation in the amino acid base pairs is the most common pathway for acquired resistance in *Enterococcus*. Mutations in the 23S rRNA gene, amino acid changes, and point mutations are frequently seen. Systems called pheromones are only employed in plasmid transmission. Mutations in the 23S rRNA gene in regions like the V domain have been linked to resistance to linezolid. These alterations alter the ribosome binding site, decreasing the antibiotic's potency (Hasman et al., 2019). Other genes, including *cfr*(B), *cfr*(D), *optr*A, and *poxt*A, are also associated with developing resistance against oxazolidinones and phenicol and phenicol-

oxazolidinone-tetracycline (Wang et al., 2024). DNA mismatch repair (MMR) protein-encoding mutations in mutS and mutL can result in hypermutator phenotypes. It may promote the development of mutational antibiotic resistance in bacteria (Willems et al., 2003). Point mutations to the ribosome or ribosomal mutations can cause resistance to streptomycin (Hollingshead and Vapnek, 1985). Point mutations in the genes *gyr*A, *gyr*B, and *par*C encode DNA gyrase and topoisomerase IV, respectively, are associated with fluoroquinolone resistance (Huang et al., 2024). These changes make it more difficult for the antibiotic to target these enzymes, essential for replicating and repairing bacterial DNA. High-level penicillin resistance in *E. faecium* is most commonly associated with the accumulation of point mutations in the penicillin-binding region of PBP5 (Zapun et al., 2008). In addition, genes encoding a novel ion pump, antibiotic inactivation by acetylation, or disruption of the 50S ribosome binding site are the three ways that Enterococci develop resistance to lincosamides and streptogramins (Iancu et al., 2023).

Contributing factors to the transmission of resistance genes

Horizontal gene transfer

Horizontal gene transfer (HGT) can transmit resistance genes from one organism to another, controlled by a pheromone-based conjugative plasmid. In Enterococci, the discovery of transferable antimicrobial resistance began in the 1970s, with high-level vancomycin resistance in *E. faecium* being molecularly identified in the late 1980s, sparking interest in their resistance mechanisms and transmission routes (Jacob and Hobbs, 1974; Uttley et al., 1988). It has been demonstrated that plasmids containing several resistance genes to vancomycin, erythromycin, tetracycline, and aminoglycosides can be conjugated between Enterococci (Conwell et al., 2017). This is concerning because drug resistance may spread from food isolates to Enterococci, which live in humans.

Horizontal gene transfer is divided into conjugation, transduction, and transformation. However, due to the rigid cell wall, transformation is unusual for Enterococci to acquire foreign DNA (Krause et al., 2022). Therefore, it is not possible to spread the resistance genes. On the other hand, conjugation and transduction are common in Enterococci. Pheromone-inducible plasmid transfer is a prevalent mechanism of plasmid transfer in *E. faecalis*, although it is less common in *E. faecium* (Sterling et al., 2020). Transduction, which involves the bacteriophage's introduction and possible integration of DNA into the host cell, is another method of HGT. Clinical MDR *E. faecium* harbors more mobile genetic elements (MGEs), likely via phages, and *E. faecalis* VRE isolate V583 has seven prophage-like elements enhancing virulence (Krause et al., 2022).

Because of this horizontal gene transfer, Enterococci have become one of the primary sources of hospital-acquired infections (Brinkwirth et al., 2021). It has also been demonstrated that enterococcal mobile genetic elements can transmit resistance determinants to more harmful bacteria, like *Staphylococcus aureus* (Hegstad et al., 2010). One of the main reasons *E. faecalis* and *E. faecium* have become common hospital infections is their capacity to absorb mobile genetic components like plasmids and transposons. These mobile elements often encode antibiotic resistance and virulent factors, enabling these bacteria to survive and thrive in healthcare environments (Palmer et al., 2011). These characteristics have developed in particular genetic lineages linked to hospital epidemics, including *E. faecalis* CC2 and *E. faecium* clonal complex (CC) 17 (Leavis et al., 2007; Mcbride et al., 2007). These lineages are particularly concerning because they exhibit multidrug resistance and enhanced ability to cause infections in vulnerable patients. Peptide pheromones generated by recipient strains lacking plasmids trigger the conjugation process in Enterococci, promoting DNA transfer and intercellular communication. This is exemplified by pheromone-responsive plasmids like pAD1, pAM373, pCF10, pRUM (*E. faecium*), and RepA_N (*E. faecalis*) (Hegstad et al., 2010; Weaver et al., 2009). Transposons elements such as IS3, IS6, IS30, IS256, ISL3 IS4, IS66, IS110, IS200/IS605, IS982, IS1182 and IS1380 are commonly found in *Enterococcus* (Hegstad et al., 2010). The spread of antibiotic resistance can be facilitated by these mobile genetic elements, which can carry and insert antibiotic-resistant genes onto plasmids, chromosomes, or other transposons. A study by (Li et al., 2019) concludes that the *van*A gene was transmitted via a Tn1546-like transposon, identified in a 142,988-bp multidrug-resistant plasmid, facilitating

horizontal gene transfer between *Enterococcus* strains (Moubareck et al., 2005) described that in dibiotic mice, human *E. faecalis* can acquire the *van*A resistance gene from animal *E. faecium*. However, compared to intraspecies transfer, interspecies transmission is less frequent; *van*A transfer was not found in mice connected with human fecal flora. *Listeria monocytogenes* and *L. innocua* can acquire tetracycline and streptomycin resistance from *E. faecium* via transposon-mediated gene transfer, highlighting the role of Enterococci in spreading antibiotic resistance in foodborne pathogens (Jahan and Holley, 2016). Another study by (Agersø et al., 2006) highlighted that Enterococci harbor diverse *tet*(M) genes on various mobile elements, including Tn5397-like transposons, underscoring their role in the evolution and dissemination of antibiotic-resistance genes. The *tet*(M) gene in *E. coli* may have been transferred from other intestinal bacteria, likely Enterococci, where it is commonly linked to conjugative transposons such as Tn916, Tn5397, and Tn5801 (Jurado-rabadán et al., 2014). (Chotinantakul et al., 2020) demonstrated for the first time that meat-derived Enterococci can conjugate to spread the *aad*E gene, which causes streptomycin resistance.

Biofilm community

Biofilms enhance conjugation by minimizing shear forces and promoting close cell-to-cell contact, facilitating the transfer of plasmids encoding multidrug resistance and supporting the spread of antimicrobial resistance (Donlan, 2002). HGT can quickly spread antibiotic resistance genes, and it has been demonstrated that biofilms experience this more frequently than planktonic cultures (Michaelis and Grohmann, 2023). *Enterococcus* is one of those pathogens that can form biofilm and exchange genetic materials within the biofilm community. A great variety of accessory genes is identified in *E. faecium* (Thammavongs et al., 1996). A result of the *Enterococcus* cassette chromosomal (ECC) element, which serves as a focus for genetic exchange in a community (Suriyanarayanan et al., 2018). Also, the biofilm matrix provides a protective environment that shields DNA and plasmids from degradation, thereby maintaining their stability and increasing the likelihood of successful gene transfer (Figure 1).

Stability in the harsh environment

The genus *Enterococcus* is characterized by its metabolic adaptability and durability in various settings. Enterococci are well-suited to withstand the harsh conditions required to pass through the gastrointestinal tract and return to the environment. Additionally, they may adapt to various stresses (Table 1) to survive in the environment, leading to resistant gene transfer. The relationship between transmission, disease production, and stress tolerance is related.

Table 1: Summarizes stress management enzymes and their function according to Gaca and Lemos (2019).

Enzyme	Function
Catalase	Catalyzes the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen, protecting cells from oxidative stress
NADH peroxidase (Npr)	Reduces H_2O_2 to water using NADH, particularly effective against low levels of H_2O_2
Thiol peroxidases	Reduces a broad range of hydroperoxides to water, contributing to peroxide tolerance
MnSOD	Converts superoxide radicals (O_2^-) into H_2O_2 and oxygen, mitigating oxidative damage
Methionine reductases (MsrA and MsrB)	Repairs oxidized methionine residues in proteins, restoring their structure and function
DnaK	Assists in the correct folding of proteins under heat stress
GroEL	Molecular chaperone aiding in protein folding under stress conditions like heat
Clp proteins (ClpP, ClpC, ClpE, ClpB)	Degrade misfolded proteins and assist in protein refolding during heat stress
RNA binding proteins (e.g., CspR)	Maintain proper RNA structure and facilitate gene expression under cold stress
Na+/H+ antiporters	Regulate intracellular pH by exchanging sodium ions for protons, which is critical during alkaline stress

MnSOD=Manganese superoxide dismutase, DnaK=DNA-binding chaperone K, GroEL=GroE large subunit, Clp=Caseinolytic Proteases, ClpP=Caseinolytic protease subunit, ClpC=Caseinolytic ATPase+chaperone, ClpE=Caseinolytic ATPase+chaperone, ClpB=Caseinolytic disaggregase, chaperone.

Enterococci's strong stress tolerance mechanisms allow them to endure harsh settings, elude host defenses, and continue to exist in reservoirs such as the gut and hospitals. Together, these elements raise the possibility of interactions with other microbial populations and encourage the horizontal transfer of AMR genes, accelerating the spread of antibiotic resistance. The overall view of *Enterococcus*'s role in the spread of AMR is presented in (Table 2 and Figure 1).

Table 2: Overall view of *Enterococcus*'s role in the spread of AMR.

Category	Factors	Ways and causes
Intrinsic	• Reduced penicillin-binding protein affinity to antibiotics • Increased production of enzymes that are naturally present triggers the development of resistance • Presence of species-specific resistance gene • Low permeability of the cell membrane • Two-component regulatory pathway • Regulation of efflux pump • Reduced drug uptake	1. These processes aid in their resistance to numerous drugs, eventually spreading to other bacteria via horizontal genes. 2. Serve as a genetic repository for genes that provide resistance to other bacteria. 3. Facilitate the development of multidrug resistance and its dissemination across the environment.
Acquired	• Mutation of amino acids • Gaining resistance genes from another bacteria helps to increase the specific enzymes required for resistance • Modifications and protection of the target sites. • Methylation of 16s rRNA • Acquiring resistance genes through horizontal gene transfer	1. Transfer through pheromone-based conjugative plasmid to the donor cell 2. Transposon and integrons-based resistance gene transfer.
Biofilm community	• Build a complex bacterial community through virulent genes • Making high antibiotic tolerance ability. • Harbor antibiotic resistance organism	1. Horizontal gene transfer to other organisms in a biofilm community 2. Provide nutrients for the growth of the resistant organisms
Environmental durability and dispersal	• Presence of peroxidase enzymes • Presence of superoxide dismutase • Contains methionine • Ability to show heat shock response by heat shock proteins, such as chaperones and protease • Presence of ion exchange pore	1. Helps to survive in stressful conditions and ends up with the transfer of resistance genes.

Mechanism of biofilm formation by Enterococci

A self-produced matrix of extracellular polymeric substances (EPS) encases an organized population of bacteria called a biofilm, which aids in their survival in harsh conditions such as desiccation, antibiotics, and immunological reactions. The capacity of Enterococci to create a biofilm that can withstand critical conditions is a reason for their notoriety. The most prevalent species that can form biofilms, a population of cells permanently affixed to various biotic and abiotic surfaces, are *E. faecalis* and *E. faecium*. *E. faecalis* and *E. faecium* have been shown to produce biofilms on a variety of biomaterials. Their ability to attach to a range of medical devices, including silicone gastrostomy devices, ureteral stents, biliary stents, intravascular catheters, and ocular lens materials, has been linked to their capacity to form biofilms (Dautle et al., 2003; Dowidar et al., 1991; Keane et al., 1994; Sandoe et al., 2003). There are four stages: attachment, microcolony formation, maturation, and dispersal of biofilm formation in Enterococci, which are regulated by several virulent genes.

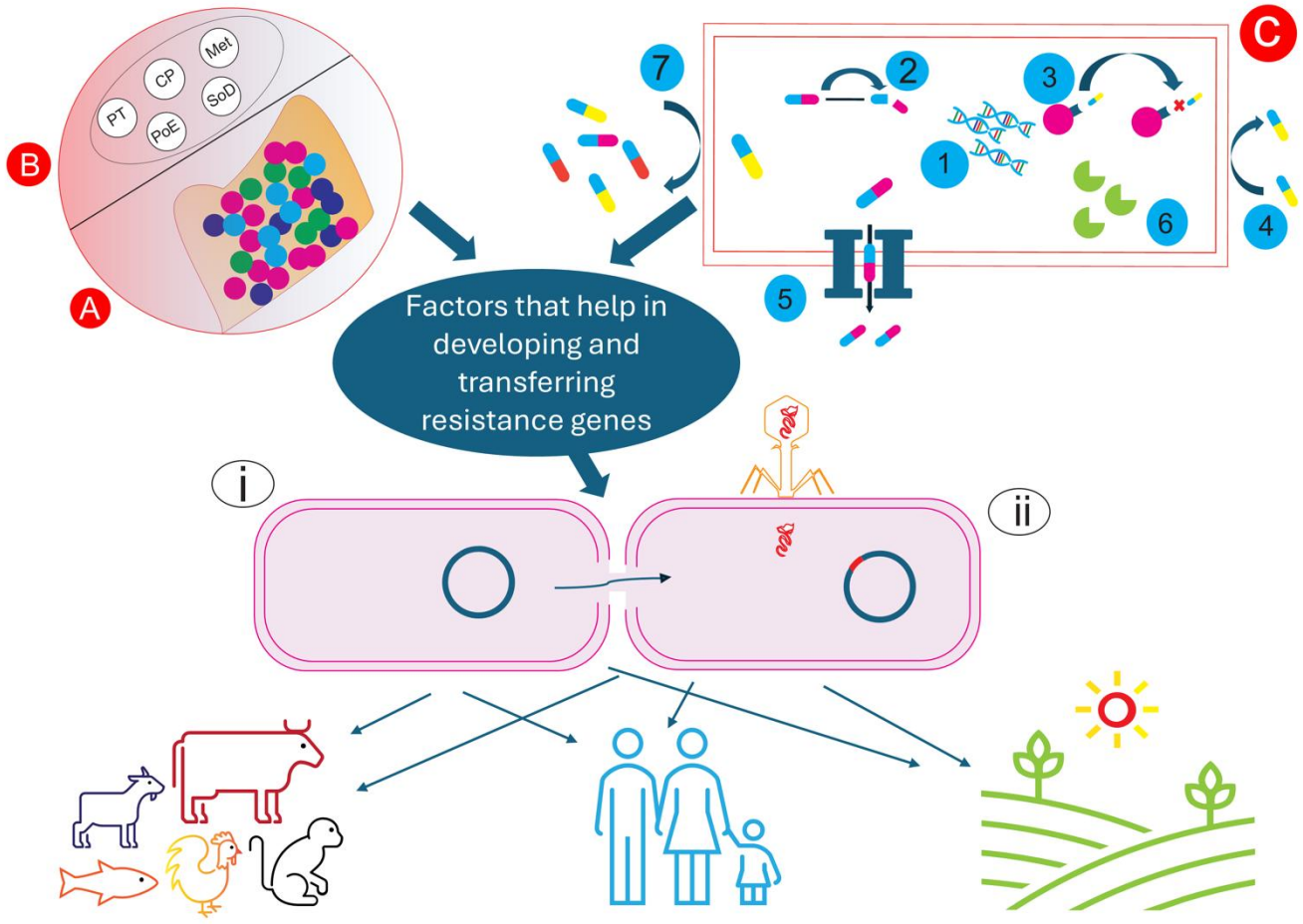

Figure 1: Overall mechanism of resistance gene transfer. A indicates its biofilm community, B indicates stress management enzymes, and C indicates its antibiotics resistance mechanism in where 1(acquiring resistance genes), 2(break down of drugs), 3(inhibit to bind medications in a specific site), 4(inhibit to act in), 5(pump out antibiotics through efflux pump), 6(produce specific enzymes to be resistant to antibiotics), and 7(reduce drug uptake).

Attachment

Several Enterococci components, including pili, Enterococci surface proteins (ESP), Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs), and aggregation compounds, mediate the initial stage of biofilm formation, known as bacterial surface attachment. The first initial attachment stage, adhesion, is facilitated by pili expression on the bacterial cell surface. In *E. faecalis* and *E. faecium*, pili are essential for bacterial adhesion to host cells and the formation of biofilms, which can protect the bacterium from antibiotics and the host's immune system. The Ebp (endocarditis and biofilm-associated pili) proteins, more especially Ebp (A, B, and C), have been shown to be essential for the *in vitro* formation of biofilms related to endocarditis and urinary tract infections (Nallapareddy et al., 2006; Singh et al., 2009). Despite being present in all *E. faecalis* isolates, Ebp pilus's expression fluctuates significantly within a cell population (30–72%) based on growing conditions (Nallapareddy et al., 2011). However, a single *E. faecium* bacterium has two distinct pili on its surface, *pil*A and *pil*B, both of which aid in the production of biofilms (Hendrickx et al., 2008).

The large, surface-anchored protein known as enterococcal surface protein is primarily present in *E. faecalis* and certain strains of *E. faecium*. It enhances biofilm formation, especially on abiotic surfaces, and contributes to persistence in hospital environments and medical devices. A significant correlation between *E. faecalis* esp and the ability to produce biofilms was demonstrated by early research that found that 93.5% of *E. faecalis* esp-positive isolates developed biofilms on polystyrene, whereas none of the esp-negative isolates did (Toledo-arana et al., 2001). *Ace, acm*, and *scm* are examples of MSCRAMMs that help colonize host tissues and help bacterial cells adhere to abiotic surfaces indwelling and coated in the host-produced extracellular matrix. The first of three MSCRAMMs thoroughly studied in Enterococci was the adherence of *E. faecalis* (Ace),

which has been demonstrated to bind to collagen type I, collagen type IV, laminin, and dentin (Kowalski et al., 2006). The virulence of *E. faecium* is significantly influenced by the acm protein, which adheres to the bacteria's collagen. The N-terminal A domain of acm, composed of N1, N2, and N3 subunits, facilitates high-affinity binding to collagen via the "collagen hug mechanism," essential for host tissue adherence (Nallapareddy and Sillanpa, 2007). Scm's high affinity for collagen type V, abundant in the intestinal submucosa, aids *E. faecium* in resisting clearance and persisting in the GI tract (Rasheed et al., 2021). The other two are SgrA, which binds to nidogen-1, nidogen-2, and fibrinogen, contributing to *E. faecium* biofilm formation, while EcbA mediates binding to collagen type V (Hendrickx et al., 2009).

Microcolony formation

Quorum sensing, which is essential for controlling biofilm development, includes the change from planktonic cells to organized communities like microcolonies and affects the formation of microcolonies in Enterococci. Histidine kinase and its related response regulator comprise the two-component signal transduction system in well-characterized microbial species. According to a genomic sequence analysis, *E. faecalis* V583 has 17 paired two-component systems and an extra orphan response regulator (Hancock and Perego, 2004). The fsr locus in *Enterococcus* species, comprising four operon genes (*fsrA, fsrB, fsrD*, and *fsrC*), and peptide pheromones (e.g., Cpd, Cob, Ccf), regulate biofilm formation. The staphylococcal Agr system, in which a histidine kinase *(fsrC)* phosphorylates the cognate response regulator *(fsrA)* in response to the 418 Enterococci buildup of a quorum peptide (GBAP), is somewhat similar to the fsr quorum system of *E. faecalis* (Novick and Geisinger, 2008). *fsrD* is processed by *fsrB* into GBAP, which activates the sensor kinase fsrC, leading to phosphorylation of the response regulator *fsrA* (Galloway-pen et al., 2011). This triggers the transcription of genes like *gelE* (gelatinase), *sprE* (serine protease), and others, including *bopD* (key for biofilm formation) (Hufnagel et al., 2004). Lastly, autoinducer 2 (Al-2) is generated by S-ribosyl homocysteine lyase (LuxS) and plays a role in the biofilm production of *E. faecalis*.). Next, through lysis mediated by *GelE* and *Spr*E, which have conflicting effects on autolysis, the fsr quorum-sensing system controls the growth of *Enterococcus* biofilms (Thomas et al., 2008). While SprE stabilizes a single active AtlA-An endogenous lytic enzyme is essential for the division of cells into daughter cells to form that is resistant to *Gel*E, *Gel*E facilitates lysis by converting the autolysin *Atl*A into several active forms (Qin et al., 1998). The chaining phenotype that results from mutations in both genes highlights the interrelated roles of *Atl*A and gelE in cell wall dynamics.

Maturation

The EPS and microcolonies in established, mature biofilms have developed into large, known as microcolonies. LTA, polysaccharides, extracellular DNA (eDNA), and extracellular proteases are examples of extracellular matrix constituents that must actively multiply and synthesize for biofilms to form. With minimal lysis, metabolically active cells release eDNA, a crucial component of the biofilm matrix, through autolysin AtlA. It was also discovered that MprF2, a paralogue of multiple peptide resistance factors (MprF), stimulates the release of eDNA and the production of biofilms (Kristich et al., 2008).

Biofilm dispersion

Several processes in *Enterococcus* cause biofilm dispersion, which releases bacterial cells into the surrounding environment by breaking down the extracellular matrix and decreasing adhesion. Quorum sensing-influenced population density in a biofilm leads to the biofilms' spread from one place to another (Hashem et al., 2017). The Biofilm formation mechanism of Enterococci is presented in (Figure 2). Protease activities such as *gel*E and *spr*E reduce the load and help to spread the biofilm community. Alterations in nutrient availability, pH, or osmotic stress can influence biofilm stability and lead to natural dispersion.

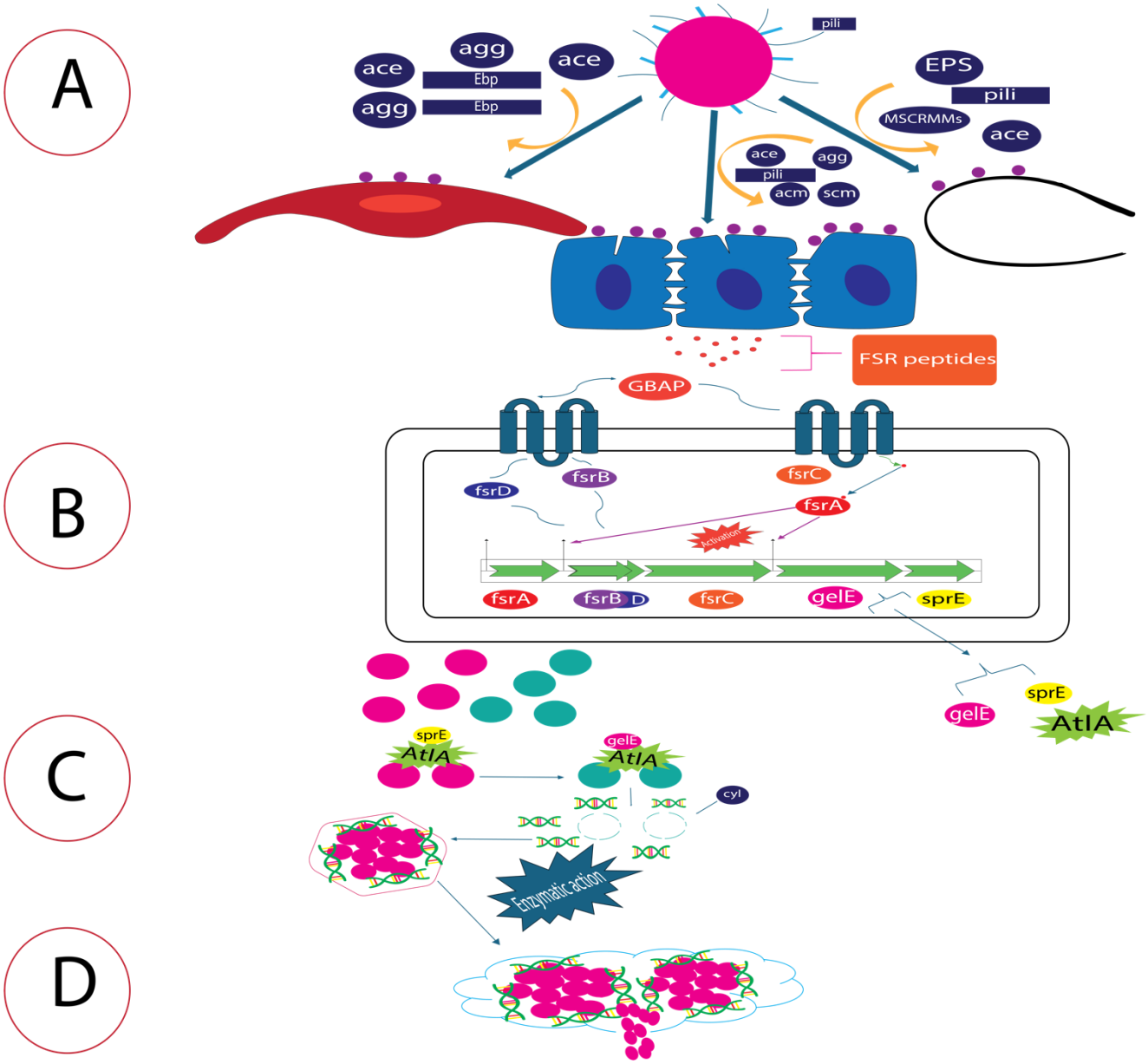

Figure 2: Biofilm formation mechanism by *Enterococci*, A=initial attachment, B=microcolony formation, C=biofilm maturation, D=dispersal.

Public health significance of Enterococci

The genus *Enterococcus* comprises bacteria with beneficial and detrimental implications for public health. Both humans' and animals' digestive systems and the environment (soil, water, etc.) are home to these bacteria. Even while *Enterococcus* benefits human health, it also has a high potential for pathogenicity, particularly in hospital settings where it can result in nosocomial infections of the respiratory, cardiovascular, urogenital, and digestive systems. Diseases caused by Enterococci are presented in (Table 3).

Environmental and water samples often reveal the presence of Enterococci (Paul et al., 1995). Sewage and non-sewage systems allow large animal and human waste to enter the environment. Enterococci have been used to identify fecal contamination in human food and water for over a century (Paul et al., 1995). Discharging treated effluent from sewage wastewater treatment plants is the primary source of pathogenic bacteria detected in ambient surface waterways. MDR bacteria may enter the food chain through treated sludge, a wastewater treatment that contains human and animal feces. Effective wastewater management presents unique issues for Southeast Asian nations. These issues include a lack of sewage treatment facilities, poor sanitation in rural regions, and inadequate sewerage network coverage (McIntosh & Asian Development Bank., 2014). Environmental water sources, coastal

waters, rivers, and canals predominantly contain *E. faecalis* and *E. faecium* as fecal contaminants. However, other enterococcal species may also be detected (Gilmore, 2002). It has been proposed that the water cycle serves as a conduit for spreading antibiotic resistance, mainly when insufficient incentives exist to monitor water quality and the possibility of directly releasing untreated sewage into riverine and coastal ecosystems.

Table 3: Disease caused by Enterococci.

Body system	Diseases	References
Digestive system	Diarrhea, inflammatory bowel disease (ulcerative colitis, Crohn's disease), colorectal cancer (CRC), peritonitis	Karlinger et al. (2000); Siegel et al. (2018)
Respiratory system	Pneumonia, thoracic empyema/pleural Empyema	MacEachern et al. (2005); Tornos et al., (1984); Vanschooneveld et al. (2009)
Cardiovascular system	Endocarditis	Arias and Murray, (2008)
Urogenital system	Upper UTI (bacteremia), lower UTI (cystitis, prostatitis, and epididymitis	Arias and Murray (2008); Lutters and Vogt-Ferrier (2008); Rosen et al. (2007)
Others	Enterococcal meningitis, intra-abdominal and pelvic abscesses and wounds, soft tissue infections, hematogenous osteomyelitis, septic arthritis	Buchs (1977); Durand et al. (1993); Eigler et al. (1961)

The two most common *Enterococcus* species in the human gastrointestinal tract are *E. faecalis* and *E. faecium.* 80–90% of hospital infections linked to Enterococcal disease are caused by *E. faecalis*, whereas 10–15% are caused by *E. faecium* (Jett et al., 1994). Other *Enterococcus* species, such as *E. avium, E. casseliflavus, E. cecorum, E. dispar, E. durans, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus, E. saccharolyticus, E. seriolicida*, and *E. solitarius* are mainly found in the gastrointestinal tracts of various animals. Still, they are occasionally associated with human infections (Gilmore, 2002). Approximately 100 times more significant than *E. faecium* is the increased incidence of *E. faecalis* among clinical isolates in the gastrointestinal system. Although this is the case, recent epidemiological trends over the past 20 years show that *E. faecium* is becoming more common in hospitals in Europe and the United States.

Enterococci have become important healthcare-associated pathogens in recent decades. Urinary tract infections, bacteremia, intra-abdominal infections, and endocarditis are among the hospital-acquired illnesses caused by *Enterococcus* species, especially *E. faecalis* and *E. faecium* (Kristich et al., 2014). Enterococci have emerged as significant multidrug-resistant pathogens in healthcare settings. In the late 1970s and 1980s, these bacteria showed a marked rise in resistance to various antibiotic classes (Galloway-Peña et al., 2009; Gilmore et al., 2013; Huycke et al., 1998).

Following antibiotic exposure, resistant *Enterococci* often establish dense populations in the gut, potentially disrupting the balance of protective intestinal microbiota (Donskey et al., 2000; Taur et al., 2012; Ubeda et al., 2010). Increased use of antibiotics in healthcare environments is used to spread these resistant Enterococci, making them one of the leading causes of hospital-associated infections (Hidron et al., 2008).

Inflammatory bowel diseases (IBD) affect millions of patients and are primarily manifested in two clinical forms: 1) Ulcerative colitis, an inflammatory disorder limited to the colonic and rectal mucosa, and 2) Crohn's disease, which involves both the small and large intestines. The second or third decade of life is when inflammatory bowel disease typically first appears, with a secondary peak in the 60s. In terms of gender differences, Crohn's disease is somewhat more common in women, but ulcerative colitis shows a slight male predominance (Karlinger et al., 2000).

In many wealthy nations, CRC ranks third; in the US, an estimated 135,430 new cases were expected in 2017 (Siegel et al., 2018). The rising incidence in developing countries appears to be closely linked to lifestyle changes (Das Neves et al., 2005; Ferlay et al., 2010). Interestingly, only

15% of patients with this condition have a hereditary genetic predisposition, whereas 85% are attributed to sporadic occurr ences (Macfarlane and Stover, 2007). *E. faecalis* can act as a pathogenic agent, and some authors have reported its potentially harmful effects on CRC development, primarily due to its capacity to damage the DNA of colonic epithelial cells (Macfarlane and Stover, 2007).

Pleural empyema caused by *Enterococcus* species is rare and typically occurs in individuals with compromised immune systems (MacEachern et al., 2005; Tornos et al., 1984; Vanschooneveld et al., 2009). Pleural empyema has not been extensively documented, with a few studies and case reports from Western countries addressing this condition (Alfageme et al., 1993; Bergman et al., 2009; Cotton and Packer, 2018; Fetene et al., 2024; MacEachern et al., 2005; Tornos et al., 1984; Vanschooneveld et al., 2009). *Enterococcus* species were found in 18 individuals, or 16% of the positive cultures, in a retrospective review of 102 patients with thoracic empyema at the Royal Melbourne Hospital in Australia, which was carried out over 14 years from January 1976 to December 1989 (Smith et al., 1991). A search of Medline for English-language studies on enterococcal empyema since 1966 uncovered only two studies from North India (Goyal et al., 2014; Malhotra et al., 2007) and one from Northeast India (Dass et al., 2011). Beyond these reports, individual cases of enterococcal empyema have been noted in Taiwan (Chien et al., 2017), China (Chen et al., 2006; Lian et al., 2017), and Japan (Tsubochi et al., 2009). Patients with weakened immune systems, such as those with hematologic illnesses, cancer, solid organ transplants, HIV, cirrhosis, alcoholism, and smoking, are more likely to experience empyema linked to *Enterococcus* species (Lian et al., 2017; MacEachern et al., 2005; Tornos et al., 1984; Vanschooneveld et al., 2009). There have been recent reports of cases with unusual associations and comorbidities, including living liver donors (Chien et al., 2017), nephrotic syndrome (Chen et al., 2006), and asplenia (Cotton and Packer, 2018), which emphasizes the importance of recognizing further risk factors. In the present instance, there was no discernible indication of serious immune system damage, even though the patient had hypochromic anemia and an underlying thyroid condition. However, it is still unclear how coexisting illnesses affect the development of respiratory disease. *E. faecalis*, *E. faecium*, and infrequently *E. casseliflavus* are the key species involved (Bergman et al., 2009; Cotton and Packer, 2018; MacEachern et al., 2005; Tornos et al., 1984; Vanschooneveld et al., 2009). *E. faecium* is known to have a greater rate of resistance pattern than *E. faecalis* among the two main species. However, it has been reported that extremely drug-resistant strains of *E. faecalis* (resistant to linezolid) and *E. faecium* (resistant to vancomycin) both cause empyema (Chien et al., 2017; Cotton and Packer, 2018). Although not typical, it is essential to consider *Enterococcus* species as possible contributors to thoracic empyema, especially in adults who present with this condition from the community.

These bacteria account for around 66,000 infections in the United States yearly (Hidron et al., 2008). These organisms often recover from mixed infections in the pelvis, abdominal region, and soft tissues (Agudelo and Huycke, 2014). While the specific role of Enterococci in these infections is not always well understood, they are frequently treated with antibiotics. In rarer instances, Enterococci may be implicated in more severe infections such as meningitis and septic arthritis, particularly in patients with pre-existing health conditions or weakened immune systems (Agudelo and Huycke, 2014). *E. faecalis* is frequently detected in cases of acute urinary tract infections (Lutters and Vogt-Ferrier, 2008; Rosen et al., 2007) and patients with non-dysuric lower urinary tract symptoms (Khasriya et al., 2013). It is also a common pathogen in catheter-associated and chronic urinary tract infections (Guiton et al., 2013; Poulsen et al., 2012). In recent years, *Enterococcus* spp. has attracted considerable attention, mainly as an opportunistic uropathogen, due to the involvement of multidrug-resistant strains in infections acquired in healthcare settings. Additionally, with the rapid emergence of antibiotic resistance and the ability to form biofilms (Chai et al., 2007; Mohamed and Huang, 2007) and the natural capacity to thrive in the urinary tract, infections caused by *Enterococcus* spp. difficult to eradicate (Felmingham et al., 1992; Guiton et al., 2013; Hanin et al., 2010; Nichol et al., 2006; Swaminathan and Alangaden, 2010).

Enterococci ranks as the second most common cause of healthcare-associated

bacteremia, with an associated mortality rate of approximately 33% (Hidron et al., 2008; Wisplinghoff et al., 2004). *E. faecalis* and *E. faecium* are the third most common cause of bacteremia in Europe and America, accounting for 11% and 13% of cases (Ammerlaan et al., 2013; de Kraker et al., 2013). The onset of enterococcal bacteremia is typically preceded by extensive colonization of the gastrointestinal tract, which can facilitate the translocation of these bacteria into the bloodstream (Berg, 2000; Ubeda et al., 2010). Additionally, the impairment of mucosal immunity and disruption of the intestinal barrier have been correlated with enterococcal bacteremia. Key risk factors for this condition include mucositis, *Clostridium difficile* infection, and neutropenia (Kuehnert et al., 1999; Lautenbach et al., 1999; Roghmann et al., 1997). Bloodstream infections caused by Gram-positive bacteria are common, with Enterococci accounting for approximately 45% of these cases (Silva Lopes et al., 2005; Willems and Bonten, 2007).

Enterococcal meningitis is rare, representing only 0.3% to 4.0% of bacterial meningitis cases (Buchs, 1977; Durand et al., 1993; Eigler et al., 1961). It is primarily documented in patients with neurosurgical issues, including head injuries, the presence of shunt devices, or cerebrospinal fluid leaks. Still, it can also develop as a "spontaneous" infection following distant enterococcal infections, including endocarditis or pyelonephritis. Due to the limited number of reported cases (Bayer et al., 1978; Jang et al., 1995; Stevenson et al., 1994). The epidemiology, clinical features, best therapeutic approaches, and prognostic factors associated with this disease remain poorly understood.

Conclusions

Enterococcus, particularly *E. faecalis* and *E. faecium*, poses a formidable public health concern due to its role in disseminating AMR genes and disease-causing ability. The intestines of humans and other monogastric animals, green plants, silage, milk, and soil are among the many environments where it is commonly found. It also regularly coexists with insects, birds, and other species. It works as the second most potential nosocomial infection-causing pathogens. Inappropriate use of antibiotics and poor hygiene management make matters worse by contributing to AMR and other disease conditions. To lessen the impact of multidrug-resistant enterococcal infections and public health impact, immediate action is required in response to their increasing prevalence. Comprehensive strategies, including strict infection control procedures, careful use of antibiotics, and intense AMR surveillance, should be guaranteed to address that situation. To overcome the therapeutic issues faced by resistant *Enterococcus*, research should also concentrate on developing novel therapies, such as bacteriophage therapy, antimicrobial peptides, and alternative techniques. Health agencies and research organizations should collaborate to find a sustainable solution. This review highlights the global burden of Enterococci and the necessity for a coordinated, interdisciplinary approach to battle *Enterococcus*'s involvement in the worldwide AMR epidemic.

Article Information

Funding. This research received no external funding.

Conflicts of Interest. The authors declare no conflict of interest.

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Authors contribution. M.T.R: Conceptualization, N.A.I.F, R.I.M, S..S and MA.E.H: Literature search, review, and initial draft of the manuscript, N.A.I.F: figure, N.A.I.F, R.I.M, S.S, S.A.P, and M.A.E.H: review and editing, M.T.R, S.A.P and A.M.T.R critical review, M.T.R Supervision. All authors read and approved the final version of the manuscript.

Data availability. Not applicable

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