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Mechanism of Drug Resistance in *Enterococci* and Therapeutic Options -A Review

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Abstract

Enterococci exhibit an array of ways for constitutional and extrinsic resistance to primary antimicrobial agents available for clinical use. Susceptibility of these agents to β lactams, aminoglycosides or glycopeptides antibiotics or low susceptibility to combination of these agents, monomicrobial or polymicrobial nature of the infection, the involvement of heart valves or other endovascular structures affects therapy of Enterococcal infection. Vancomycin-resistant phenotypes A and B are most prevalent among medically important Enterococci isolates. Due to poor uptake of aminoglycosides, moderate level inherent resistance was reported in Enterococci. Gentamicin and Streptomycin are among the aminoglycoside antibiotics recommended for synergistic combination therapy with a cell wall acting agent. *Enterococci* isolates display inherent resistance to beta-lactam antibiotics due to less affinity penicillin-binding proteins, class B. Resistance to macrolides, due to erm B genotype and efflux proteins are common in Enterococci. Fluoroquinolone resistance due to genetic changes in chromosomal resistance determining regions has been observed in *Enterococci* isolates. Despite studies on good invitro action of Daptomycin, Linezolid and Tigecycline on Enterococci, their use may be limited due to shortage of clinical data and emergence of drug resistance. Thus optimal therapeutic option for Multidrug-resistant Enterococci infection is based on empirical observation, higher doses and combination therapies. This article reviews the antimicrobial resistance mechanism in Enterococci and available therapeutic options.

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INTRODUCTION

Enterococci are non-obligatory anaerobes oval in morphology, appear as a single cell, pairs or short chains, on a smear. *E. faecalis* had been reported more prevalent than *E. faecium* in humans. The most frequently caused infection by these organisms includes Urinary tract infections (UTI's), wound infections, followed by bloodstream infection (Sood *et al.*, 2008). The rarely encountered infections include neonatal, central nervous system and respiratory tract infections. These organisms display intrinsic low-level resistance to Penicillins, Cephalosporins, Clindamycin, Nalidixic acid and Aminoglycosides (Arias and Murray, 2012; Kristich *et al.*, 2014).

The primary threat involved in the selection of therapeutic agents against severe infection caused by these agents is their ability to exhibit innate resistance to antibiotic groups. The basis towards the

appearance of decreased susceptibility to multiple drugs in Enterococci are due to

1. Innate potential to display decreased susceptibility to various antibiotics.
2. Acquisition of antimicrobial resistance genes through translocation on plasmids and chromosomes.
3. The ability for an interchange of low susceptibility genes (Shantala and Nagarathnamma, 2015).

Glycopeptides group of antibiotics

These group of antibiotics integrate with peptides and develop complexes, which ends in Acyl D-alanyl alanine. This combination impedes cell wall biosynthesis, which results in a defect of cell wall integrity.

Isolates of *Enterococci* resistant to Glycopeptides develops modified mucopeptide precursor in which dipeptide ends are converted to either lactate or serine which results in decreased affinity to bind to mucopeptide precursors and make them incapable of preventing cell wall biosynthesis (Miller *et al.*, 2014).

Based on phenotypic and genotypic characters, nine distinct gene clusters from Van A to Van N are reported in *Enterococci* (Shantala and Nagarathnamma, 2015; Sujatha and Praharaj, 2012).

Vancomycin-Resistant Phenotype A

These phenotypes are linked with Minimum Inhibitory Concentration of $\geq$ 128 microgram/ml for vancomycin and $\geq$ 8 microgram/ml for Teicoplanin. Isolates of Enterococcus from a urine sample with MIC of $\geq 32\mu$g/ml for glycopeptides have been observed in our study (Rajan *et al.*, 2017c).

The following genes define the generation of modified mucopeptide precursors:

1. Van gene A: This ligase generates the depsipeptide ending in D-alanyl lactate which binds Vancomycin with decreased affinity.
2. Van gene H: This Dehydrogenase generates D-lactate from pyruvate.
3. Van gene X: This dipeptidase splits dipeptide, of Chromosomal origin and creates glycopeptides –sensitive pentapeptides termini to incorporate into growing murein chain.
4. Van gene Y: This carboxypeptidase removes alanine units from dipeptide termini and supplement dipeptidase action.
5. Van gene R / S: This system detects glycopeptide presence and prompts van genes expression. Van S serves as a detector of vancomycin presence and transfers a signal to response regulator R thereby activating generation of van H, van A and van X involved in resistance mechanism (Depardieu *et al.*, 2007).
6. Van gene Z: Contributes to low Teicoplanin susceptibility.

Vancomycin-resistant phenotype B

Enterococci with resistant phenotype display Minimum Inhibitory Concentration of $\geq$8 microgram/ml to vancomycin but not activated by glycopeptide, Teicoplanin. This resistant phenotype is acquired on extrachromosomal genetic elements or host chromosome. This phenotype encodes Van R_B and Van S_{B-a} two-component system, homologs of Van H, D –Alanyl alanine ligase (Van X) and carboxypeptidases (Van Y).

Vancomycin dependent Enterococci

Resistant Isolates of Enterococci dependent on vancomycin for growth have evolved in Phenotypes A and B. These *strains* shut off dipeptide synthesis and needs dipeptide alternative for growth. Dipeptide, D-alanyl-D-alanine is not required for the biosynthesis of the cell wall in resistant isolates while vancomycin is available. Additionally, in the absence of dipeptide, D-alanyl-D-alanine or D-alanyl-D-lactate, the cell cannot resume to develop or reproduce (Kristich *et al.*, 2014).

Aminoglycosides

In *E. faecium*, inherent resistance is amplified by rRNA methyltransferase encoded on a chromosome, EfmM gene that uses S-adenosyl methionine, methylation of specific residue on 16S rRNA by methyl donor (Galimand *et al.*, 2011). The deactivation of efmM enhances the ability of these organisms to become susceptible to Aminoglycosides, Tobramycin and Kanamycin. Low-level intrinsic resistance to aminoglycosides is chromosomally encoded on $^{'}$N –acetyltransferase.

Low susceptibility to aminoglycosides are acquired and are encoded on aminoglycoside modifying enzymes. The bifunctional gene encoding, acetylase and phosphotransferase enzyme confer high-level resistance aminoglycosides excluding Streptomycin (Hollenbeck and Rice, 2012). Our study reported more than 70% urinary isolates with MIC$\geq$500 μg/ml for High-level Gentamicin and 30% isolates with MIC$\geq$2000 μg/ml for High-level Streptomycin (Rajan *et al.*, 2017a).

Resistance to Streptomycin is either ribosomal or due to adenyl transferase enzyme. Most isolates of *E. faecium* produce 6′ acetylase transferase enzyme, thereby these isolates develop inborn resistance to aminoglycosides (Hollenbeck and Rice, 2012).

In isolates with MIC of 62 - 500 microgram/ml (moderate level resistance) to Streptomycin, the low permeability observed may overcome with the use of cell wall acting agents. In strains with MIC of more than 2000 microgram/ml, cell wall acting agents enhance absorption of Streptomycin.

In isolates that produce inactivating enzymes, high-level resistance to Streptomycin is controlled at a concentration of 16, 000 microgram /ml. In contrast, ribosomal resistance is not affected at higher concentration of 128, 000 microgram/ml of Streptomycin (Miller *et al.*, 2014).

In isolates with MIC of 256 microgram/ml for Gentamicin, and <500 micrograms/ml for Streptomycin, combination with cell wall acting agent can benefit therapy. Prevalence of acetylase transferase enzyme and subclass APH - (3') - III an encoding gene contributes to Kanamycin resistance (High level) and nullify Amikacin synergism. Nephrotoxic potential of these drugs restricts its use in severely affected patients (Hollenbeck and Rice, 2012).

Beta-lactam antibiotics

The decreased affinity of Penicillin Binding Proteins (PBP's) is accountable to diminished Penicillin susceptibility in Enterococci. Consequently, minimum inhibitory concentration is higher in Enterococci than in Streptococci. Thus cessation of PBP's does not progress to antibacterial activity in isolates. Exposure of susceptible isolates of E.faecalis to Penicillin lead to mutant strains with enhanced production of Penicillin Binding Protein 5.Strains of E.faecium display enhanced intrinsic resistance to Penicillin with MIC of 16-32 microgram/ml than isolates of E.faecalis with MIC of 2-4 microgram /ml.

In *E. faecalis* plasmid-mediated bla genes encoding β lactamases production have been reported *Enterococci* isolates display inherent resistance to beta-lactam antibiotics as a result of low tendency penicillin-binding proteins, belong to class B. Due to less inclination for beta-lactam drugs, chromosomal Penicillin Binding Protein accomplishes mucopeptide biosynthesis at drug concentration which saturates other Penicillin Binding Proteins. In *E. faecium* clinical isolates, decreased susceptibility to β lactamase enzyme is connected with a genetic variation or Penicillin Binding Protein 5 hyper-production with Minimum Inhibitory Concentration of more than 256 milligram/L for Ampicillin in few isolates. In *E. faecalis* β lactamase enzyme producers will respond to the inhibitor of β lactams and aminoglycoside combination. *E. faecium* isolates may respond to Ampicillin with Minimum Inhibitory Concentration of $\leq$64 mg/L (Arias *et al.*, 2010).

The genetic determinants responsible for inherent resistance to cephalosporins in isolates of *Enterococcus faecalis* are less affinity Penicillin Binding Proteins (Miller *et al.*, 2014; Kristich and Little, 2012).

Penicillin Binding Protein$_5$ modifications are linked with enhanced beta-lactam resistance such as ampicillin. Conjugative transfer of chromosomally coded PBP$_5$ genetic determinants is transmitted among *E. faecium strains*. In *E. faecalis* strains, the mutation in Penicillin Binding Protein$_5$ may result in augmented Imipenem and Ampicillin resistance (Kristich *et al.*, 2014). In Enterococci, the Minimum Inhibitory Concentration of amoxicillin or ampicillin is one dilution lesser than Penicillin.

Macrolides, Lincosamide and Streptogramin

Acquired resistance to macrolides, due to erm B genotype lessen the ability of these drugs to bind 50S ribosomal subunit. Low-level resistance to Macrolide is due to efflux gene,mefA which drive macrolides of the cell (Garrido, 2014)

E. faecalis is inherently resistant to Quinupristin-Dalfopristin (Q-D), due to the presence of chromosomal genetic determinant ABC transporter (Lsa). Quinupristin drug has been proved as an effective therapeutic agent for resistant isolates *of E. faecium*. Combination therapy of Quinupristin-Dalfopristin (Q-D) with Doxycycline, Gentamicin, Rifampicin and Ampicillin is utilised for *E. faecium* endocarditis and Vga genes, in *E. faecium*, an ABC transporter which exports the antibiotic from the cell (Miller *et al.*, 2014).

Linezolid

Linezolid impedes bacterial growth by hindering synthesis of protein through translational initiation complex interaction (Shantala and Nagarathnamma, 2015; Bourgeois-Nicolaos *et al.*, 2007). Linezolid is accounted as an alternative drug for CNS infections by Vancomycin-resistant isolates of *E. faecium*. This drug is described as curative for endocarditis and bloodstream infection, Urinary Tract Infection and wound infections caused by vancomycin-resistant isolates. The clinical success rate of Linezolid therapy for Vancomycin-resistant bloodstream infection ranges from 58 % to 78 %. Long term therapy with linezolid can lead to anaemia, neutropenia and thrombocytopenia in VRE patients (Chong *et al.*, 2010). In our study among clinical isolates of Ente-

rococci, more than 89% isolates showed susceptible MIC of $\leq 2\mu$g/ml (Rajan *et al.*, 2017b).

Daptomycin

Daptomycin, display extensive spectrum activity against bacteria such as *Staphylococcus aureus* and *Enterococci* and have been used for skin and underlying tissue infections. Daptomycin acts by membrane insertion, depolarisation, the release of ions and results in cell death. A high dose therapy with Daptomycin should be considered for endovascular infections by Enterococci (Kristich *et al.*, 2014; Smith *et al.*, 2015). Combination therapy with 8mg/kg of Daptomycin, Gentamicin and Ampicillin/Rifampicin is proved to be effective in cases of endocarditis by resistant isolates of Enterococci (Hindler *et al.*, 2015).

Quinolones

Fluoroquinolone resistance due to mutation inhibits affinity of enzymes DNA gyrase and topoisomerase IV to Fluoroquinolones which allows DNA replication to continue notwithstanding the presence of quinolone (Leavis *et al.*, 2006).

In *E. faecalis*, inactivation of Qnr homolog contributed to the decline in resistance to quinolones resistance and hyperexpression of Qnr leads to enhanced resistance (Arsène and Leclercq, 2007). Fluoroquinolones are recommended for uncomplicated Urinary Tract Infections due to its in-vitro susceptibility against *Enterococci* isolates. Nitrofurantoin and Fosfomycin have been considered as alternative drugs useful for uncomplicated lower urinary tract infections management. Though the susceptibility pattern of VRE towards Doxycycline and Fluoroquinolones varies, these drugs have been proven for the treatment of infections of the skin and underlying tissues and urinary tract (López *et al.*, 2011; Werner *et al.*, 2010).

Rifampicin

In *Enterococci*, rifampicin binds to β subunit of RNA replicase enzyme and blocks mRNA transcription (Kristich and Little, 2012). Rifampicin resistance emerges by a variety of genetic changes in rpoB protein. In isolates with rpoB mutation, decreased susceptibility to cephalosporins is observed (Kristich *et al.*, 2014). In rare strains of E. faecium, rifampicin resistance can be reversed by including a less inhibitory concentration of Daptomycin.

Tetracyclines and Glycylcycline

In clinical isolates of *Enterococci*, tetracycline resistance is generally linked to protein tet (M) and efflux proteins, tet (K) and tet (L) (Garrido, 2014; Chopra and Roberts, 2001).

Tigecycline exhibit extensive activity and potency against Gram-positive organisms with Minimum Inhibitory Concentration $_{90}$ value of $\leq$ 12 microgram/ml. The effectiveness of Tigecycline in therapy with antibacterial agents such as glycopeptide, aminoglycoside, Rifampicin or Daptomycin is reported (Shantala and Nagarathnamma, 2015). Our study reported 88% isolates with susceptible MIC of $\leq 0.12\mu$g/ml (Rajan *et al.*, 2017b) for Tigecycline.

Lipoglycopeptides

Lipoglycopeptides display activity against Vancomycin susceptible isolates of *Enterococcus.* Lipoglycopeptides such as oritavancin telavancin and dalbavancin disintegrate membrane potential, enhances the antibacterial activity and cell death.

Telavancin is recommended for the therapy of complicated skin infections. Oritavancin is active against Van A resistant Phenotype of Enterococci (Linden *et al.*, 2001).

CONCLUSION

The unique nature of Enterococci to harbour and transfer resistance genes and the evolution of multidrug resistance poses enormous challenges to clinicians. A better knowledge of resistance mechanism and treatment options will aid in the control of epidemiologic spread. Combination therapy with novel antibiotic groups such as Tigecycline and Daptomycin are promising treatment modalities for severe enterococcal infection.

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Conflict of Interest

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