Enterococcal Infections and Drug Resistance Mechanisms

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Abstract

Multidrug resistance of pathogens is a serious issue which is emerging these days and causing a serious threat to mankind. The species belonging to *Enterococcus* genera, namely *Enterococcus faecium* (*E. faecium*) and *Enterococcus faecalis* (*E. faecalis*) are next to *Staphylococcus aureus* in causing severe health issues. The

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emerging resistance of *Enterococcus* species towards various antibiotics is posing a serious challenge in the therapy of diseases. *E. faecium* and *E. faecalis* exhibit resistance (intrinsic, acquired, and tolerance) against antibiotics. Moreover, the exchange of genetic material between or within the genus is the major concern for the proliferation of antibiotic resistance. Apart from the above the strains isolated from different geological niches exhibited varying pattern of resistance. However, the combination of cell wall active drugs with aminoglycosides acts as an alternative therapy in very few cases like *E. faecalis* associated endocarditis. With this knowledge, this chapter highlights the infection caused by *Enterococcus* species and its consequences. The sensitivity and resistance of *Enterococcus* species against different types of antibiotics will be discussed. The underlying molecular mechanism, genes responsible for resistance, and the treatment approaches for susceptible versus resistant strains will also be emphasized.

Keywords
Enterococcus species · Pathogen · Infection · Antibiotics · Resistance

9.1 Introduction

Hospital-associated *Enterococci* species are the major cause of infections like urinary tract infections (UTI), bacteremia, and endocarditis (Goh et al. 2017). Systemic infection of *Enterococci* starts from dense colonization in the gastrointestinal tract (GI), and gains access to various sites through the bloodstream (Goh et al. 2017). *Enterococcus faecium* and *E. faecalis* are the two strains reported with increased frequency of human infection as compared to other *Enterococcus* species (Gordon et al. 1992). Intestinal colonization of drug-resistant *Enterococci* is favored due to the long-term exposure of antibiotics in hospitalized patients. Infectious *Enterococci* are capable of forming biofilm and are often resistant to antibiotics like ampicillin, vancomycin, and aminoglycosides (Sydnor and Perl 2011; Van Harten et al. 2017). Global epidemiology report demonstrates that *Enterococcal* infections are the major cause of nosocomial diseases worldwide due to their antibiotic-resistant nature (Goh et al. 2017; Sydnor and Perl 2011). The standard line of evidence suggests that *Enterococcus faecium* is the most common bacteria acquiring intrinsic resistant on exposure to various antibiotics as compared to other *Enterococcal* species (Hollenbeck and Rice 2012). Emerging resistance to specific antibiotic was controlled through synergistic therapy. But the mechanism of synergistic bactericidal activity due to a combination of antibiotic remains a mystery. This chapter reviews the literature behind the genetic transfer of antibiotic resistance, a summary of various risk factors involved in enterococcal infection, virulent determinant, and the mechanism of antibiotic resistance along with possible treatment options.

9.1.1 Historical Overview of *Enterococcal* Antibiotic Resistance

Enterococcus is a common microbial flora of human and animal intestine (Dubin and Pamer 2014). The discovery of *Enterococcus genus* comes to attention through bacterial isolates from infective endocarditis in 1899, until then kept under the subset of *Streptococci* species (Schleifer and Kilpper-Balz 1984). Only after 1984, *Enterococcus* strains were proved to exhibit genetic diversity from *Streptococcus* species and allocated in to a separate genus (Dubin and Pamer 2014; Schleifer and Kilpper-Balz 1984). It acts as early gut colonizers in many animals including humans. It is the most common genera of fecal content in birds, reptiles, mammals, and humans (Gilmore et al. 2013). In human feces, *E. faecalis* and *E. faecium* were the most predominant species reported so far (Fisher and Phillips 2009). The genus of *Enterococcus* are mostly commensal in nature and some of the species are the leading cause of nosocomial infections in hospitalized patients next to *Staphylococci* (Gilmore et al. 2013). The antibiotic penicillin was found to be remarkably effective against *Streptococcal* infectious (Williamson et al. 1983). However, penicillin was less effective against *Enterococci* infections due to the inherent resistance (Handwerger et al. 1993). Later, several research findings suggest that the synergistic effect of these antimicrobial agents with aminoglycosides results in significant improvement in endocarditis caused by *Enterococcal* infection (Eliopoulos and Moellering 1982; Serra 1977). Noteworthy, multiple courses of antimicrobials usage in clinical care seem to evolve *Enterococcus* with antibiotic-resistant determinants. Thus, by the twentieth century, there is an incredible increase in multidrug resistant (MDR) *Enterococci* just by acquiring drug-resistant genes (Van Harten et al. 2017). In addition, the modern-day MDR *Enterococci* exposed to multiple antibiotics come into sight of diverged genetic clade with increased transposable elements, rapid mutation, and metabolic alterations (Gilmore et al. 2013). For instance, enterococci recruit vancomycin resistance with improved biochemical machinery of various gene clusters (Van Harten et al. 2017). These resistances were transmitted to most pathogenic bacteria like methicillin resistance *Staphylococcus aureus* (MRSA), making very serious public health concern. It is well documented with emerging resistance of enterococci against all the available gram-positive agents like quinupristin, daptopristin, linezolid, and tigecycline (Van Hoek et al. 2011; Hollenbeck and Rice 2012; Binda et al. 2014).

9.2 Risk Factors Associated with *Enterococcal* Infection

9.2.1 Stable Colonization

It was reported that *Enterococcus* strains are less-abundant bacteria inhabited in the intestine of human (Van Harten et al. 2017). However, the density of colonization increases with nosocomial infection of resistant *Enterococci* species. It was reported that the administration of multiple antibiotics to the hospitalized patients induces substantial change in the gut microbiota, thereby facilitating the colonization of resistant strains of *Enterococci* species (Ubeda et al. 2010). For instance, gram-negative

commensals lipopolysaccharide and flagellin induce the paneth cells to produce REGIIIγ which are active against gram-positive bacteria. Thus, the decrease in population of gram-negative commensals due to antibiotic treatment facilitates an increase in the population of resistant *Enterococci* (Kinnebrew et al. 2010). Notably, the use of higher minimal inhibitory concentration of antibiotics like neomycin, vancomycin, and metronidazole in hospitalized patients displayed preferential growth of vancomycin-resistant *Enterococci* (Brandl et al. 2008).

9.2.2 Nosocomial Transmission

The dissemination of resistant *Enterococci* among the hospital settings is widely due to its adaptation to extreme environmental conditions prevailing in the surroundings. Hence, it can remain active for longer periods in surgical units, including surfaces of medical devices, doorknobs, bed rails, and so on (Sydnor and Perl 2011). The major reasons for the development of nosocomial infection caused by *Enterococci* species were due to the multi-dose regimen, long-term hospitalization, transmission through physical proximity of infected patients, organ transplantation, and co-morbidities like catheter-associated infections, and hemodialysis (Bradley and Fraise 1996). In the hospitalized patients, spreading of resistant *Enterococci* is widely through the nurse handling different patients suffering from various diseases (Sydnor and Perl 2011; Bradley and Fraise 1996).

9.2.3 Virulence Determinants of Enterococcal Infections

9.2.3.1 Secreted Virulence Factor

Secretions of virulent proteins were more prevalent in clinical isolates of *Enterococci* (Benachour et al. 2009). Cytolysin, one of the important virulent factors released outside the cell, is divided into two subunits, CylL-L and CylL-S. Cytolysins are capable of lysing RBC and WBC of humans, rabbits, and horses (Shokoohizadeh et al. 2018). Moreover, the virulence nature of cytolysin was due to membrane damage in host tissue. Gelatinase-E (gelE) is another important virulent factor with protease activity mainly degrading the misfolded proteins (Benachour et al. 2009). GelE gene expression is controlled by Fsr quorum sensing system, as GelE is regarded as the most frequent virulent determinants expressed (48.5%) in *E. faecalis* (Shokoohizadeh et al. 2018). Perhaps, GelE activates the peptidoglycan-degrading enzymes such as autolysin, in turn, the release of extracellular DNA and so mediates pathogenesis through biofilm formation. SagA is a major secreted antigen from *E. faecium* accomplishing broad-spectrum extracellular matrix binding (ECM) like fibrinogen, fibronectin, laminin, type-I and type-II collagen (Kropec et al. 2011).

9.2.3.2 Cell Surface Determinants

Cell surface determinants are another putative virulent factor comprising of an array of components which endorse colonization and biofilm formation for

Enterococcal infection (Kang et al. 2013; Soheili et al. 2014). The surface protein in *Enterococcus* is encoded by esp gene which helps in cell adhesion and commonly found in clinical isolates of patients suffering from endocarditis or urinary tract infections (UTIs) (Soheili et al. 2014). The surface of *E. faecalis* is comprised of protein, namely aggregate substance (AS), which aids in binding to cultured renal epithelial cells, colon cell internalization, and tolerant to immune response (polymorphonuclear neutrophils) (Sava et al. 2010). In addition, at the early stage of infection, microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) recognize the microbial surface which help in adhering to LPxTG (Leu-Pro-x-Thr-Gly) type surface anchored proteins and pili gene cluster (PGC) proteins helps in *Enterococcal* binding to host extracellular matrix (Kang et al. 2013; Hendrickx et al. 2009). In this way, two reputed adhesins such as Ace and Acm which help in binding to collagen produced by *E. faecalis* and *E. faecium* were broadly studied in *Enterococcus* species (Kang et al. 2013). Ace adhesin provisionally expressed in clinical isolates of *E. faecalis* during its growth conditions in collagen was found to be a major contributor in endocarditis (Kang et al. 2013). On the other hand, Acm facilitates the binding of *E. faecium* to the collagen (Kang et al. 2013; Soheili et al. 2014). Acm gene expression was more frequently found in hospital-acquired *E. faecium* compared to the community (animal and human) acquired strains (Soheili et al. 2014). In *E. faecalis*, ElrA, an enterococcal leucine-rich protein-A, sustains the strain from evasion of macrophage recognition and adhesion (Nunez et al. 2018). Two important enterococcal proteins origin from pilus loci proficiency in biofilm formation of *E. faecalis* such as *Ebp* (endocarditis biofilm-associated pili) and *Bee* (Sava et al. 2010). *Ebp* helps in causing enterococcal pathogenesis in experimental endocarditis and UTI. Similarly, *Bee* an *Enterococcal* biofilm enhancer from pilus loci of *E. faecalis* mediates biofilm-associated pathogenesis (Hendrickx et al. 2009). Critical components of membrane structures like capsular polysaccharide, lipoteichoic acid (LTA) can promote skirting of *Enterococci* from phagocytosis (Hendrickx et al. 2009). For instance, Epa (Enterococcal polysaccharide antigen) a rhamnose containing polysaccharide has the ability to enhance the biofilm formation and translocation across enterocyte monolayer. The adhesion and biofilm formation is higher in mutant strains of *E. faecalis* as compared to wild-type strain due to higher production of lipoteichoic acid (LTA) and glycolipids in cell membrane (Hendrickx et al. 2009; Sava et al. 2010). Distribution of various virulent determinants expressed in *Enterococcal* cell wall is mentioned in Fig. 9.1.

9.2.3.3 Phosphotransferase System (PTS)

The role of PTS system is to translocate the phosphorylated carbohydrate across the bacterial cell membrane (Peng et al. 2017). Mostly in clinical samples of *E. faecium*, PTS system is associated with virulence and stress response (Paganelli et al. 2016). Virulent types of PTS system were widely distributed in clinical isolates and lack in commensal strains (Peng et al. 2017; Paganelli et al. 2016). Among the PTS system, the most notable one is PTS permease BepA, which plays a significant role in endocarditis (Paganelli et al. 2016).

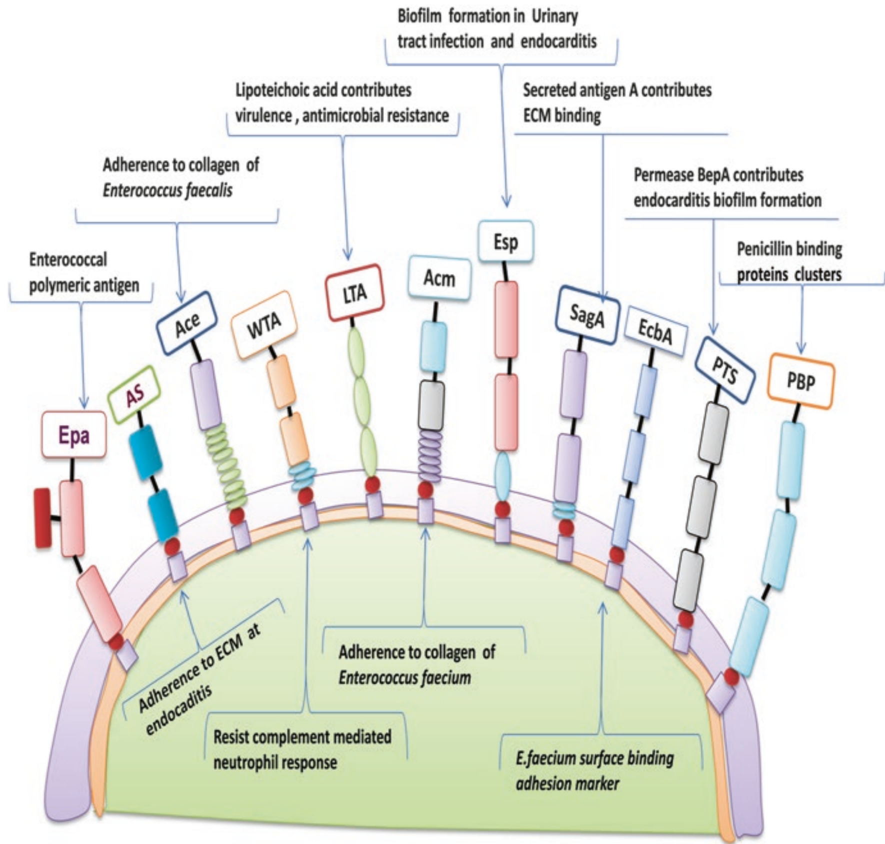

Fig. 9.1 Overview of enterococcal cell surface determinants responsible for virulence expression

9.2.4 Dissemination of Antibiotic Resistance Among Enterococci Species Through Horizontal Gene Transmission

The unrestrained practice of broad-spectrum antibiotics instigates the survival mechanism of certain gut microbial flora by promoting multidrug resistance in them (Chajęcka-Wierzchowska et al. 2019). The gastrointestinal tract of humans is the hot spot for antibiotic resistance. The gastrointestinal tract is colonized with diverse bacterial flora through biofilm formation and regarded as the hot spot region for acquisition of resistance (Schjorring and Krogfelt 2011). The contemporary disquiet in clinical microbiology is the acquired antibiotic resistance among *Enterococci* that colonizes gut lumen of human, which is highly difficult to medicate (Manson et al. 2010; Palmer et al. 2010). Consuming the fermented food, and ready to eat edible foods from various sources rich in bacterial population will act as a donor and/or recipient of antibiotic resistance (Togay et al. 2010; Chajęcka-Wierzchowska et al. 2019). The transition of *Enterococci* from harmless commensals to pesky

pathogen has emerged over decades through various mechanisms such as the acquisition of resistant genes against several antibiotics from other organisms, gene mutations, and modification of bacterial surface molecules (Bourgeois-Nicolaos et al. 2006; Schjorring and Krogfelt 2011). However, these characteristics can be divided into internal and acquired resistance through horizontal gene transfer (HGT). Among these, HGT is the leading cause of infections in hospitals by gaining resistance against commercially available antibiotics (Chajęcka-Wierzchowska et al. 2019). Surprisingly Polidori et al. (2013) found that about 25% genome of *E. faecalis* V583 is integrated from other organisms which made this strain to resist the last-line antibiotic vancomycin. Few years before to this study, the total genome analysis of *E. faecalis* OG1X strain showed that there was no trace of foreign DNA in it (Bourgeois-Nicolaos et al. 2007). This clearly indicated that how selective pressure of antibiotics would coerce commensal to acquire genetic elements from their surroundings through an array of mechanisms. Moreover, *Enterococcus* species resistant to various antibiotics are emerging from diverse environmental sources which could probably enter our system and cause adverse effects (Conwell et al. 2017).

9.2.4.1 Antibiotic Resistance Mechanism in *Enterococci* Species

It is proven that gram-negative bacteria of the gut flora are more prone to accomplish antibiotic-resistant gene and might transfer it to other members of the microbiome (Bourgeois-Nicolaos et al. 2006). There is a more chance of this acquisition in the hospital environment where antibiotics are targeted frequently against gram-negative bacteria (Schjorring and Krogfelt 2011; Lerminiaux and Cameron 2019). Acquired resistance in *Enterococci* generally takes place by the exchange of pheromone-responsive genes or plasmids, or transposons. The most pathogenic species in *Enterococci* are *E. faecalis* and *E. faecium* which possess resistance towards several antibiotics (Palmer et al. 2010; Quinones et al. 2018). Most of the *Enterococci* strains hold multiple plasmids and transposons with the ability to resist antibiotics and exhibit virulence factors (Palmer et al. 2010). Some common virulent genes encoded in plasmids and transposons are enlisted in Table 9.1.

9.2.4.2 Plasmids

The antibiotic-resistant genes are present in the extra chromosomal elements called plasmids, which is transferred to neighboring species through bacterial conjugation (Clewell 2011). Among several genera that colonizes the gut lumen, plasmid characterizations are studied extensively in *Enterococci* bacteria (Di Sante et al. 2017). Based on the mode of transfer and host organisms the plasmids are categorized into two major classes: (a) pheromone-related plasmid and (b) broad host range plasmids (Weaver 2019).

9.2.4.2.1 Pheromone-Responsive Plasmids

These plasmids are transformed into recipient cells from donor cells in response to pheromone signals received from the environment (Clewell 2011). It was detected in *Streptococci* that pheromone signals are transferred through plasmids by conjugation (Tomura et al. 1973). In the entire *Enterococci* family, *E. faecalis*

Table 9.1 Plasmids and transposons in *Enterococcal* antibiotic resistance

S. No	Plasmid/ chromosome type	Antibiotic resistance gene	Resistance mechanism	References
1	pAD1	*vanB, ermB*	Carrier of resistant determinant	Clewell (2007)
2	pCF9	*tetM, uvrA*	Carrier of tetracycline A-resistance protein	Hirt et al. (2018)
3	pAMβ1	*ermB*	B-subunit, a carrier of resistant determinant	Clewell (2011)
4	pIP501	*cat, ermB*	Carrier of resistant determinant	Palmer et al. (2010)
5	pLG1	*vanA*	Carrier of vancomycin A-resistance protein	Laverde Gomez et al. (2011)
6	Tn5281	*aac-6′-aph-2″*	Aminoglycoside modifying enzyme	Feizabadi et al. (2008)
7	Tn5384	*ermB*	*β-lactamase* production	Bonafede et al. (1997)
8	Tn5385	*tetM, ermAM, aadE, Bla*	*β-lactamase* production	Rice and Carias (1998)
9	Tn*917*	*ermB, tnPR, mefE, tnPA*	Ribosome methylase activity, efflux action	Rice (2014)
10	Tn*552*	*Bla, aacA-aphD*	*β-lactamase*	Sarti et al. (2012)
11	Tn*1546*	*aac6′-aph2″*	Aminoglycoside modifying enzyme	Simjee et al. (2002)
12	Tn916	*tetM*	Ribosomal protection	Santoro et al. (2014)

predominantly possesses these pheromone-responsive plasmids and transforms it into other strains (Clewell 2011). It was witnessed that these kinds of plasmids could not replicate in the host cells other than *Enterococcal* species. Because, *Enterococci* chromosome expresses several peptides that could act as pheromones which stimulates specific pheromone-responsive plasmids in the target cells (Dunny 2013). Initially, the extra cellular signals produced by recipients will induce donors to initiate conjugation. During this transfer, recipient cells synthesize the chromosomally encoded lipoprotein fragment, which will be detected by donors. This process initiates the cascade reaction and stimulates the production of aggregation substances like *Asa1* and *PrgB* in the plasmids of the donor (Hollenbeck and Rice 2012). Then, these peculiar substances bind with the enterococcal binding element on the recipient cell surface and instigate conjugation. Experiments suggest that co-cultivation of donor and receipt strains enhances the conjugal exchange of pheromone-related plasmids (Dunny et al. 1978; Dunny and Clewell 1975). Though the transfer rate was minimal in the initial hours, the ratio of transconjugants and the parental strain was increased within a few hours from the startup and visible cell clumping started appearing in the medium. The efficiency of genetic transformation between *E. faecalis* strains is 10^{-3} donor cells during 4 h of matting (Kristich et al. 2014). More evidently, when the cell-free filtrate of recipient cells which presumed to contain the clumping inducing agent (heat resistance and enzyme sensitive

compound) was added to the medium containing donor cells carrying conjugative plasmids induced cell aggregation (Dunny 2013). These signaling molecules were later called as bacterial sex pheromones. The widely considered pheromone-related plasmids among *Enterococcus* are pAD1 and pCF10 which encode for antibiotic resistance and virulence factor (Kristich et al. 2014). Transfer of plasmids among species was majorly controlled by two competitive molecules, namely pheromones and inhibitors.

9.2.4.2.2 pCF10

pCF10 is a 67.7 kb, a plasmid that habitually carries antibiotic resistance gene (tetracycline) and certain virulence genes (hemolysin and bacteriocins) which is efficiently disseminated among *Enterococci* of the different environment (Hirt et al. 2018). Over 25 genes from pCF10 are responsible for cell aggregation; matting channel formation and conjugal plasmid transfer have been reported. Conjugal transfer of this plasmid is majorly controlled by two peptides, i.e., cCF10 (c-pheromone, released by recipient cells) and iCF0 (i-inhibitor, expressed by donor cells), where conjugation is induced by cCF10 and inhibited by counteracting peptide iCF0. cCF10 peptide from recipient cell induces the pCF10 plasmid of contributor cells to initiate the conjugal transfer. In the absence of receiver, so as to avert the conjugal transfer, pCF10-encoded iCF10 will be expressed and halt the plasmid transmission (Kohler et al. 2019).

Upon external stress, bacteria will produce cCF10 in search of donor cells for conjugal transfer of plasmids. Thus, this cCF10 peptide is imported into a donor cell by surface receptor *PrgZ* and accumulates in the cytosol (Hirt et al. 2018; Breuer et al. 2018). There it competes with iCF10 for transcriptional factor *PrgX* in order to regulate the transcription of pCF10 plasmid by modifying the PQ promoter of the operon PrgQ and downstreaming genes (Kozlowicz et al. 2004; Chen et al. 2017). The negative regulator of PQ is reported to be *PrgX* which is derepressed by cCF10 and enhanced by iCF10. *PrgX* is a tetramer protein which could bind both cCF10 and iCF10 and interact with the operator sites of the PQ region contrarily. The structural difference in between these two peptide complex molecules either suppresses by inhibiting the RNA polymerase binding (iCF10) or enhances (cCF10) the expression of conjugation genes (Dunny 2013). The schematic representation for the spreading of antibiotic resistance in *Enterococcus* species is demonstrated in Fig. 9.2.

PrgQ operon (26 kb) encodes *PrgA*, *PrgB*, *PrgC* cell wall binding proteins which are responsible for cell aggregation, the formation of mating junctions, plasmid transfer into the recipient cell (Bhatty et al. 2015). In addition to this, the current results show that *PrgA* is the significant component involved in biofilm formation and binding of *Enterococci* into abiotic surfaces in the symbiotic environment (Bhatty et al. 2015). In some mammalian infection models these genes also expressed virulence factors. Regulation of *PrgQ* operon is majorly controlled by the relative concentration of peptides in the donor cells, which in turn, enhance the plasmid transfer in recipient cells (Chen et al. 2017; Kohler et al. 2019). In the GI tract, beyond plasmid transfer, acquisition of antibiotic, virulence genes, and other factors that aid in the interaction of enterococci with its mammalian host might lead

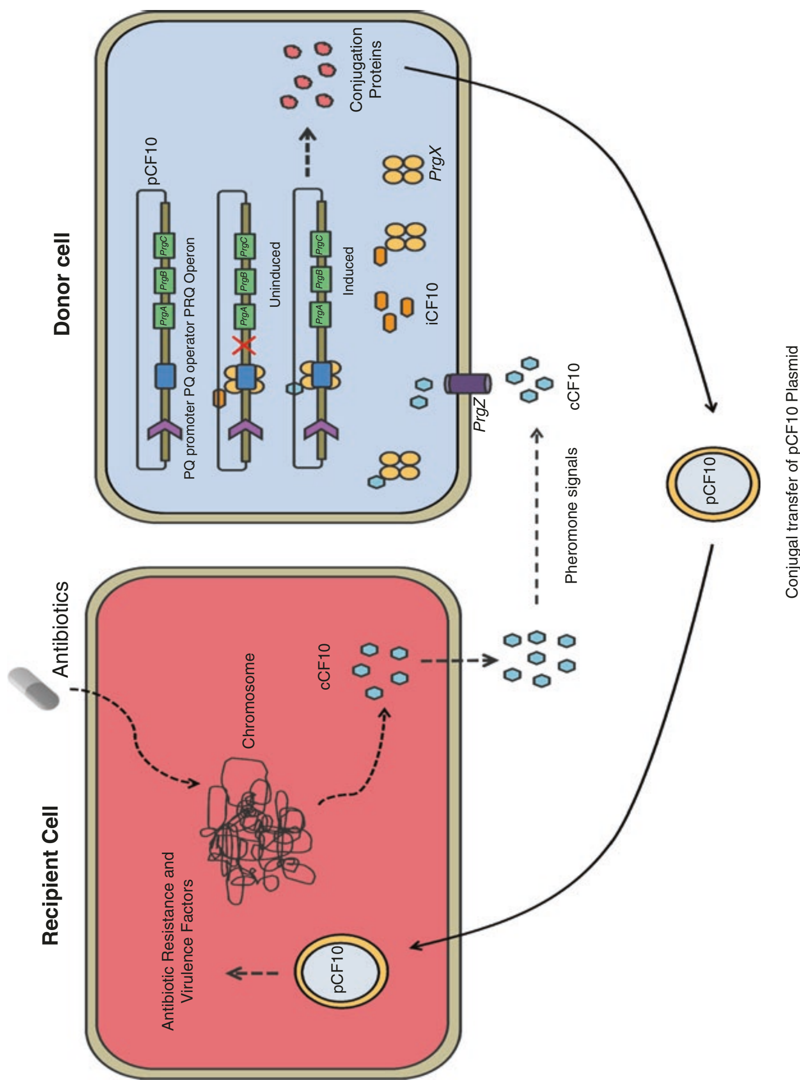

Fig. 9.2 Pheromone-responsive conjugal transfer of Pcf10 plasmid from donor to recipient cells

to the unsolicited effects. For instance, the expression of surface adherence molecules could negatively influence the host innate immune response and lead to adverse effects.

9.2.4.2.3 pAD1

It was reported that pAD1 is a 60 kb pheromone-related plasmid which is commonly reported in clinical samples of *E. faecalis* (Francia et al. 2004; Weaver 2019). In reply to the sex pheromone peptide cAD1 formed by receiver cell, pAD1 plasmid will encode mating responsive elements for its efficient own transfer reported in *E. faecalis* DS16 (Dunny and Berntsson 2016; An and Clewell 2002). pAD1 encodes various factors such as hemolysin and bacteriocins which trigger virulence in the mammalian host and develop resistance against several gram-positive bacterial species (Clewell 2007). There are 87 open reading frames (ORF) in the pAD1 plasmid among 9.2 kb segment that codes the genes responsible for cytolysin and two origins of replication, namely *oriT1* and *oriT2* responsible for conjugal transfer (Clewell 2011). In addition, this strain holds 28 kb pAD2 plasmid that encodes antibiotic resistance (erythromycin, streptomycin, tetracycline, and kanamycin). However, pAD2 plasmid does not encode any conjugative elements that make its own transfer (Showsh et al. 2001).

9.2.4.2.4 Wide Host Range Plasmids

These plasmids are involved in transferring genetic information to other gram-positive and gram-negative organisms. Among them, the extensively studied is mismatched group 18 also called as Inc18 (Kohler et al. 2018; Zhu et al. 2010). However, the transmission efficiency of this plasmid is very low when compared to the other type. These Inc18 plasmids are copious in *E. faecium* and their well-characterized prototypes are pAMβ1 (encodes resistance against macrolides, a class of antibiotics that includes erythromycin, roxithromycin, azithromycin, and clarithromycin) and pIP501 (encodes resistance against chloramphenicol in addition to macrolides) (Kohler et al. 2019). The *Enterococcal* strains that contain these plasmids transfer antibiotic-resistant genes to *Streptomyces*, *Leuconostoc*, *Listeria*, *Lactococcus,* and *E. coli* species, respectively (Kohler et al. 2018). In *Enterocccus* species tra genes encodes the plasmid transfer genes which help in easy transfer of plasmids. Tuohy et al. (2002) reported the transmission of wide host range plasmid pAMβ1 from *Lactobacillus lactis* to *Enterococcus* species in an animal model which contains the genetic material that could resist erythromycin and lincomycin. Interestingly this team observed that the bacterium could not transfer it into other bacteria like *Lactobacillus* and *Bifidobacterium*.

9.2.4.2.5 Transposons

Mobile genetic elements, i.e., transposons have been reported to hold and spread virulence factors and antibiotic resistance in *Enterococci* (Hegstad et al. 2010). The sequencing results reveal that mobile genetic elements are scattered within the genome of *Enterococci*. Transfer of these elements will lead to the emergence of numerous phenotypes, such as pathogenicity, ability to colonize the host cells, and

communication between cells. So far, three types of transposons have been reported to transfer genetic materials in *Enterococci* such as Tn3, composite, and conjugative transposons (Hegstad et al. 2010). Complementation is difficult in transposons.

9.2.4.2.6 Tn3 Family Transposons

These are the widespread group of replicative transposons which is associated with dissemination of multidrug resistance. There are several Tn3 family transposons that have been reported in *Enterococci*, including Tn*917*, Tn*552*, and Tn*1546* (Nicolas et al. 2017). Tn*917* is the first Tn*3* family transposon identified in the *Enterococci* that confer resistance to macrolides, streptogramin B, and lincosamides antibiotics (Shaw and Clewell 1985). The *ermB* gene (RNA methylase), *mefE* (macrolide efflux genes *mef*), and mel that are found within this 5 kb element regulate their resistant mechanism. These elements have a broad host range such as *Bacillus*, *Enterococcus*, *Lactococcus*, *Listeria*, *Paenibacillus*, *Staphylococcus*, and *Streptococcus*. In addition, mutant libraries have been successfully developed using this transposon in *S. aureus* and *S. epidermidis* (Rice 2014).

Tn*552* is a β-lactamase encoding transposon identified from *S. aureus* (Rowland and Dyke 1990). Penicillin exposure among *Staphylococci* induces the production of β-lactamase to confer resistance. It is a very rare type of Tn*3* transposon that was detected in *Enterococci* where *bla* gene encodes the resistant element. In the majority of the *E. faecalis* strains, the regulative elements are present in the upstream region of the β-lactamase gene that is either truncated or deleted, as a result the expression might affect the isolates negatively. Since the regulatory genes are non-functional, the expression of β-lactamase was found to be constitutive in these strains and expressed at a very low level (Ono et al. 2005; Tomayko et al. 1996). This could be the reason why Tn*552* transposons are rare in *Enterococci* which exhibits a low level of resistance. However, β-lactamase flanking the complete regulatory genes is recognized in several *E. faecium* strains (Sarti et al. 2012).

Tn*1546* is an 11 kb transposon element regularly seen in the clinical samples of *E. faecium.* These elements confer resistance to vancomycin and are called vancomycin-resistant enterococci (VRE) (Novais et al. 2008; Sletvold et al. 2010; Freitas et al. 2013). Among the genotypes *vanA* and *vanB*, *vanA* is predominant and leads to the development of glycopeptide-resistant *Enterococci* worldwide. The *vanA* cluster comprises of seven genes which include *vanA*, *vanH*, *vanR*, *vanS*, *vanX*, *vanY*, and *vanZ* (Arthur et al. 1993). This transposon is often associated with plasmids and chromosomes, for instance, pIP816, a 4-kb conjugative plasmid present in VRE is known to have this transposon (Kristich et al. 2014). The occurrence of Tn*1546* element isolated from *E. faecium* of the distinct geographical region suggests its rapid spread through HGT. Interestingly, several mutations (deletions and insertions) have been described within Tn*1546*. These alterations result in the multi-resistant *Enterococci* (Kristich et al. 2014). For instance, the insertion of *fosB* gene within the Tn*1546* made the *E. faecium* to resist fosfomycin in addition to the vancomycin (Sun et al. 2017). In contrast, such mutation might cause nil effects, sequence result of Tn*1546* from the clinical isolates of *E. faecium* revealed several modifications in the cluster including 889 bp deletions in the ORF1. Sequence analysis of Tn1546 has revealed that, ORF1

had an 889-bp deletion and an IS1216V insertion at the 5′ end and an IS1251 insertion between *vanS* and *vanH* (Simjee et al. 2002). In the future, the diversity of Tn*1546* transposon will further increase by the sequencing of several *Enterococci* from various environmental sources.

9.2.4.2.7 Composite Transposons

These transposons are flanked by insertion sequences (IS) elements as their mobility is determined by its presence. These are flanked at both the ends and are oriented as direct or as inverted repeats (Hegstad et al. 209). Tn*1547* is the composite transposon that confers the VanB resistance in *Enterococcal* species (Garnier et al. 2000). This 64 kb element is transferred through the chromosome of *E. faecalis* to the hemolysin plasmid. This transposon is bordered by two addition sequences in straight alignment, namely IS256-like and IS16 (Quintiliani and Courvalin 1996).

Tn*5281* element is the next commonly observed composite transposon, which provides resistance against all aminoglycoside antibiotics excluding streptomycin in *E. faecalis* and *E. faecium* (Feizabadi et al. 2008). However, this transposon containing *Enterococci* strains prevalently resist the antibiotic gentamycin. The gene responsible for aminoglycoside modifying enzyme *aac (6′)-aph (2″)* is bordered by inverted replicas of IS*256* insertion sequence (Behnood et al. 2013). Interestingly, this transposon is similar to the composite transposon (Tn*4001*) of *Staphylococcus*, bordered by an inverted copy of IS256.

Tn*5385* is a multi-resistant composite element that is frequently found in the hospital strains of *E. faecalis* (Rice and Carias 1998). Tn*5385* could resist wide variety of antibiotics such as erythromycin, gentamicin, streptomycin, tetracycline, minocycline and penicillin. It is a 65 kb transposable element flanked by directly repeated copies of IS1216. Interestingly, Tn*5385* is composed of various transposons and transposon-like elements which include conjugative transposon Tn*5381*, composite transposon Tn*5384*, and indistinguishable elements belonging to *Staphylococcus* transposons Tn*4001* and Tn*552* (Rice et al. 2007, 2009). Importantly, various regions of Tn*5385* are linked by several IS elements like IS256, IS257, and IS1216. The genetic exchange of these transposons takes place through homologous recombination by the involvement of either neighboring sequences or inner portable elements.

9.2.4.2.8 Conjugative Transposons

Tn*916* is one of the most extensively characterized conjugative transposon from *E. faecalis* which is about 18 kb in size (Roberts and Mullany 2011; Rice 1998). This has the ability to resist tetracycline and minocycline by the production of *tet (M)* gene. Interestingly, Tn*1545* from *S. pneumonia* is similar and is even identical in many respects to this Tn*916* transposon (Santaro et al. 2014). These conjugative elements transpose to their target sites from their origin without any replication. Simply, they excised from the genomic material of donor cells to form a circular structure and transferred via conjugation finally integrated into the target genome by site-specific recombination (Celli and Trieu-Cuot 1998). The recent whole-genome sequencing results reveal that Tn*916* transposons are distributed among numerous

strains of *Enterococci* family. The first completely described Tn*916* family transposon is Tn*5382* which confers resistance to *VanB* glycopeptide-resistance gene cluster (Garnier et al. 2000). This transposon could be transferred among different *Enterococcal* strains. However, emerging genomic sequences of *Enterococci* from diverse geographical regions would further widen the diversity of transposon across the *Enterococci* species and lead to further detailed classification.

9.3 Mechanism of Antibiotic Resistance in *Enterococcus* Species

Extensive research study on *Enterococcus* genus opens up the distribution of antibiotic resistance over the commensals and pathogenic forms. The diverse range of antibiotic-resistant mechanism observed in enterococci is depicted in Fig. 9.3.

9.3.1 Cell Wall Active Agents

9.3.1.1 Ampicillin/Penicillin Resistance

For β-lactam antimicrobial agents, inhibition of peptidoglycan synthesis has been a primary target in many gram-positive bacteria. Moreover, these kinds of antibiotics were highly approved in the initial stage because of no evidence of toxicity profile in the eukaryotic cell wall which lacks peptidoglycan (Fair and Tor 2014). However, clinical isolates of *Enterococci* species possess intrinsic tolerance to β-lactam antibiotics due to production of penicillin-binding proteins (PBPs) or due to the release of β-lactamases (Hollenbeck and Rice 2012). PBPs is a repository of a cluster of enzymes involved in cell wall synthesis which are categorized into two groups. One with bifunctional activity (transglycosylase) in "Class A" group while the another one with monofunctional activity (D, D-transpeptidase) in "Class B" group (Kong et al. 2010). Six putative PBPs genes were confirmed through DNA sequencing of *E. faecalis* and *E. faecium* with three genes each in class A and class B (Duez et al. 2004). The antibiotic resistance Pbp5 gene with independent operon structure is present in *E. faecalis* and *E. faecium*. PBP5 enzyme poses less affinity to ampicillin, besides higher antibiotic concentration is required to saturate enzyme active site (Fontana et al. 1996). Additionally, a mutation in the protein sequence with the substitution of Met → Ala at position 485 near the active serine residue provides resistance to ampicillin binding. Therefore in *E. faecium*, PBP5 sequence variations were observed between hospital-acquired variants with higher resistance (Pbp5-R) and from community-associated variants (Pbp5-S) with lower MIC results (Rice et al. 2004). Hence, in *Enterococci*, the mechanism of antibiotic resistance is coupled with overexpression and mutation in PBP5 thus shows greater degree of resistance towards ampicillin (Duez et al. 2001; Ono et al. 2005). In accordance with β-lactamases, both *E. faecalis* and *E. faecium* promote β-lactam ring cleavage (Murray 1992). *Enterococci* gene coding β-lactamases were found as *blaZ*, which is predicted to be originated from *Staphylococci*. But unlike *Staphylococci*, expression of *blaZ* in *Enterococci* is at lower level which is correlated with inoculum

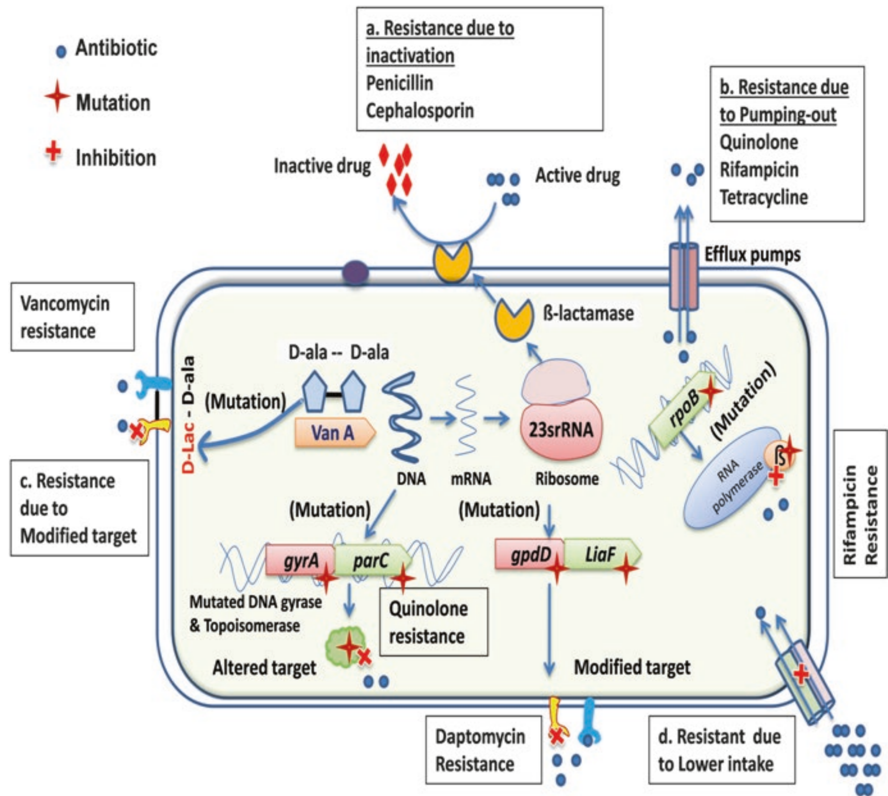

Fig. 9.3 Mechanism of antibiotic resistance in *Enterococcal* infections: (**a**) drug inactivation *Enterococci* were intrinsically resistance to certain antibiotics due to the production of modifying enzymes like β-lactamases. (**b**) Efflux pumps. Those pumps with resistance modulator will secrete the antibiotic outside the cell. (**c**) Resistance mutation. Mutation in the specific gene capable of altering the antibiotic binding site. For example, mutation in gyrA and parC inhibits binding of quinolone to DNA gyrase and topoisomerase. Similarly, rpoB mutation protects RNA polymerase from Rifampicin and gpdD LiaF mutations inhibit cell binding of daptomycin

volume (Hackbarth and Chambers 1993). The responsible enzyme for antibiotic-resistant came into view with the higher bacterial inoculums in *Enterococci*. On the other hand, the addition of β-lactamases inhibitor restores the efficiency of antibiotic activity against *Enterococci* (Sarti et al. 2012).

9.3.1.2 Cephalosporin Resistance

Intrinsic resistance to cephalosporin is a most familiar trait in enterococci which occurs through low binding affinity with Pbp5 (Rice et al. 2009). Some of the research findings suggest that *E. faecalis* and *E. faecium* resistance towards cephalosporin was linked to either PonA or PbpZ of class-A PBPs system (Arbeloa et al. 2004; Rice et al. 2009). This phenomenon illustrates that there is ideal cooperation between pbp5 and one or more class-A PBPs system to convey resistance

mechanism against cephalosporins. Study with sequential deletion of class-A PBPs in both the strains reveals that there is an activation of the differential regulatory pathway in response to a cephalosporin (Rice et al. 2009). The intrinsic resistance to cephalosporin is controlled by two-component regulatory system (TCS) like CroR/S (Le Breton et al. 2007). Correspondingly, IreK, MurAA were also found to be an important regulatory system associated with cephalosporin resistance. Cognate response sensor (CroS) can phosphorylate itself by histidine kinase and has the ability to transfer the phosphate group to cognate response regulator (CroR) (Comenge et al. 2003). Activated CroR response regulator is capable of modifying DNA transcription in order to adapt cephalosporin stress conditions (Muller et al. 2006). However, some of the identified genes such as SalB, glutamine transporter with CroR regulation are not obvious in cephalosporin resistance. IreK is a eukaryotic type serine/threonine kinase coupled with extracellular PASTA domain through a transmembrane segment (Kristich et al. 2014). IreK exhibit kinase activity which help in maintaining the cell wall integrity by sensing the damaged peptidoglycan and signaling through autophosphorylation of threonine (Labbe and Kristich 2017). Subsequent deletion of mutant analyses in *E. faecalis* illustrates the significant role of IreK in cell envelop stress is an indicative of intrinsic cephalosporin resistance (Hall et al. 2013). MurAA gene (encoding for UDP-N-acetylglucosamine 1-carboxyvinyl transferase) is responsible for providing resistance to *E. faecalis* against cephalosporin by initiating the peptidoglycan synthesis which is not due to its homolog protein Mur AB (Vesic and Kristich 2012). Individual deletion mutants of either Mur AA or Mur AB capable of executing the enzyme activity for converting UDP-N-acetylglucosamine to UDP-N-acetyl glucosamine-enolpyruvate in the presence of UDP-N-acetylglucosamine 1-carboxyvinyl transferase is the major step in peptidoglycan synthesis. However, with the underlying physiology of *E. faecalis* Mur AA phenotype is more expressive in cephalosporin resistance rather than Mur AB with least.

9.3.1.3 Resistance Against Glycopeptide

Glycopeptide antibiotics like teicoplanin and vancomycin avoids the interaction between the peptidoglycan chain by adhering to D-alanine-D-alanine (D-Ala-D-Ala) terminal moiety of peptidoglycan precursor (Hollenbeck and Rice 2012). The pattern of resistance is highly relevant to the type of amino acid change occurred in precursor moiety. For instance, change in terminal pentapeptide amino acids from D-Ala-D-Ala to D-alanine-D-Lactate (D-Ala-D-Lla) reduces the binding attraction of vancomycin to peptidoglycan chain by 1000-folds, thus conferring a high level of resistance (Courvalin 2006). Minimal level of resistance was due to the modification of amino acid terminal to D-alanine-D-serine (D-Ala-D-Ser) that can exhibit a seven-fold reduction in binding affinity (Hollenbeck and Rice 2012). So far nine different vancomycin resistance gene clusters were identified in *Enterococcus* species (Courvalin 2006). Based on the genes encoded by this cluster, the enzymes are categorized into three groups: (1) enzymes that destroy precursor of terminal amino acids moiety (D-Ala-D-Ala); (2) enzymes for remodeling the new peptidoglycan precursor; (3) enzymes for two component regulatory systems (TCS) (Binda et al. 2014).

Vancomycin-resistant in *Enterococci* is commonly mediated by *vanA* cluster under the control of two promoters. The first promoter controls the expression and function of VanA by initiating VanR/S transcription (Depardieu et al. 2007). VanS is a transmembrane protein comprising of histidine kinase domain that can sense the glycopeptide signal and phosphorylate the VanR regulator (Binda et al. 2014). The triggered VanR initiates the transcription of the second promoter situated near the upstream of resistant genes (Depardieu et al. 2007). Vancomycin resistance begins from the transcription of *vanH* encoded dehydrogenase involved in the conversion of pyruvate to D-Lactate (Hollenbeck and Rice 2012). Next, the addition of D-Lac to D-Ala with the help of *vanA* encoded ligase results in pentapeptide structure. Then this pentapeptide integrates into the peptidoglycan structure by cross-linking. VanX and VanY work together in removing the usual D-Ala-D-Ala structure from the growing cell wall (Binda et al. 2014). Hence, the crucial stage in glycopeptide resistance is D-Ala clearance. The other group of enzymes which are similar to *vanA* is *vanB* cluster, involved in the regulation of resistance in *Enterococci* species followed by $VanS_B$ (sensor kinase) and $VanR_B$ (response regulator) posing distinct homolog to v*anA* cluster (Depardieu et al. 2007). VanC is reported in chromosomal DNA of *Enterococcus gallinarum* and *E. casseliflavus*. VanC promotes resistance to vancomycin through the incorporation of D-Ala-D-Ser to the terminal dipeptide, thus confers low-level resistance pattern (Binda et al. 2014).

9.3.1.4 Resistance to Daptomycin

Daptomycin (DAP) is a lipopeptide antibiotic whose mode of action is similar to that of cationic antimicrobial peptides (CAMPS from the immune system). It requires calcium ions for insertion into the bacterial cell membrane (CM) (Steenbergen et al. 2005). DAP starts with preferential binding at division septal plane for membrane entry. At the inner membrane space, presence of phospholipid (phosphatidylglycerol) stimulates the oligomer formation in DAP at the surface of the CM (Muraih et al. 2012). The oligomers of DAP enter the inner membrane by pore formation by the action of cardiolipin (CL), a membrane-associated phospholipid (Hollenbeck and Rice 2012). Thus, it disrupts the membrane integrity and functionality leading to cell death (Zhang et al. 2014). However, some of the recent research findings provides evidence of genetic changes for resistance mechanism in *Enterococci* based on three gene expression such as *liaF*, *gpdD*, and *cls*. *LiaF* is a member of three-component regulatory system (LiaFSR) in many gram-positive bacteria that govern the organized cell envelop response to stress conditions (Munita et al. 2012). The other two genes for DAP-R are *gpdD* and *cls* that encode for glycerol-phosphodiester phosphodiesterase and cardiolipin synthase which are involved in phospholipid metabolism (Arias et al. 2011). The DAP resistance mechanism is varied among the strains of *Enterococci*. For instance, in *E. faecalis* the redistribution of CL domain from the septal to non-septal areas within the CM is found to be a major reason for resistance phenotype. Based on the experimental evidence it was hypothesized that remodeling of CL microdomain was associated with change in LiaFSR system (Miller et al. 2013). Moreover, an in vitro study performed in *E. faecalis* has proved that changes in phospholipids contents of CM also plays a significant role in providing resistance against DAP insertion (Mishra

et al. 2012). Prevention of DAP oligomerization occurs at phosphotidylglycerol deficiency, thereby CL domain traps/prevents the moment of monomeric DAP substance from reaching the inner leaflet of CM (Arias et al. 2011). Instead of DAP resistance based on septal diversion, DAP repulsion from the cell surface is considered as a prominent mechanism in *E. faecium*. In case of *E. faecalis*, the genetic basis of DAP resistance is due to change in *liaFSR* system. Mutation in *liaFSR* gene cluster with corresponding changes in TCS mediates DAP resistance in enterococci (Miller et al. 2013).

9.3.2 Agents Interfering Protein Synthesis

9.3.2.1 Aminoglycosides

Enterococci mediate intrinsic tolerance to various aminoglycoside through two important factors such as poor uptake and covalent modification in aminoglycoside molecule. Lack of sufficient concentration of antibiotics inhibits the protein synthesis and promotes resistance against aminoglycoside (Galimand et al. 2011). Naturally occurring *Enterococci* enzymes are capable of decreasing the binding affinity of aminoglycoside molecule with the ribosomal target by inducing changes in hydroxyl or amino group of aminoglycoside by covalent modification (Hollenbeck and Rice 2012). Similarly, *Enterococci* with *EfmM* encoding ribosomal RNA methyltransferase can induce alteration in ribosomal targets against aminoglycosides. Mostly, the enzymes encoded by chromosomes such as 6′ acetyltransferase (AAC (6′)-li) and APH (3′)-IIIa with phosphotransferase action are capable of modifying antibiotics like kanamycin, amikacin in *E. faecium* (Galimand et al. 2011).

Hence, among the aminoglycosides, gentamycin and streptomycin which are unaffected by intrinsic enzymes were selected for treating the enterococcal infection. The resistance arises for this compound when there is an increase in minimum inhibitory concentration (MIC) value. The antibiotics kanamycin and streptomycin with MIC of 900 and 500 μg/ml were sufficient to provide resistance against *Enterococcus* species (Hollenbeck and Rice 2012). A greater degree of resistance is mediated by streptomycin inactivation through the acquisition of streptomycin adenyl transferase in *Enterococci*. However, a bifunctional modifying enzyme like both AAC (6′)-li and APH (3′)-IIIa acts synergistically in providing resistance to almost all kind of aminoglycosides (Galimand et al. 2011).

9.3.2.2 Oxazolidinones

Linezolid is the FDA approved antibiotic employed for curing drug-resistant *Enterococcus* species (Hollenbeck and Rice 2012). It is a bacteriostatic agent that inhibits the initiation complex for protein synthesis, thereby affects the mRNA translation process (Bourgeois-Nicolaos et al. 2007). It mainly interacts with 23S rRNA and inhibits the peptide elongation in the A-site of the ribosome (Van Harten et al. 2017). Hence, a mutation in 23S rRNA encoding gene develops resistance by preventing linezolid binding. Substitution of uracil to guanosine at 2567 domain of 23S rRNA indicates mutation in patients suffering from linezolid resistant

enterococcal infection (Hollenbeck and Rice 2012). Similarly, methylation of adenine at 2503 domain by enzymatic modification of 23S rRNA is another form of mutation which confers resistance mechanism in *Enterococci* (Van Harten et al. 2017).

9.3.2.3 Tetracyclines

Tetracycline promotes antibacterial activity by 30S subunit ribosomal binding and interfering with the docking of aminoacyl-tRNA (Van Hoek et al. 2011). Based on two general strategies such as antibiotic efflux and ribosome protection, multiple genes in enterococci develop resistance against tetracycline. Efflux pump is a transmembrane domain that confers tetracycline resistance (Fluit et al. 2005). The resistance determining genes (tetM, tetO, and tetS) encoded by chromosomes poses similar homology to elongation factors (EFs) which can hydrolyze GTP, thereby promotes remodeling of ribosome conformation to display tetracycline resistance (Celli and Trieu-Cuot 1998).

9.3.3 Agents Interfering Nucleic Acid Synthesis

9.3.3.1 Quinolones

DNA topoisomerase IV and DNA gyrase are targeted by quinolone (Van Hoek et al. 2011). Generally, DNA gyrase induces negative supercoil in double strand DNA and allows initiation of replication followed by topoisomerase IV which unwinds the newly replicated DNA that allows segregation to occur (Hawkey 2005). Both the process requires enzyme/DNA stable complex which can be interrupted by quinolones, thereby arrest the replication process. DNA gyrase and DNA topoisomerase are tetrameric in nature comprised of two different subunits (GyrA and GyrB) and (ParC and ParE) (Van Hoek et al. 2011). *Enterococci* mediate a higher degree of resistance towards quinolones, which has been reported through several mechanisms. Mutation of genes *qyrA* and *parC* in *E. faecalis* and *E. faecium* changes the binding affinity of quinolones to the target site (Lopez et al. 2011). These sites were named as quinolones resistance determining regions. Quinolone externalization through efflux pump encoded in NorA is a well described mechanism in *E. faecium* (Hawkey 2005). In *E. faecalis* presence of pentapeptide repeats encoded by *qnr* decreases quinolone binding to DNA (Van Hoek et al. 2011), hence protects DNA gyrase from the complex formation with quinolone.

9.3.3.2 Rifampicin

The primary target of rifampicin is a β-subunit of DNA-dependent RNA polymerase inhibiting enterococcal mRNA transcription (Enne et al. 2004; Deshpande et al. 2007). Mutation in rpoB gene encoding β-subunit of RNA polymerase is the major reason for the development of resistance against rifampicin (Kristich and Little 2012). Specific mutation at rpoB loci H486Y in *E. faecalis* and *E. faecium* mediates differential transcription of genes capable of producing cell wall resistance agents against cephalosporins (Enne et al. 2004). Despite mutation in rpoB gene rifampicin resistance was observed through efflux pump or enzyme inactivation (Kristich and Little 2012).

9.3.3.3 Trimethoprim

Bacterial enzymes involved in folate synthesis are the primary target for trimethoprim (Hollenbeck and Rice 2012). In particular, trimethoprim acts on enzymes involved in dihydrofolate production; therefore, conversion of tetrahydrofolate is limited (Grayson et al. 1990). Folate plays a vital role in many cellular functions such as thymidine synthesis. Due to insufficient uptake of exogenous folate from the environment, several bacteria have been reported to synthesize folate from p-amino benzoic acid (Hollenbeck and Rice 2012). However, *Enterococci* bypass the inhibition effect of trimethoprim just by utilizing exogenous folate, which was confirmed by in vivo studies (Grayson et al. 1990).

9.3.4 Treatment for Drug Resistance Enterococci Infection

Many alternative therapeutic strategies were revealed to combat antibiotic-resistant enterococcal infections that are listed in Table 9.2. Some of the approaches were found to be more effective and efficient under in vivo studies: (a) A novel class of bacteriocins produced by intestinal microbiota exerting potential antimicrobial activity and specificity are of clinical importance which does not entail harmful effects against commensal microbiota (Millette et al. 2008). (b) Investigational antibiotics such as cadazolid exhibit the development of lower propensity of antibiotic resistance among enterococcal infections (Seiler et al. 2015). (c) Active molecules such as RegIIIγ, stimulate the pheromone response in commensal bacteria and works in concert with mucosal immunity to impede the vancomycin-resistant *Enterococci* (VRE) outgrowth (Brandl et al. 2007; Gilmore et al. 2015). (d) Moreover, phage endolysin is an important therapeutic approach for efficiently clearing the *E. faecalis* clearance (Duerkop et al. 2012).

9.4 Conclusion

With the constant upsurge in the resistance against *Enterococcal* species towards various antibiotics, it is now hard to treat the disease with conventional antibiotics. The genetic versatility and antibiotic resistance ability makes the enterococcus species to excel other bacteria in ecological region. Though with a diverse array of virulent determinants, genes encoding surface proteins such as *ace, epa, agg* are potential factors for enterococcal infections. Thus, it ensued as a primary target for vaccination against strains with drug resistance. Moreover, understanding the membrane physiology of resistance varieties brings a new avenue of antibiotic discovery for cell wall active agents. Bacteriocins are one of the best therapeutic options in clearing resistant Enterococci upon specific targets, and capable of showing synergistic action with traditional antibiotics. Moreover, steps should be taken to limit bacteriocins resistant development and transfer of resistant trait among the pathogens. Horizontal gene transfer mechanism played a leading role in spreading the antibiotic resistance among this genus in the diverse niche. Many plasmids and transposable elements are

Table 9.2 Strategies employed for the treatment of drug-resistant enterococcal infection

S. No	Active compounds	Mode of transfer	Activity	Treatment options for	References
1	Nisin and pediocin	Administration of bacteriocins encoded lactobacillus	Colonization resistance	Vancomycin-resistant enterococci	Millette et al. (2008)
2	Cadazolid	Oral administration	Colonization resistance	Vancomycin-resistant enterococci	Seiler et al. (2015)
3	Barnesiella species	Fecal microbiota transplantation	Colonization resistance	Prevent infection of enterococci	Ubeda (2013)
4	RegIIIγ, a C-type lectin from paneth or intestinal cells	Oral administration of LPS or flagellin stimulates the production	Bactericidal activity	Inhibits the growth of resistant enterococci	Brandl et al. (2007)
5	Intact mucin production	Stimulation of goblet cells by commensal bacteria	Maintaining intestinal barrier integrity	Prevents invasion of infectious enterococci over the intestinal epithelium	Johansson et al. (2015)
6	Bacteriocin (BCN) production	Oral administration of BCN producing *Lactococcus lactis* MM19	Antimicrobial activity	Elimination of MDR enterococci	Borrero et al. (2015)
7	Pheromones, responsive conjugative plasmid	Administration of commensal *E. faecalis* with bacteriocins coded plasmid	Pheromones mediated cell death in competitors	Elimination of MDR *E. faecalis* V583	Gilmore et al. (2015)
8	Endolysin of bacteriophage ϕV 1/7	Intraperitoneal injection in mice	Phage-mediated lysis of competitors	Narrow spectrum antimicrobial against *E. faecalis*	Duerkop et al. (2012)
9	Probiotics	Oral supplementation of L. rhamnosus GG	Colonization resistant	Elimination of growth of VRE through growth competitors	Manley et al. (2007)
10	ß-lactamases inhibitors	Production through *Bacteroides sartorii* and *Parabacteroides distasonis*	Inactivate ß-lactamases	Replenish the ß-lactamases producing ampicillin sensitive strains	Caballero et al. (2017)

recognized to transfer such traits among *Enterococci* species. In addition to the reported class of transposable elements, mutations involving addition and deletion of nucleotides further increase its classification and increase its resistance to new antibiotics, thus cause devastating effects in humans unless this natural genetic transmission is hammered. CRISPR system can be considered to remove the antibiotic resistance genes from *Enterococcus* species.

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