Chapter 18
Enterococcal Infections, Drug Resistance, and Application of Nanotechnology

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Contents

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Abstract Enterococci are Gram-positive facultative anaerobes that have changed over epoch as highly modified representer of the gastrointestinal (GI) consortia of an extensive array of organisms like insects, birds, reptiles, mammals, and human. These commensal microorganisms have grossed resistance to all the antimicrobial drugs that currently exist. Multidrug-resistant (MDR) enterococci shows an extensive repertoire of mechanisms of drug resistance including drug target modification, overexpression of efflux pumps, inactivation of antibacterial agents, and cell membrane adaptive response that helps to persist in the body of the host and nosocomial atmosphere. MDR enterococci are renewed to persist in the GI environment and predisposing to invasive infections in those patients who are severely ill and immunocompromised. This chapter mainly focuses the resistance mechanisms of antimicrobial drugs and also role of certain new antimicrobial genes like *optr*A and *cfr* in enterococci. Moreover different strategies to control and therapeutic approaches for controlling MDR enterococci especially using nanotechnology are also highlighted.

Keywords Enterococci · Pathogenesis · Antibiotics · Multidrug resistance (MDR) · Endocarditis · Cephalosporin

18.1 Introduction

Enterococci are a primitive genus of microorganisms that are highly adapted to surviving in heterogeneous and harsh environmental conditions. In the ending of nineteenth century, a saprophytic and infectious cocci found in intestine was described as "*Enterococcus*" (Thiercelin 1899). MacCallum and Hastings also characterized an enterococcal organism, *Enterococcus faecalis*, from a fatal endocarditis case, and provide first comprehensive picture of its pathogenesis (MacCallum and Hastings 1899). Early report attested that enterococcal pathogens are basically commensal opportunist (Lebreton et al. 2014). With the development of genomic technologies, an array of enterococcal species has explored. Enterococci are the principal

causes of healthcare-associated infections (HAIs) all around the globe. The last few decades have witnessed the development of multidrug-resistant (MDR) enterococci which extensively complicates this issue and also enhances the chance of treatment failure and sometimes leads to death. In the last decade, antibiotic-resistant enterococci have become familiar as the prime cause of nosocomial bacteremia, postsurgical wound infection, urinary tract infections, and device-associated infections (García-Solache and Rice 2019; Prabaker and Weinstein 2011; Emori and Gaynes 1993; McDonald et al. 1997).

In this section, we will explain the overall characteristics of the genus *Enterococcus* species, diseases induced by it, and the historical viewpoint behind the creation of MDR enterococci as pathogens and current knowledge of the molecular foundation of drug resistance in *Enterococcus*. Finally we addressed briefly the necessity to advance new drug targets, development of new approaches of nanobiotechnological methods against these dangerous and insubordinate organisms as well as difficulties and opportunities for the future.

18.1.1 Features of Enterococcus *Genus*

Enterococcus species are catalase negative Gram-positive bacteria, are natural inhabitants, and can be isolated easily from their habitats. They are also an important intestinal microfloral component of humans and animals (Van and Willems 2010). The basic physiological and morphological characteristics of all enterococcal strain include Gram-positive, ovoid/spherical cells organized in pairs/chains; among them a few strains exhibit pathogenic potential (Thiercelin 1899). Different salient feature of genus streptococci is represented in Fig. 18.1. They are obligatory fermentative chemoorganotrophs and non-spore-forming facultative anaerobes. They usually grow at an optimal temperature of 35 °C and can growth in the range of 10–45 °C. They normally have an optimal growth in medium having 6.5% sodium chloride (Facklam 1973). They are generally unable to produce catalase and all cytochromes. A few species are able to produce nominal catalase with weak effervescence. Usually they are homofermentative and produce only lactic acid as end product by fermenting glucose (Klein 2003).

18.1.2 Phylogenetic Diversity of Enterococcus *Genus*

In recent decades, knowledge regarding the biology, ecology, virulence, and genetics of the genus *Enterococcus* has sharply increased. The enterococci's taxonomy has changed considerably since the end of the twentieth century when the genus had only 20 species. As a consequence of improvements in differentiation techniques coupled with enhanced interest in enterococci, many fresh species have been well documented. Being ubiquitous, three *Enterococcal* species, namely, *E. durans, E. faecalis*, and *E. faecium*, were documented before 1950. *E. faecium and E. faecalis*

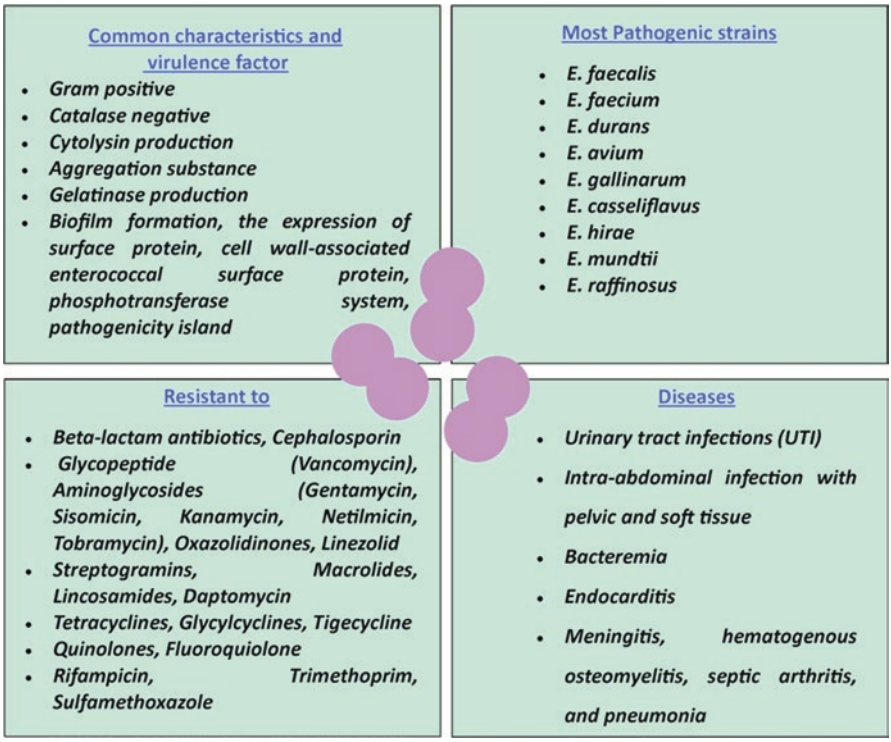

Fig. 18.1 Different salient features of Enterococci

account for most of the enterococcal diseases (Malani et al. 2002). Other species like *E. avium*, *E. casseliflavus*, *E. hirae*, *E. gallinarum*, *E. mundtii*, and *E. raffinosus* have also been isolated from human infection (Devriese et al. 1994; Hammerum 2012; Murray 1990; Lebreton et al. 2014). In the era of 1992–2012, about 30 species of *Enterococcus* were documented, and only four of them were associated with human infection and pathogenesis (*E. sanguinicola*, *E. pallens, E. gilvus*, and *E. canintestini*). Till date, there are 52 species available that belong to *Enterococcus* genus.

18.1.3 Enterococci-Associated Infections

Over the last couple of eras, enterococci emerged as significant pathogens (Arias and Murray 2012). The variety of diseases caused by streptococci becomes devastating which is attributed to their tendency to become increased antibiotic resistance. Although other microorganisms are often isolated from the source site with

enterococci, it is often not well understood and remains a paradox that the enterococci are directly associated with the manifestations of the diseases or whether they are avirulent and opportunistic one and suppose to play an insignificant role in the manifestation of diseases (Higuita and Huycke 2014). Among the several types of enterococcal infection, endocarditis and bacteremia are the leading life-threatening disease.

18.1.3.1 Urinary Tract Infections (UTI)

Urinary tract is the most susceptible area of enterococci infection. Lower urinary tract portions, especially cystitis, prostatitis, and epididymitis, are the frequent sites of UTI caused by enterococci in older man. Young women are also affected by uncomplicated cystitis, infected by enterococci. Occurrences of bacteremia in upper UTI are most often reported in older men. Enterococci-induced UTIs are more likely to be acquired in hospitals or in long-term settings, making them more resistant to antibiotics. Moreover, ICU setting also contributes to 15% of healthcare-associated UTI. Among the ICU patients, enterococci resistant to vancomycin have become the major urinary tract pathogens associated with healthcare (Hidron et al. 2008).

18.1.3.2 Intra-abdominal Infection with Pelvic and Soft Tissue

Intra-abdominal infection with pelvic and soft tissue is also the site of enterococcal infections. Enterococci are isolated from these samples often associated with other microbial flora and infrequently cause mono-microbial infection at the above sites. Bacteremia caused by enterococci is mainly associated with abscesses and wounds in the intra-abdominal and pelvic regions (Graninger and Ragette 1992; Maki and Agger 1988). Though most of the physician routinely follows antibiotic regimens to treat such type of infections, drainage of abscesses and debridement of wounds are also essential adjuncts to antibiotic therapy. Moreover, conjunction of liver cirrhosis or patients receiving chronic peritoneal dialysis most often suffered from an infection called peritonitis. Peritonitis mainly occurs in the abdominal lining. Moreover, abdominal or pelvic mixed aerobic-anaerobic infections should be considered separately. Though, in this type of cases, enterococci show monomicrobial infection, *Escherichia coli*, coagulase-negative *Staphylococci,* and *Staphylococcus aureus* are also responsible for bacterial peritonitis and dialysis-associated peritonitis. Over and above, enterococci are also often isolated in cultures from decubitus and foot ulcers. However, their roles in causing such site-specific infections are not clearly understood till date.

18.1.3.3 Bacteremia

Enterococci are presently one of the major causes of bacteremia associated with healthcare. Over the last couples of years, bacteremia is usually associated with gastrointestinal tract, although sources of bacteremia also reported from biliary and intra-abdominal regions, indwelling central lines, or infections in soft tissues. Polymicrobial bacteremia is associated mainly with enterococci, though there are also other microorganisms partially involved in the occurrence of such type of infection. Enterococci-associated bacteremia causes metastatic abscesses. The rate of overall mortality in enterococci associated bacteremia is varied (Maki and Agger 1988; Patterson et al. 1995; Higuita and Huycke 2014). Several scientific reviews regarding bloodstream infections clearly reported that enterococci is the only Gram-positive bacteria associated with high risks of death. Moreover, higher mortality rate was reported in the case of *E. faecium*-associated bacteremia than *E. faecalis* (Noskin et al. 1995a, b; Higuita and Huycke 2014). The chances of occurrence of enterococci-associated bacteremia are higher in the case of elderly people with multiple underlying diseases like malignancy, diabetes mellitus, cardiovascular diseases, transplantation, and postsurgery infection.

18.1.3.4 Endocarditis

Among different types of infection caused by enterococci, endocarditis is the most fatal enterococcal infections. The alimentary or urinogenital tract is the primary bacteremia source which leads to endocarditis. In reality, left-sided participation is much more prevalent than right-sided participation. Prosthetic valve enterococcal endocarditis has been increasingly marked. This is mainly associated with increasing application of prostheses in aged persons who have higher risks for bacteremia caused by enterococci (Anderson et al. 2004; Rice et al. 1991). Enterococcal endocarditis is more common in men compared to women (McDonald et al. 2005). Several retrospective analysis reported that between 15 and 39% of enterococcal endocarditis are healthcare associated (Anderson et al. 2004; McDonald et al. 2005). Endocarditis associated with enterococci is a subacute infection followed by cardiac failure, rather than an embolic effect (McDonald et al. 2005). Though death rates are low (9–15%) in enterococcal endocarditis in comparison to other infective endocarditis (McDonald et al. 2005; Rice et al. 1991; Wilson et al. 1984; Higuita and Huycke 2014), selection of effective therapy against the multidrug-resistant enterococci is definitely a challenging task.

18.1.3.5 Uncommon Infections

Meningitis, septic arthritis, hematogenous osteomyelitis, and pneumonia are the less common or rarely seen infections caused by enterococci. Pneumonia is quite rare even in the presence of ventilators, and it is reported in significantly weakened

or in immunocompromised patients who have received antibiotic drugs of a broad spectrum. Antibiotic-resistant enterococci (VRE) are likely to be responsible for such types of infection than antibiotic-susceptible enterococcal isolates.

18.2 Expansion of Antibiotic Resistance

With the innovation of antimicrobial drugs discovery and understanding the microbiological foundation of diseases, infection became remediable with remarkable recovery. Clinicians, however, quickly understood that certain microbes appear to be less effective in responding to specific antimicrobials and that's why generations of antibiotics had come. It was also documented that penicillin and aminoglycoside are less effective against many enterococcal species, and conjugation of aminoglycosides with penicillin was prescribed which showed synergistic response that improved enterococcal endocarditis cure rates from 40 to 88% (Robbins and Tompsett 1951). Thus the particular combination of a cell-wall-active agent (i.e., penicillin/ampicillin) along with an aminoglycoside will be the solution for the treatment of deep-rooted *Enterococcus*-associated diseases, and this combination remains effective (Baddour et al. 2005).

Unknowingly the seeds of the resistance of enterococci against an array of drug were already being sown and propagated. By the help of comparative genomics, it was documented that the modern MDR *Enterococcus faecium* is a part of a genetic class that seems to have divergent root of ancestry from animal-adapted *E. faecium* strains in clinical practice approximately 75 years ago, corresponding to the introduction of antibiotics (Lebreton et al. 2013). This was achieved by various means which include an upsurge in horizontal gene transfer, metabolic bypass, and hypermutability in the enterococcal strains. The acquisition of genes for vancomycin resistance is one of the utmost examples of this adaptability. Vancomycin-resistance enterococci (VRE) was first time documented in 1988, and within two decades, more than 80% of *E. faecium* acquired the said property in the USA (Arias and Murray 2012). Of particular concern, *E. faecium* is also increasingly reported to cause nosocomial infection, which now occurs as often frequently as *E. faecalis* (Hidron et al. 2008). Recently, enterococci have also reported to share the vancomycin-resistant gene clusters with potential pathogens (such as methicillin-resistant *Staphylococcus aureus*) through horizontal gene transfer, which is matter of a great health risk (Chang et al. 2003; Ray et al. 2003). Enterococci have adapted rapidly despite the abundance of anti-Gram-positive antimicrobials, and the emergence of resistance against these agents has been theorized. This becomes a clinical challenge to treat enterococcal MDR infections. The following sections give a picture of the mechanisms and prevalence of antimicrobial resistance in enterococci which is summarized in Table 18.1.

Table 18.1 Antibiotics and the resistance mechanism of enterococci against them

Class/name of antibiotics	Basic mode of action	Specific gene(s)/ operon responsible for resistance	Possible mechanism of antibiotic resistance
Ampicillin, Penicillin, Mezlocillin, Piperacillin	Inhibit the synthesis of peptidoglycan	*ponA*, *pbp F*, *pbpZ*, *pbp5*, *pbp A*, *pbpB*	Reduced susceptibility for the antibiotic
Cephalosporin		*pbp5*, *ponA*, *pbpZ*	Reduced binding affinity for the antibiotic
Glycopeptide (vancomycin)	Prevent cross-linking of peptidoglycan	*vanA*, *vanB*, *vanD*, *vanM, vanC, vanE, vanG, vanL, vanN, vanT, vanXY, vanT*	Reduced affinity for the antibiotic
Aminoglycosides (gentamicin, sisomicin, kanamycin, netilmicin, tobramycin)	Create pores in the cell membrane of the bacterial cell	*aac (6')-Ii, aph(2')-Ic, aph(3')- IIIa, aph(2')-Id, aph (2')-I*	Modification of the aminoglycoside structure
Oxazolidinones, linezolid	Inhibit the peptide delivery	*cfr* or *cfr(B)*	Methylation of 23S rRNA and reducing affinity to the antibiotics due to mutations
Streptogramins, macrolides, lincosamides	Dissociation of peptidyl-tRNA, preventing binding of aminoacyl-tRNA to the ribosomal and the formation of the peptide bond	*isa, msrC, eatA, msr(A), linB, mef(A), vgb(A), vat(D), vat(E)*	Efflux pump to eliminate antibiotics
Daptomycin	Alterations in the cellular membrane characteristics	*liaFSR* operon	Mutation in the specific gene to exclude the effect of antibiotics
Tetracyclines, glycylcyclines, tigecycline	Interfere with the docking of aminoacyl-tRNA in the ribosome	*tetM*, *tetO*, *tetS tetL*	Efflux mechanism, ribosomal protection overexpression of genes
Quinolones, fluoroquinolone	Disrupt DNA strand continuity as well as stop replication	*gyrA, parC, qnr*	Mutation in the specific gene, efflux pump
Rifampicin	Inhibit the process of transcription	*rpoB*	Reduced affinity due to point mutation
Trimethoprim, sulfamethoxazole	Inhibit folate biosynthesis pathway	–	Gain ability to utilize exogenous folate

18.3 Biofilm Formation in Enterococcal Infections

Biofilm is multicellular community of microbes attached on abiotic and biotic surfaces or interfaces, enclosed in a hydrated self-produced extracellular polymeric matrix (Costerton 2001). Development of biofilm is a multistep phenomenon which includes surface attachment, immobilization, cell-cell interaction, microcolony formation, confluent biofilm formation, and subsequently three-dimensional biofilm formation (O'Toole et al. 2000). Biofilms are the reservoir of many chronic infections and extremely difficult to eliminate (Mohamed and Huang 2007). As per the National Institutes of Health, about 4/5 share of all bacterial infection in the body associated with biofilm formation (Lewis 2001). Biofilm containing bacteria are phagocytosis resistant, and therefore it is an extremely challenging task to eliminate from the host or infected individual (Lewis 2001). Biofilms are reported to form in/on a broad range of medically used devices like pacemakers, catheters, orthopedic appliances, and prosthetic heart valves, which is correlated with multiple pathogenic consequences (Costerton et al. 1999).

Biofilm producing enterococci are extremely antibiotic resistant and therefore the impact of biofilm development is very crucial. Perusal of literature attested that enterococci were found to form biofilm in an array of infection like UTI, wounds, GI dysbiosis, endocarditis, etc. Though exopolymeric matrix and antibiotic resistance are the two major hurdles to eradicate enterococci, the foremost problem is the dissemination of the genetic trait of antibiotic resistance to other microbes (Ch'ng et al. 2019). As like as other biofilm-forming bacteria, adherence and biofilm formation by *E. faecalis* and *E. faecium* on diverse biomaterials and numerous medical apparatus (biliary stents, intravascular catheters, silicone gastrostomy devices, ureteral stents, etc.) have been documented (Joyanes et al. 2000; Distel et al. 2002; Dowidar et al. 1991; Sandoe et al. 2003; Dautle et al. 2003; Keane et al. 1994). Formation of enterococcal biofilm of on ocular lens has also been demonstrated (Kobayakawa et al. 2005).

18.3.1 Factors Contributing Formation of Biofilm in Enterococci

18.3.1.1 Biofilm Formation in *E. faecalis*

Development of biofilm generally consists of four phases: initial attachment, formation of microcolony, maturation of biofilm, and, finally, dispersal. There are multiple factors that influence formation of biofilm in enterococci within or outside their host condition (Dunny et al. 2014); however, the dispersion mediators have yet to be identified (Table 18.2).

Adherence to the surface is the early stage for the establishment of biofilm. Various factors like surface adhesins, glycolipids, and proteases perform significant

Table 18.2 Some nanoparticle and their effects on enterococci

Type of nanoparticles	Antibiotic resistance type/special feature	Mechanism of action	References
AgNPs	Vancomycin resistance	In combination with vancomycin causing bacterial death	Saeb et al. (2014)
	Erythromycin resistance	Cell surface damage and loss of the chain integrity	Otari et al. (2013)
	Multidrug resistance	Modification of physicochemical properties of the cell	Cavassin et al. (2015)
	Multidrug resistance	Combined effect with gentamicin and chloramphenicol	Katva et al. (2018)
Graphene oxide NPs	Multidrug resistance	UV irradiation leads to reactive oxygen species generation, multiple toxic mechanisms	Govindaraju et al. (2016)
Magnetite NPs	Biofilm forming	Effective aminoglycoside antibiotic carrier	Chifiriuc et al. (2013)
Calcium hydroxide NPs	Multidrug resistance	–	

tasks in the first step of biofilm formation. The multi-subunit (viz., A, B, and C) endocarditis and biofilm-associated pilus (Ebp) encoded by e*bpABC* facilitates surface adherence both in vivo and in vitro (Nielsen et al. 2012; Nallapareddy et al. 2011a; Singh et al. 2007). The role of Ebp in the early development of biofilm was showed by in vivo models of UTIs, catheter-associated UTI, and infective endocarditis (Nallapareddy et al. 2006, 2011a, b; Nielsen et al. 2013). Several in vivo experiments in cultured human cell also described the significance of surface adhesins in formation of biofilm (Mohamed et al. 2006; Rozdzinski et al. 2001; Sussmuth et al. 2000; Sillanpaa et al. 2010). It was also demonstrated that biofilm-associated glycolipid synthesis A influences in vitro surface adherence and subsequent biofilm development (Theilacker et al. 2009).

Initial attachment followed by formation of microcolony in which bacteria divided repeatedly and produce minute sizes of biofilm which subsequently get aggregated (Monds and O'Toole 2009). In vitro findings have clearly showed that microcolony formation is the mature stage of biofilm development, and this is significant for gut colonization. An enterococcal polysaccharide antigen gene cluster (*epaOX*) encodes a glycosyltransferase which is associated with the production of rhamnopolysaccharide associated with cell wall, and mutant *E. faecalis* for the particular trait showed a reduction in biofilm reduction (Ch'ng et al. 2019; Xu et al. 2000).

Maturation of *E. faecalis* biofilm is associated with the vigorous growth and development of extracellular matrix materials like extracellular DNA, polysaccharide, glycoprotein, modified lipid, lipoteichoic acid, etc. (Ch'ng et al. 2019; Fabretti et al. 2006). Deletion of *atl*A reduces the release of extracellular DNA, thus decreasing biofilm formation (Guiton et al. 2009). In vitro deletion of *dltABCD* operon causes inhibition of biofilm development by Gram-positive bacteria by reducing the production of D-alanine esters of lipoteichoic acid.

Biofilm formation is also contributed by population density-dependent signaling mechanism like quorum sensing and peptide pheromone signaling which upgrade expression of genes towards biofilm formation by enterococci (Krasteva et al. 2012; Camilli and Bassler 2006; Cook and Federle 2014; Li and Tian 2012; Cook et al. 2011). Recently, transfer of plasmid DNA between *E. faecalis* cells in GI tract has been documented which encourages biofilm formation (Chen et al. 2017; Hirt et al. 2018). *Eep* (Chandler and Dunny 2008), *fsrABC* (Ali et al. 2017), *bopABCD, gelE, sprE* (Dunny et al. 2014), and *AI-2* (Shao et al. 2012) are also involved in quorum sensing system of enterococcal biofilm formation.

18.3.1.2 Biofilm Formation in *E. faecium*

Multiple genes are responsible for the development of biofilm in *E. faecium* like *atlA*, *ebpABC*, *esp*, *fsrB*, *luxS*, *spx*, *acm*, *scm*, *sgrA*, *pilA*, *pilB*, *ecbA*, and *asrR* (Dunny et al. 2014; Lim et al. 2017; Sava et al. 2010; Hendrickx et al. 2009; Sillanpaa et al. 2008). Among these genes, *atlA*, *ebpABC*, *esp*, *acm*, and *asrR* are responsible to cause biofilm-associated infection in in vivo condition (Dunny et al. 2014; Sava et al. 2010). The cell surface adhesin, Esp, and EbpABC perform a crucial task in the initial attachment of *E. faecium*, followed by biofilm development in the case of UTI and infective endocarditis model (Montealegre et al. 2016a, b; Almohamad et al. 2014). Deletion of the gene *esp* and *ebpABC* operon reduced the chances of biofilm formation by the organism (Heikens et al. 2011). There are similarities in the occurrence of biofilm formation in the case of *E. faecium* and *E. faecalis* (Ch'ng et al. 2019). AtlA-dependent release of extracellular DNA plays a crucial role in biofilm formation in vitro in both the species (Paganelli et al. 2013). Several reports suggest that upregulation of gene like *ebpABC* and downregulation of genes like *fsrB*, *luxS*, and *spx* might regulate biofilm-forming potential of *E. faecium* (Lim et al. 2017). Moreover, deletion of asrR gene involves in growth and maturation of biofilm and also influences biofilm-associated infections (Lebreton et al. 2012).

18.4 Mechanism of Antimicrobial Drug Resistance in Enterococci

18.4.1 Mechanism of Resistance of β-Lactam Derivatives (Cell-Wall-Active Agents)

18.4.1.1 Resistance to β-Lactams

Penicillin and ampicillin are the foremost pronounced β-lactams which competitively block peptidoglycan (PPG) biosynthesis which is basic and the most common component of the bacterial cell wall. However, the lack of analogous structural

component in eukaryotes excludes the lethality of these agents and makes them an ideal against bacterial infection as therapeutics. Penicillin-binding proteins (PBPs) are the flagship of the cell wall biosynthesis machinery which is broadly subdivided into two classes: class A, which exhibits bipartite enzymatic activity, namely D,D-transpeptidase and transglycosylase, and class B, which exhibits transpeptidase activity towards other enzymes.

Enterococci are inherently resistant to most β-lactams and hence less prone to restricted by the antibiotics. This is due to the expression of one kind of PBPs which have low affinity towards β-lactam antibiotics. Consequently, the minimum inhibitory concentration (MIC) of penicillin is higher in enterococci in contrast with streptococci or other Gram-positive bacteria, which do not produce chromosomally encoded low affinity PBPs. Lower MIC values of penicillin were documented for *E. faecalis* strains than *E. faecium*.

Every enterococci have at least 5 PBPs, and 6 putative PBP genes were recognized by studying the genome of *E. faecalis* and *E. faecium* (class A, *ponA*, *pbp F*, *pbpZ*; class B, *pbp5*, *pbp A*, *pbpB*) (Miller et al. 2014). Inherent tolerance against the β-lactam antibiotics is linked with the expression of species-specific *pbp5* gene (class B PBP) that minimizes binding affinity cell wall with the antibiotics. In *E. faecium*, the *pbp5* gene is a part of operon which has three genes (including *pbp5*) that take part in cell wall synthesis (*psr* and *ftsW*) (Miller et al. 2014). Enhanced resistance against β-lactam antibiotics has frequently been noticed among clinically isolated *E. faecium* but rarely noticed in the case of *E. faecalis*. High-level ampicillin resistance of *E. faecium* (MIC>128 μg/ml) has been correlated with concomitant production of Pbp5 or with specific amino acid modifications in its sequence, which minimizes affinity of the same with penicillins resulting in less vulnerable to be inhibited. The substitutions of amino acid at or near the active-site cavity (Ser-Thr-Phe-Lys, Ser-Asp-Ala, and Lys-Thr-Gly motifs) seem to be the utmost significant ones (Rybkine et al. 1998; Zorzi et al. 1996). Combinations of specific amino acid alterations in the carboxyl-terminal transpeptidase domain of PBP5 (substitution Met-485-Ala/Thr, Ala-499-Ile/Thr, Glu-629-Val and Pro-667-Ser) and the insertion of serine or aspartate after position 466 have been related to ampicillin resistance of *E. faecium* isolates (Montealegre et al. 2016a, b; Jureen et al. 2003; Poeta et al. 2007; Klibi et al. 2008; Arbeloa et al. 2004; Rice et al. 2004).

Alongside, β-lactam antibiotic resistance is also facilitated by a β-lactamase enzyme which restricts the antibiotic action by cleaving the β-lactam ring. The phenomenon was documented in both *E. faecalis* and *E. faecium* (Rice and Murray 1995; Murray 1992; Coudron et al. 1992). Selected strains of *E. faecalis* produce a plasmid-mediated β-lactamase that is similar to the enzyme produced by *Staphylococcus aureus*, encoded by the *blaZ*gene, although some polymorphisms in this gene have also been detected in some isolates (Hollenbeck and Rice 2012; Murray et al. 1992).

18.4.1.2 Resistance to Cephalosporin

As like as β-lactam antibiotic resistance, the intrinsic resistance of enterococci is correlated with a decline in the affinity of binding of cephalosporin with enterococcal PBPs, especially Pbp5 (Rice et al. 2009; Arbeloa et al. 2004). It was documented that expression of either *ponA* or *pbpF* gene in *E. faecalis* and *E. faecium* is required to exhibit cephalosporin resistance, and PbpZ alone is incapable of offering the transglycosylation property.

An array of regulatory pathways manifested by two-component system is responsible for showing cephalosporin resistance by enterococci. Downstream effector like CroRS was publicized to be imperative for the same. Besides, two-component system implicated a role in resistance also relayed by a serine/threonine kinase, namely, IreK and IreP (phosphorylated). IreB was proven as target of both the aforementioned proteins and in turn upgrade the expression of cephalosporin resistance (Comenge et al. 2003; Muller et al. 2006; Kristich et al. 2007; Hall et al. 2013). MurAA protein involved at the downstream of the IreK signaling pathway and catalyzes the first committed step in PPG biosynthesis (Miller et al. 2014).

18.4.1.3 Resistance to Glycopeptide

Vancomycin and teicoplanin belongs to glycopeptide family employed for the treatment of severe human diseases. Glycopeptides actually bind with the terminal D-alanyl-D-alanine of the pentapeptide of PPG precursors that subsequently inhibit cross-linking of PPG chains and thus restrict the bacterial cell wall synthesis. The mechanism underlying the glycopeptide resistance of enterococcal strains is the alteration of the PPG synthesis pathway. The terminus D-alanyl-D-alanine with which vancomycin binds is modified to D-alanyl-D-lactate (high resistance, MIC >64 μg/ml) or to D-alanyl-D-serine (low resistance, MIC >4–32 μg/ml). This kind of alteration in the cell wall precursors leads to reduced binding affinity of the glycopeptide with the former (Miller et al. 2014; Ahmed and Baptiste 2018; Shlaes et al. 1989; Arthur et al. 1993).

Vancomycin-resistant enterococci are formed by *van* operons, which encode the modified PPG precursors. Nine *van* operons have been recognized so far in enterococci-mediating vancomycin resistance (for D-alanyl-D-lactate modification, *van*A, *van*B, *van*D, *van*M, and for D-alanyl-D-serine modification, *van*C, *van*E, *van*G, *van*L, and *van*N) (Miller et al. 2014; Courvalin 2006; Depardieu et al. 2015). The *van*A and *van*B are the most common genotypes among VRE with acquired resistance mechanisms of humans and animals, mostly among *E. faecalis* and *E. faecium* (Ahmed and Baptiste 2018). VanC operon is the fundamental component of *E. gallinarum* and *E. casseliflavus* that helps to produce PPG precursor with terminal D-alanyl-D-serine residue reported first time (Leclercq et al. 1992; Reid et al. 2001). Apart from VanC (which is a D-alanine-D-serine ligase), the enterococcal cells encode a serine racemase (VanT), combined dipeptidase-carboxypeptidase (VanXY) and regulators encoded by *vanR* and *vanS* genes which encode (cytoplas-

mic) transcriptional regulator and membrane-bound histidine kinase, respectively (Depardieu et al. 2015; Sassi et al. 2018).

The *van*A operon is associated with the transposon Tn1546 and includes seven open reading frames (ORFs) transcribed under two different promoters. Regulation is mediated by *van*S-*van*R (sensor-kinase-response regulator) two-component system, transcribed with a common promoter. The *van*H- and *van*A-encoded protein modifies the PPG precursors, whereas *van*Y interrupt the creation of the D-alanyl-D-alanine termini of the pentapeptide by its D,D-carboxypeptidase activity. Moreover, *van*Z gene is associated with teicoplanin resistance in enterococci.

Tn1547, Tn1549, and Tn5382 are the transposons associated with *van*B operon. Among the transposons, Tn1549 is widely predominant among *van*B-type enterococci located in chromosome. *van*B has two promoters and seven ORFs. *van*B enterococci represent vancomycin resistance but susceptibility towards teicoplanin (Ahmed and Baptiste 2018; Arthur and Courvalin 1993). It was well documented that a few of van operons belong to transposable genetic element which triggers the spreading of the antibiotic resistance trait.

18.4.2 Mechanism of Resistance to Protein Synthesis Interfering Antibiotics

18.4.2.1 Resistance to Aminoglycosides

Aminoglycosides are effective bactericidal chemotherapeutic agents that interfere with the protein synthesis of the bacterial cell by binding with 30S ribosomal subunit followed by misread of genetic code. The intrinsic resistance of enterococci against aminoglycosides is imparted by inactivating the aminoglycoside through covalent modification of amino or hydroxyl groups which is carried out by enterococcal enzymes.

E. faecium express 6′-acetyltransferase enzymes [AAC (6′)-Ii] which was reported to modify tobramycin, kanamycin, sisomicin, and netilmicin. Moreover, numerous isolates from clinical samples also possess the enzyme APH(3′)-IIIa which triggers the resistance against amikacin and kanamycin owing to its phosphotransferase activity (Costa et al. 1993). Alongside, in *E. faecium*, the bypassing of the aminoglycoside action was carried out by modifying the ribosomal target through the action of rRNA methyltransferase which methylates cytidine residue at 1404 position (Galimand et al. 2011).

Gentamycin and streptomycin are the aminoglycosides that are used in clinical practice reliably because these two are not readily degraded by enterococci-produced intrinsic enzymes. APH(2′)-Ic is another gene encoding phosphotransferases reported in *E. gallinarum*, *E. faecium*, and *E. faecalis* which counteracts against gentamycin (Chow et al. 1997) and tobramycin but not in against of amikacin, whereas APH(2′)-Id, isolated from *E. casseliflavus* and *E. faecium*, confers gentamycin resistance but not against amikacin. Moreover the presence of another gene,

aph (2')-Ib, in *E. faecium* causes amino-glycoside resistance except for amikacin and streptomycin (Eliopoulos et al. 1984; Courvalin et al. 1980).

18.4.2.2 Resistance to Oxazolidinones and Linezolid

Bacteriostatic agent linezolid binds to the 23S rRNA of Gram-positive bacteria and causes disruption in the docking of charged tRNA in ribosomal A site, followed by inhibition in the peptide delivery and elongation of the polypeptide chain subsequently (Shinabarger et al. 1997; Leach et al. 2007; Locke et al. 2009; Mendes et al. 2008). The mechanism of linezolid resistance is the gene mutation which generally encodes 23S rRNA, an important ribosomal drug-binding site (Marshall et al. 2002; Chen et al. 2013; Diaz et al. 2012, 2013). Moreover, linezolid resistance develops in enterococci through acquirement of methyltransferase gene followed by modification of A2503 in the 23S rRNA (Kehrenberg et al. 2005; Vester 2018; Wang et al. 2015). Many copy of the 23S rRNA gene present in enterococci, and as much as the gene becomes mutated, the resistance property is increased concomitantly (Boumghar-Bourtchaï et al. 2009; Bourgeois-Nicolaos et al. 2007; Toh et al. 2007).

18.4.2.3 Resistance to Streptogramins, Macrolides, and Lincosamides

Unlike *E. faecium, E. faecalis* is resistant to pristinamycin derivatives, streptomycin A and B.

In *E. faecalis* genome, *Isa* gene encodes an ATP-binding cassette (ABC) transporter protein necessary for efflux pump which eliminates the action of lincosamide and streptogramin A (Singh et al. 2002). Similar type of pumps coded by *msrC* has also been reported to act in removing the streptomycin A and B (Portillo et al. 2000). An intrinsic resistance mechanism of chromosome towards macrolides by *msr*(A) and to linosamides by *linB* in *E. faecium* has been documented (Portillo et al. 2000; Bozdogan et al. 1999). Several other genes in *Enterococcus* genus are also responsible for conferring resistance like gene *mef*(A), causing resistance to macrolides; *vgb*(A), causing resistance to lincosamide; and *vat*(D) and *vat*(E), causing resistance to streptogramins.

18.4.2.4 Resistance to Daptomycin

Daptomycin binds with cellular membrane facilitated by calcium that causes alterations in its characteristics and function. It is a cyclic lipopeptide that primarily interacts with phosphatidylglycerol and, in the presence of calcium ions, aggregates and enters into the cell membrane and reaches to the inner leaflet. This causes leakage of ions, and also formation of pores occurs on the cell membrane. It also causes lipid aggregation on the membrane surface by "lipid extraction effect." Daptomycin-

resistant enterococci are reported and it is achieved by means of mutations. Report suggests that *E. faecium* repulses daptomycin from its cell surface by changing membrane phospholipids which is commonly associated with mutation in *liaFSR* operon (García-Solache and Rice 2019; Miller et al. 2016). Mutation in *liaFSR* system causes synergism between ampicillin and daptomycin in daptomycin-resistant *E. faecium* (Mishra et al. 2012).

18.4.2.5 Resistance to Tetracyclines and Glycylcyclines

Tetracyclines exhibit bacteriostatic effect by interfering with the aminoacyl-tRNA docking in the ribosome. Enterococci-acquired tetracycline resistance by ribosome shielding mechanism is facilitated by *tet(M)*, and antibiotic efflux mechanism is facilitated by *tet(L)* genes (García-Solache and Rice 2019). Several other genes like *tetO* and *tetS* confer resistance to doxycyclines, minocyclines, and tetracyclines and are transferred via the Tn*916* transposon. The encoded proteins of the above-mentioned genes hydrolyze GTP in the presence of ribosome and cause alteration of ribosomal conformation and finally displace bound tetracyclines (Rice 1998; Speer et al. 1992).

Tigecycline belongs to glycylcycline which is a broad-spectrum antibiotic used as therapeutics in severe infections in skin, soft tissues, and abdomen. It binds with the 16S rRNA and causes inhibition in the association of aminoacyl-tRNA. In tigecycline-resistant *E. faecium,* increased expressions of *tet*(M) and *tet*(L) genes were reported to confer tigecycline resistance (Fiedler et al. 2016).

18.4.3 Mechanism of Resistance to Antibiotics That Interfere in Central Dogma

18.4.3.1 Resistance to Quinolones

For the onset of cell division, starting of replication and transcription of DNA is important. Quinolones generally target two enzymes like DNA gyrase and topoisomerase IV. Those enzymes are responsible for the replication and transcription process. Administration of quinolones causes disruption of strand continuity, stopping replication process (Hawkey 2003). This antibacterial compound shows broad-spectrum effect on numerous bacteria by targeting the two said enzymes. Reduction of antibacterial activity of fluoroquinolones against *Enterococci* has also been reported (Oyamada et al. 2006). Though enterococci acquire low levels of quinolone resistance, sometimes it can also confer high-level resistance by several mechanisms (López et al. 2011; Werner et al. 2010; Yasufuku et al. 2011). Mutations in the *gyrA* and *parC genes* are responsible for the acquisition of resistance (in the case of levofloxacin and moxifloxacin) in *E. faecium* and *E. faecalis* (Tankovic et al. 1999; Kanematsu et al. 1998). EmeA and NorA like efflux pumps have also been

reported for conferring the resistance of *E. faecalis* and *E. faecium* against quinolones, respectively (Hooper 2000). Another gene, *qnr*-encoded protein, is also responsible for the formation of quinolone-gyrase complex, protecting DNA gyrase, and in this way it confers resistance in *Enterobacteriaceae* (Arsène and Leclercq 2007; Tran et al. 2005).

18.4.3.2 Resistance to Rifampicin

Rifampicin binds with the β-subunit of DNA-dependent RNA polymerase and thus inhibits the process of transcription. Rifampicin-resistant *E. faecium* is developed due to substituted mutation in *rpoB* gene (H486Y) which encodes the said enzyme (Kristich and Little 2012). Moreover, *rpoB*-mutated *E. feecium* and *E. faecalis* show elevated resistance to cephalosporin (Enne et al. 2004; Rand et al. 2007).

18.4.3.3 Resistance to Trimethoprim and Sulfamethoxazole

Trimethoprim and sulfamethoxazole are the two notable antibacterial compounds that mainly target the enzymes associated with folate biosynthesis. Folate is synthesized from the *p*-amino benzoic acid and essential for synthesis of nucleic acids. The aforementioned compounds decrease the production of dihydrofolate and also blocked the conversion of tetrahydrofolate by inhibiting several enzymes in folate biosynthesis pathway. Though in vitro susceptibility is present, in vivo reports showed that these two antibiotics are ineffective against enterococci as they have gained the ability to utilize exogenous folate (Chenoweth et al. 1990; Grayson et al. 1990).

18.5 Alternative Strategies for Combating Multidrug-Resistant *Enterococcus*

The evolution of MDR enterococci has boosted interest towards alternative therapies to alleviate the disease causing potentiality of enterococci. Though virulence factors do not directly confer resistance, it will help bacteria to withstand in an unfavorable environmental condition and resist host defense mechanisms. Host biomacromolecules associated with the cell surface of *Enterococcus* and release of these molecules into the extracellular matrix inhibit the antimicrobial drugs from reaching their targeted sites (Otto 2006). Cyclic-AMP (cAMP) as an important mediator of innate immune system imparts antimicrobial activity by disturbing PPG biosynthesis and cytoplasmic membrane structure (bacterial) as well as promotes autolysins which collectively help to keep microbial populations within threshold level. However, coevolution of cAMPs and their bacterial targets is well docu-

mented (Kandaswamy et al. 2013; Gilmore et al. 2013). Exploitation of host adaptive immunity is also targeted through vaccination for the production of antibodies against enterococci. In this context, the lipoteichoic acids and diheteroglycans present over the cell walls of enterococci are marked as an epitope as they will help to induce an antibody response. This will protect the host (mouse bacteremia model) against *E. faecalis* (Theilacker et al. 2011). Application of antibodies against those specific enterococcal antigenic motifs could be a possible therapeutic to combat MDR strains in the future.

18.6 Application of Nanotechnology Against Enterococcal Infections

Development of multidrug-resistant enterococci becomes a most pressing concern in community health worldwide. The WHO (World Health Organization) and CDC (Center for Disease Control) have already expressed major concern about the gradual increase in the formation of multidrug-resistant bacteria (Baptista et al. 2018). This has boosted researchers to develop potent strategies for drug delivery and, finally, targeting bacteria. Nanostructured materials (e.g., organic, inorganic, metallic, carbon nanotubes, etc.) are being synthesized to circumvent such types of drug resistance as they easily convey antimicrobials, assist novel drug delivery, exert antimicrobial activities, and inhibit biofilm development (Baptista et al. 2018).

Several attempts were made for the synthesis of potent nanoparticles and subsequent effective delivery of the same against multidrug-resistant enterococci (Katva et al. 2018). Silver is a nontoxic, safe antimicrobial inorganic agent, and silver nanoparticles (AgNPs) have obtained much more attention as compared to other metal-based nanoparticles due to its strong antimicrobial activity. AgNPs are the utmost encouraging inorganic nanoparticles that can be applied for the alleviation of enterococcal infections. It was demonstrated that AgNPs in combination with vancomycin exhibited excellent antibacterial potential against vancomycin-resistant *E. faecalis*. Likewise, a mixture of gentamycin, chloramphenicol, and AgNPs could be promising to treat MDR *E. faecalis* infection than both the above-mentioned antibiotics separately (Katva et al. 2018). The antibacterial efficiency of AgNPs was also evaluated by Wu et al. (2014) against *E. faecalis* biofilm. Otari et al. (2013) also showed the effect of AgNPs on the erythromycin-resistant *E. faecalis*. It was suggested that AgNPs inhibit bacterial growth and proliferation by adhering on the cell wall of bacteria, leading to cell wall modification followed by penetration of AgNPs into the bacterial cell, which consequently damages the DNA leading to cell death (Aziz et al. 2015, 2016; Kumar et al. 2016; Saini et al. 2019).

Khiralla and El-Deeb (2015) developed biogenic spherical selenium nanoparticles using cell-free supernatant of *Bacillus licheniformis* which imparted paramount antimicrobial and antibiofilm potential against *E. faecalis*. Likewise, biogenic palladium nanoparticles were prepared by using flower extract of *Moringa oleifera*

which showed significant antibacterial effect against the same bacteria (Anand et al. 2016).

Graphene oxide (GO) has unique physicochemical characteristics and has therefore attracted attention for antibacterial use (Hu 2010). The GO nanosheets exhibit antibacterial activity through direct interaction with bacteria and increased the reactive oxygen species (ROS) level within the cell (Akhavan and Ghaderi 2010). Govindaraju et al. (2016) demonstrated that UV-irradiated form of glucosamine-gold nanoparticle-graphene oxide composite exhibited paramount antimicrobial activity against *E. faecalis* which is better than kanamycin, and several functional groups (like carboxyl, hydroxyl, and epoxy) present in the GO-based nanomaterial are responsible for the activity. Nanocomposite of indocyanine green and GO was also reported to exhibit potential antibacterial effect against *E. faecalis* during photodynamic therapy (Akbari et al. 2017).

In order to treat vancomycin-resistant *Enterococcus*(VRE), Zhou et al. (2018) prepared Au/Ag bimetallic NPs and demonstrated that it has immense potential to be a good anti-enterococcal agent. Both in vitro (bacterial surface-enhanced Raman scattering imaging) and in vivo (mouse infection assays) results clearly revealed the effectiveness of this newly developed nanocomposite against VRE.

Chifiriuc et al. (2013) also investigated the capability of magnetic nanoparticle for a sustained and controlled release of drug which subsequently increases the effectiveness of antibiotics against resistant opportunistic pathogen, *E. faecalis*. They also suggested that magnetic nanoparticles might be a potent carrier for delivery of amino-glucoside antibiotics.

The antibacterial efficacy of calcium hydroxide nanoparticle (NCH) showed better result against *E. faecalis* in dentin block model. The MIC determination and agar diffusion test revealed that low concentration of the NCH inhibited *E. faecalis* than the native form of calcium hydroxide which is due to the enhancement of surface area due to smaller size which encourages the penetration of the NPs into the deeper layers of dentin which subsequently inhibits *E. faecalis* growth (Dianat et al. 2015).

Despite the expected potential of newly reported nanoparticles against multidrug-resistant *Enterococcus*, there are still few shortfall related to their safety when they are used in long-term basis in human. Therefore, in-depth assessment of the physical, chemical, and biological compatibility must be addressed. Experimental proof is also desirable for establishment of mechanism of action against the targeted enterococci in vivo. Moreover, the fruitful translation of the R&D work into real-life large-scale production of the newly discovered nanoparticles needs comprehensive guidelines, and effort is needed.

18.7 Conclusions and Future Challenges

Enterococcal species can colonize and survive in different biological and environmental niches. Owing to their biofilm-forming ability, multiple-drug resistance, and tendency of transfer of resistant trait to other enterococci, it became a great burden

in healthcare sectors. Among the several species of enterococci, *E. faecalis* and *E. faecium* are associated with most clinical cases and hence they are marked as important nosocomial pathogens. Continuous exposure to prophylactic or metaphylactic and random application of antimicrobial agents by clinicians in human and animal hosts against enterococci contributed its ability to acquire and develop unique profiles of virulence and antimicrobial drug resistance. Moreover, expression of a wide variety of virulence characteristics promotes enterococci to colonize and also causes infections in the host body. Extensive tolerance to the antibacterial agents as well as their wondrous capacity to acquire resistance to marketed antibiotics becomes a great challenge to clinicians throughout the globe to combat with enterococcal pathogenesis. In the recent future, MDR enterococci will be immense clinical challenges to treat infections in hospitalized patients. Current trends in the epidemiology and population structure of antibiotic-resistant *Enterococcus* species clearly suggest that MDR enterococci may become the most common species isolated from patients in the upcoming eons. Nanotechnology is an emerging branch of science which could restrict the propagation of enterococci. Various attempts were already made worldwide to develop versatile nanomaterials that exhibited immense potentiality to limit enterococcal growth in in vitro and in vivo. However, with the advent and advancement of nanotechnology, more studies are extremely necessary to develop comprehensive strategies to limit the *Enterococcus*-associated infections and their large-scale implementation in upcoming eons.

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