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ANTIBIOTIC RESISTANCE IN *ENTEROCOCCI*: A REVIEW

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ABSTRACT

Enterococci, traditionally regarded as low grade pathogens, have emerged as an increasingly important cause of nosocomial infections in the past two decades all over the world. Their emergence as important nosocomial pathogens has coincided with increased expression of antimicrobial resistance that is intrinsic to the species or may be acquired through mutation of intrinsic genes or horizontal exchange of genetic material through the transfer of plasmids and transposons. The present paper aims at reviewed the mechanisms underlying antibiotic resistance in *enterococci*, both intrinsic and acquired with an overview on the most important mechanisms of resistance to the antibiotics that are used to treat *vancomycin-resistant enterococci.*

KEYWORDS: ***Enterococcus faecalis, Enterococcus faecium,*** **high-level aminoglycoside resistance (HLAR), nosocomial infections, vancomycin resistant *enterococci* (VRE)**

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INTRODUCTION

Enterococci are Gram-positive, facultative anaerobic organisms characterized by their ability to grow at 6.5% NaCl concentrations ,at temperatures of both 10 and 45°C, at high *p*H of 9.6 and to hydrolyze bile-esculin and Lpyrrolidonyl-B-naphthylamide (PYR). Originally considered members of Lancefield group D *Streptococcus*, studies involving fatty acid composition, nucleic acid hybridization and comparative oligonucleotide cataloguing of 16s RNA suggested that they were sufficiently different from other *streptococci* to be credited with a distinct genus.[1,2] *Enterococci* are a part of the normal human faecal flora traditionally regarded as low grade pathogens of little clinical importance, but over the past decade they have emerged as an increasingly important cause of nosocomial infections , ranking only second to *Staphylococci* in the United States(US).[3] The most common nosocomial infections produced by these organisms were urinary tract infections, followed by intra-abdominal and pelvic infections. They also cause surgical wound infections, bacteraemia, endocarditis, neonatal sepsis and rarely meningitis.[4,5,6] Both microbial and host factors contribute to the conversion of this low grade pathogen into a first-rate clinical problem.The microbial factors for the *enterococci*, appear to include their inherent resistance to antimicrobial agents, their capacity to acquire and disseminate determinants of antibiotic resistance and their malleable genomes, which may contribute to their adaptation to harsh environments (including hospitals). The host factors include the increased number of patients hospitalized in critical care units receiving multiple antimicrobial agents favouring the ability of multidrug-resistant organisms such as *enterococci* to cause infections, immunosuppresion and mechanically compromised status. [7,8] Enterococcal infections may be caused by at least 12 species, including *E. avium, E.casseliflavus, E. durans, E. faecalis, E. faecium, E. gallinarum, E. hirae, E.malodoratus, E. mundtii, E. pseudoavium, E. raffinosus,* and *E. solitaries.*[9]Most clinical infections are due to either *E. faecalis* or *E. faecium*. Until late 1970s and with the introduction of third generation cephalosporins, *Enterococcus fecalis* accounted for 90-95% of clinical enterococcal isolates. From the early 1990s with increase use of vancomycin and broad spectrum antibiotics *E.faecium* has been almost as common a cause of nosocomial infections as *E.faecalis* both in US and other parts of the world. This change in species was paramount clinical importance, as *E. faecium* is by far the more difficult of the two species to treat.[7,10] Although the resistance characteristics of these two species differ in important ways, they can generally be categorized as intrinsic resistance, acquired resistance, and tolerance. In the present Review, we discuss the mechanisms of resistance in *enterococci* with special reference to vancomycin resistance and resistance mechanisms to relevant anti-enterococcal agents used against *vancomycin resistant enterococci* in clinical practice.

Intrinsic Resistance

β-lactams and cephalosporins

β-lactams like Penicillin (or ampicillin) alone or with an aminoglycoside formed the cornerstone of treatment for enterococcal infections for more than half a century. Ampicillin resistance, which is rare in *E. faecalis*, occurs in ~90 % of modern-day hospital-associated *E. faecium* isolates. β-lactams bind covalently to penicillin binding proteins (PBPs) that are enzymes responsible for cross linking pentapeptide precursors into the peptidoglycan cellwall. The binding results in impaired cell wall synthesis and in most cases programmed cell death . *Enterococci* are resistant to most beta-lactam antibiotics because of low affinity PBPs (PBP5 in *E. faecium*, PBP4 in *E. faecalis*), which enable them to synthesize cell wall components even in the presence of modest concentrations of most beta-lactam antibiotics.[11] Due to the overproduction of low affinity PBP-5, higher level of resistance is observed in *E. faecium* in comparison to *E. faecalis* which can be inhibited by concentration of penicillin achievable in plasma . As a result, minimum inhibitory concentrations (MICs) for penicillins are typically 1–8 μg/ml for *E. faecalis* and 16-64 μg/ml for *E. faecium*.[12] The crystal structure of PBP5 indicates that specific amino acid differences may be responsible for resistance by interfering with the architecture of the active site or with β-lactam binding.[13] *Enterococci* are known to be typically tolerant to bactericidal activity of cell-wall active agents, such as β–lactam antibiotics and vancomycin. Tolerance

implies that the *enterococci* are not killed by penicillin when exposed to drug concentrations in the range of the MIC but will only be killed by concentrations far in excess of the inhibitory concentration.[14] Tolerance in *E. faecalis* has been attributed to removal of reactive oxygen species by the enzyme superoxide dismutase.[15] Tolerance may be induced when penicillin was administered by pulsed-dosing. As most *enterococci* are tolerant to cell wall active agents, penicillin or glycopeptide, alone often fail to cure serious infections like endocarditis and meningitis which require bactericidal therapy and this is achieved by synergistic effect of penicillin/ampicillin plus aminoglycoside: standard treatment for serious infection.[16]

Aminoglycosides.

Intrinsic resistance to clinically achievable concentrations of Aminoglycosides is seen both in *E. faecium* and *E. faecalis.* In *E. faecalis*, Intrinsic resistance is attributed to an inability of the aminoglycoside to enter the cell where they act by inhibiting ribosomal protein synthesis and MICs vary for the aminoglycosides,with the greatest degree of resistance seen to streptomycin (MIC up to 500 µg/ml). The combination of cell wall active agents and aminoglycosides thus results in bactericidal activity (bactericidal synergism) provided physiologic context to the long-standing observations of improved clinical outcomes with aminoglycoside-penicillin combination therapy.[17,18]Intrinsic resistance in *E.faecium* is attributed to chromosomally-encoded enzymes that increase the MIC of Aminoglycosides and prevent synergism. Ubiquitous aminoglycoside 6' acetyltransferase [aac(6')-Ii] confers resistance to tobramycin with MICs as high as 1000 µg/ml and to kanamycin, an efmM-encoded m5C methyltransferase confers low-level resistance to dibekacin, tobramycin and kanamycin. EfmM methylates the 16S rRNA resulting in a sterically-hindered ribosome target site.[19]Intrinsic enzyme-mediated high-level resistance to neither gentamicin nor streptomycin has been described in *enterococci.*As such, these drugs retain synergistic activity in *enterococci* and have consequently emerged as the drugs of choice to achieve synergism in severe infections caused by either *E. faecium* or *E. faecalis*.[20]

Lincosamides and streptogramins.

Intrinsic resistant to clindamycin (a lincosamide), quinupristin (streptogramin B class) and dalfopristin (streptogramin A class) in *E. faecalis* is conferred by expression of the *lsa* gene. Drug efflux is the possible mechanism as *lsa* gene is related structurally to ATP-binding cassette (ABC)-efflux pumps.[21] In general, for clinical resistance to quinupristin-dalfopristin to occur, the bacteria must be resistant to both streptogramin A and streptogramin B. *E. faecium* harbors a different putative ABC-efflux pump encoded by the intrinsic msrC gene. This gene, a close relative of msrA and msrB in staphylococci, confers low-level resistance (MIC 1–2 µg/ml) to streptogramin B compounds, explaining the elevated quinupristin dalfopristin MICs seen when *E. faecium* acquires a separate determinant that confers streptogramin A resistance alone.[22]

Trimethoprim-sulfamethoxazole.

The antibiotic combination trimethoprim-sulfamethoxazole inhibits two sequential steps in the tetrahydrofolate synthesis pathway, thereby inhibiting folate synthesis and synergistically killing a broad spectrum of bacterial species. *Enterococci* can absorb folic acid from the environment, thus bypass the effects of trimethoprim-sulfamethoxazole. In vitro testing of enterococcal susceptibility to trimethoprim-sulfamethoxazole in a media devoid of the folate will yield a susceptible result. Despite this apparent in vitro susceptibility, trimethoprim-sulfamethoxazole is ineffective in treating serious Enterococcus infections.[23,24]

Acquired Resistance

Acquired resistance in *enterococci* can occur through sporadic mutations or through acquisition of foreign genetic material through the transfer of plasmids, or through the movement of transposons.

β-lactams.

Acquired resistance to penicillins in *Enterococci* is through acquisition of Plasmid-mediated bla genes (encoding β-lactamases) or PBP4/5 mutations. *E. faecalis*, expressing β-lactamase enzyme with high level resistance to penicillin (HLPR) and ampicillin (MIC > 256

µg/mL) have been reported in various studies. Production of β-lactamase enzyme is plasmid-mediated and enzyme is constitutively produced. The bla genes in *enterococcus* are identical to those in *S. aureus* and are often encoded by remnants of staphylococcal β-lactamase transposon Tn552.The presence of β-lactamase is not a therapeutic challenge, as it was inhibited by β-lactamase inhibitor combinations (such as ampicillin–sulbactam), but it may pose a diagnostic challenge, as strains test positive for resistance only at high inocula.[26,27,28] High-level penicillin resistance in *E. faecium* is most commonly associated with accumulation of point mutations in the penicillin binding region of PBP5. A variety of point mutations have been described in both *E. faecium* and *E. faecalis*.Although these point mutations likely originated de novo in individual bacteria under selective pressure from antibiotics, chromosome-to-chromosome transfer of low affinity pbp5 genes have been documented in vitro and likely contributes to the dissemination of high-level penicillin resistance in *E. faecium*.[29,30,31] Resistance to β-lactams can be tested by disk diffusion method using Penicillin 10 units and Ampicillin 10 µg discs and the results interpreted as per Clinical Laboratory Standards Institute(CLSI) recommendations. The MIC Interpretive Criteria (µg/mL) for penicillin and ampicillin susceptibility is <8µg/ml and resistance is >16µg/ml respectively. The results of ampicillin susceptibility tests may be used to predict the amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin, and piperacillin-tazobactam among non β-lactamase-producing *enterococci*. Ampicillin susceptibility can be used to predict imipenem susceptibility, providing the species were confirmed to be *E. faecalis.* However enterococcal susceptibility cannot be predicted on the basis of ampicillin testing results. Testing of Penicillin is required if penicillin results are needed. *Enterococci* susceptible to penicillin are predictably susceptible to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, piperacillin, and piperacillin-tazobactam for non β-lactamase-producing *enterococci.* However, *enterococci* susceptible to ampicillin cannot be assumed to be susceptible to penicillin. . Penicillin or ampicillin resistance due to β-lactamase production is not reliably detected with routine disk or dilution methods, but is detected using a direct, nitrocefin-based β - lactamase test.[38,56]

Aminoglycosides.

Acquisition of mobile genetic elements typically underlies high-level aminoglycoside resistance(HLAR) in both *E. faecium* and *E. faecalis* resulting in MICs ranging from 2,000 µg/ml to as high as 128,000 µg/ml.This occurrence of acquired high-level resistance to all available aminoglycosides eliminates the potential for synergistic treatments of serious enterococcal infections .High-level resistance to gentamicin is usually the consequence of a transposon designated Tn5281 that encodes the bifunctional aminoglycoside-modifying enzyme(AME) aac(6′)-Ie–aph(2″)-Ia, which confers resistance to all commercially available aminoglycosides except streptomycin. Aminoglycoside-modifying enzymes catalyse the covalent modification of amino and hydroxyl groups within the aminoglycoside molecule, markedly decreasing the binding affinity between the antibiotic and the bacterial 30S ribosomal subunit . Other genes that confer gentamicin resistance, include aph(2")-Ic, aph(2")-Id and aph(2")-Ib and are known to be minor contributors when compared to aac(6′)-Ie–aph(2″)-Ia.[32,33] Their prevalence varies by geographical region. Importantly, MICs for *enterococci* harbouring aph(2")-Ic may be as low as 256 µg/ml, an MIC which would be interpreted as gentamicin-susceptible by labs that use an MIC of 500 µg/ml as a cut-off to determine highlevel gentamicin resistance. Despite the lower MIC, bacteria expressing these enzymes were resistant to the synergistic activity of cell wall active agents and gentamicin. Thus, in geographical area where aph (2")-Ic is present, laboratories should be alerted to lower the threshold MIC for gentamicin to enhance detection of *enterococci* that would be resistant to synergy.[34] High-level resistance to streptomycin results from ribosomal mutations, which is usually changed the S12 ribosomal protein, or through enzymatic modification of the antibiotic by acquisition of an aminoglycoside nucleotidyltransferase, ant(3″)-Ia or ant(6′)-Ia.[32]Resistance caused by aminoglycoside-modifying enzymes will typically have MICs in the 4,000 to 16,000 µg/ml range, ribosomal mutations result in MICs of 128,000 µg/ml.[35] Other acquired AMEs have been identified in

enterococci, including aph(3')-IIIa, an aminoglycoside phosphotransferase that confers resistance to kanamycin and ant(4")-Ia, a nucleotidyltransferase that confers resistance to tobramycin, amikacin, neomycin and kanamycin. As these enzymes did not confer gentamicin or streptomycin resistance, they are of less clinical sinificance.[36,37] According to CLSI, screening Test for detection of High-Level Aminoglycoside resistance in *Enterococcus* species is performed using high-content gentamicin (120 µg) and streptomycin (300 µg) disks. For broth and Agar dilution 500 µg/ml of gentamicin is used and 1000 µg/ml and 2000 µg/ml of streptomycin is used for broth and agar dilution respectively.[38]Mendiratta et al have reported a total 46% of enterococcal isolates with high level resistance to gentamicin and/or streptomycin by both, high content disc diffusion and agar dilution (MIC).[39] Hasani A et al in their study using multiplex PCR revealed that high-level gentamicin-resistance was related to the presence of *aac(6')Ie-aph(2")Ia*. Though an array of AMEs are responsible for HLAR status among *Enterococcus* species, Padmasini.E et al from India using multiplex PCR have demonstrated *aac(6')Ie-aph(2")Ia* and *aph(3')IIIa* genes occurring more frequently than other genes. This observation was emphasizes the restricted gene distribution and transfer of resistant genes within a geographical region.[40,41]

Glycopeptide resistance

Vancomycin was the first glycopeptide antibiotic to be discovered as early as 1950 and was the usual alternative to ampicillin for infections caused by ampicillin-resistant *enterococci* or in patients with severe allergy to β-lactams. The emergence of high-level resistance to glycopeptides in *enterococci,* first reported by Uttley et al. in 1988 from Great Britain was surprising, as vancomycin had been used for decades without the emergence of resistance. Intrinsic resistance to the glycopeptide, has been described in many bacterial species like *Erysipelothrix rhusopathiae, Lactobacillus, Leuconostoc and Pediococcus* as these organisms produced peptidoglycan precursors with decreased affinity for vancomycin, a similar resistance is seen in *Enterococcus gallinarum* and *Enterococcus casseliflavus.*[1,42,43] Glycopeptides inhibit bacterial growth by interfering with peptidoglycan biosynthesis. Normally peptidoglycan synthesis in *enterococci,* involves addition of two molecules of D-alanine joined by a ligase enzyme to form D-Ala-D-Ala, to UDP-*N* acetylmuramyl-tripeptide to form the UDP-*N* acetylmuramyl-pentapeptide .The pentapeptide is incorporated into the nascent peptidoglycan (transglycosylation), permitting the formation of cross bridges (transpeptidation) thus contributing to the strength of the peptidoglycan layer. The antibiotics form complexes with the D-Ala-D-Ala peptide termini of peptidoglycan precursors on the outer surface of the cell, which prevents the cell wall biosynthetic enzymes (i.e., the PBPs) from using them as substrates for transglycosylation and transpeptidation and, hence impairment of cell wall integrity.[44] The mechanism of Glycopeptide resistance has been extensively reviewed previously. The biochemical basis for the resistance derives from modification of the antibiotic target wherein glycopeptide-resistant *enterococci*(GRE) produce altered peptidoglycan precursors in which the D-Ala-D-Ala termini have been modified such that they terminate in either D-Ala-D-lactate or D-Ala-D-Ser. These substitutions reduce the binding affinity of the antibiotics for the peptidoglycan precursors (~1000 fold reduction for D-Ala-D-lac; ~7 fold for D-Ala-D-Ser). The altered precursors can still serve as substrates for the cell wall biosynthetic enzymes to enable the construction of functional peptidoglycan, but the reduced affinity of glycopeptides renders the drugs unable to inhibit cell wall biosynthesis. The other mechanism involves prevention or destruction of precursors that end in D-Ala–D-Ala by specific D, D-dipeptidases and carboxypeptidases.[45] Nine distinct gene clusters conferred glycopeptides resistance have been described in *enterococci* on the basis of phenotypic and genotypic criteria VanA, VanB, VanD, VanE, VanG, VanL, VanM, and VanN . VanC is an intrinsic characteristic of *Enterococcus gallinarum* and *Enterococcus casseliflavus.*[46] These determinants differ from each other, based on their physical location (encoded on a mobile genetic element or in the core genome); the specific glycopeptides to which they confer resistance (providing resistance to both vancomycin and teicoplanin,or resistance to vancomycin but not teicoplanin); the level of resistance they confer;

whether resistance is inducible or constitutively expressed; and the type of peptidoglycan precursor that is produced by their gene products.(Table 1)[46,47,48,59] The *van* gene clusters conferring glycopeptide resistance are typically referred to by the names given to the ligases they encode ;*vanA,vanB, vanD and vanM* encoding d Ala:d-Lac or *vanC, vanE, vanG, vanL* and *vanN* coding for d-Ala:d-Ser for the synthesis of peptidoglycan precursors with low affinity for glycopeptides. The *vanA* , *vanB* , and *vanD* gene clusters contain genes for a two-component regulatory system (*vanR and vanS*) that is responsible for sensing the presence of glycopeptides and activating expression of the resistance genes in inducible Van types, three resistance genes (*vanH* , encoding dehydrogenase; *vanA* ,*vanB* , or *vanD* , encoding ligase;*vanX* , encoding dd-dipeptidase); an accessory gene (*vanY*); and the *vanZ* gene, which is present in the *vanA* gene cluster, whereas the *vanW* gene is found only in the *vanB* operon. Frequently, van genes are located in plasmids or transposons, which facilitate their dissemination by means of horizontal gene transfer. The most prevalent resistance types in clnical isolates are VanA and VanB.[49]

The VanA phenotype

This phenotype represents the frequent form of glycopeptide resistance in *Enterococci* . Generally, it was associated to a high level resistance to vancomycin.

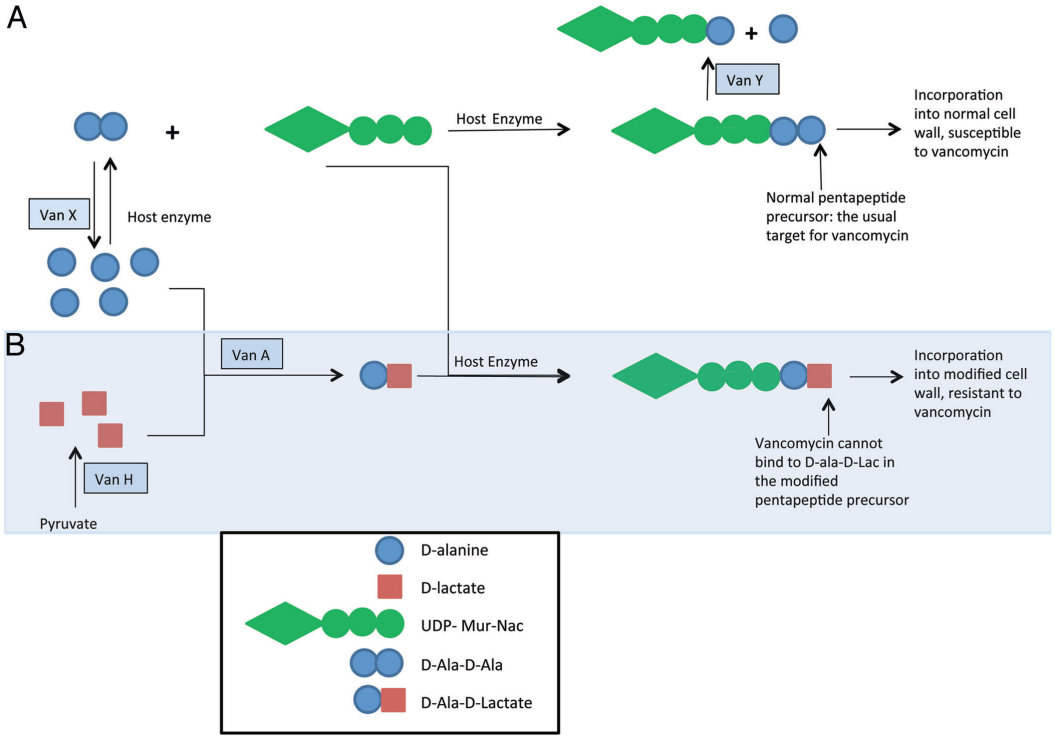

Figure 1

VanA resistance mechanism as it relates to normal cell wall synthesis. The top pathway denotes normal cell wall synthesis,and the mechanisms by which VanX and VanY disrupt this pathway. The shaded pathway denotes construction of a modified cell wall that is resistant to vancomycin. Adapted from Hollenbeck BL et al.[61]

(MIC-64 *µ*g/mL-≥1000 *µ*g/mL) and teicoplanin (MIC-16–512 *µ*g/mL MIC ≥ 8 µg/ml).This type of resistance is induced by glycopeptides (vancomycin,teicoplanin, avoparcin and ristocetin) and by other different antibiotics, like bacitracin, polymyxin B or robenidin. *vanA* is typically encoded on Tn1546 or related transposons. *vanA* encodes for the D-ala-D-lac ligase (VanA) that synthesizes the terminal dipeptide D-ala-D-lac, with much lower affinity for vancomycin. *vanH* encodes for one dehydrogenase (VanH), that produces D lactated by reduction of pyruvate. *vanX* (dipeptidase) cleaves D-Ala-D-Ala and *vanY* (D,D-carboxypeptidase) hydrolyzes the terminal D-ala from any normal pentapeptide

precursor, rendering it useless for normal cell wall construction.(Figure 1) This way, the absence of any vancomycin target in the cell wall is ensured.The mechanism by which *vanZ* augments resistance was unknown, but when present it confers decreased susceptibility to teicoplanin.[50] A two-component sensor-transducer system *vanR* and *vanS* that is part of the *vanA* operon within Tn1546 regulates the expression of the genes for *vanA, H, X, Y* and *Z* . While the specific regulatory factors are not known, the presence of glycopeptides or, more probably, some initial change caused by vancomycin on the cell wall in the environment results in activation of *vanS* through autophosphorylation. Activated *vanS* then phosphorylates *vanR*. Phosphorylated *vanR* increases *vanH, A, X, Y* and *Z* transcription through interaction with specific promoter regions. *vanR* also interacts with its own promoter region, augmenting *vanR* and *vanS* transcription. Clinical strains that harbor the *vanA* operon but contain deletions in *vanR* and *vanS* genes have been isolated and are susceptible to both vancomycin and teicoplanin suggested that *vanR* activity is required for the full expression of the *vanA* operon.[51,52,53]

The VanB phenotype

The *vanB* locus confers moderate to high-level resistance to vancomycin(MIC 4-1000 µg/ml), but most of the strains remain sensitive to teicoplanin in vitro, since this antibiotic cannot act as inducer.*vanB* is usually acquired on Tn5382/Tn1549 type transposons, which occur on plasmids or in the chromosome of the host. Genes analogous to their class A resistance counterparts are designated $vanH_B$, $vanX_B$, $vanY_B$, $vanR_B$, and $vanS_B$ .However, there is no gene counterpart of *vanZ* in these organisms and instead encodes a protein named *vanW*, whose role in resistance is not fully understood.. The regulatory system in class B strains appears insensitive to induction by teicoplanin.Teicoplanin induces the synthesis of

Table 1
Phenotypes and Genotypes of vancomycin resistant enterococci.

Phenotype	Gene clusters	Precursors end	Vancomycin resistance	Teicoplanin resistance	Type of resistance
VanA (commonly in *E.faecalis* and *E. faecium*)	*vanA* operon (*vanA, H, X, Y, Z, R, S*)	D-Ala-D-Lac	High-level resistance MIC-64 µg/mL-≥1000 µg/mL	High-level resistance MIC-16–512 µ/mL	High level inducible Resistance
VanB (commonly in *E.faecalis* and *E. faecium*)	*vanB* operon	D-Ala-D-Lac	High-level resistance MIC-4–512 µg/mL	Sensitive MIC≤ 0.5 µg/mL	High level inducible Resistance
VanC (*E.gallinarum*, *E. casseliflavus* , *E. flavescens*)	*vanC* operon	D-Ala-D-Ser	Low level resistance MIC-2 µg/mL-32 µg/mL	Sensitive MIC≤ 0.5 µg/mL	Low level constitutive Resistance
VanD (commonly in *E.faecalis* and *E. faecium*)	*vanD* operon	D-Ala-D-Lac	Moderate-High level resistance MIC-64–256 µg/mL	Low-level resistance MIC-4–32 µg/mL	Constitutive resistance
VanE (commonly in *E.Faecalis)*	*vanE* operon	D-Ala-D-Ser	Low-level resistance MIC-16 µg/mL	Sensitive MIC-≤0.5 µg/mL	Inducible resistance
VanG(commonly in *E.faecalis)*	*vanG* operon	D-Ala-D-Ser	Low level resistance MIC ≤ 16 µg/mL	Sensitive MIC≤ 0.5 µg/mL	Inducible resistance
VanL(commonly in *E.faecalis*	*vanL o*peron	D-Ala-D-Ser	Low level resistance MIC-8 µ/mL	Sensitive	Inducible resistance
VanM (commonly in *E. faecium*)	*vanM* operon	D-Ala-D-Lac	High-level resistance MIC> 256 µg/mL	High level resistance	Inducible resistance
VanN (commonly in *E. faecium*)	*vanN* operon	D-Ala-D-Ser	Low-level resistance MIC-16 µg/mL	Sensitive MIC≤ 0.5 µg/mL	Constitutive resistance

VanA-related proteins but does not induce the production of VanB related proteins. On the other hand, vancomycin induces the synthesis of the resistance proteins of both systems,

and in fact, if a teicoplanin-susceptible *enterococcus* with the *vanB* gene cluster is pre-exposed to vancomycin, the strain then tests teicoplanin resistant as well.[54,55]

Vancomycin-dependent enterococci

Some strains of VanA- and VanB-type VRE have developed a phenomenon of vancomycin dependence .These *enterococci* are not just resistant to vancomycin but now required it for growth. A likely explanation for the phenomenon of vancomycin dependence is that these *enterococci* turn off their normal production of D-Ala–D-Ala and then can grow only if a substitute dipeptide like structure is made. With most VanA- and VanB-type *enterococci*, this occurs only in the presence of vancomycin, which induces the synthesis of associated dehydrogenase (VanH) and ligase (VanA or VanB) that make D-Ala–D-Lac.The reason for the cell turning off the synthesis of D-Ala–D-Ala is that as long as vancomycin was present, D-Ala–D-Ala is not necessary for cell wall synthesis by VRE . Indeed, it is being destroyed by the action of VanX. Once the vancomycin is removed, D-Ala–D-Lac is no longer synthesized, and without either D-Ala–D-Ala or D-Ala–D-Lac, the cell cannot continue to grow or replicate. Reversion to vancomycin independence has been observed; it probably occurs by either a mutation that leads to constitutive production of D-Ala–D-Lac or one that restores the synthesis of D-Ala–D-Ala Laboratory detection of resistance to vancomycin can be performed by Kirby-Bauer disc diffusion method using vancomycin (30 *µ*g) disc and the results interpreted as per CLSI standards. Screening Test for Detection of Vancomycin Minimal Inhibitory Concentration ≥ 8 µg/mL in *Enterococcus* species can be performed using Vancomycin screen agar prepared with brain heart infusion (BHI) agar supplemented with 6 *µ*g/ml of vancomycin . For studying the MIC of vancomycin in *enterococci*, following are the MIC interpretive criteria according to CLSI to

(i) susceptible ≤4 µg/ml,
(ii) intermediate-8–16 µg/ml,
(iii) resistant ≥32 µg/ml.

E tests can also be used to detect the MIC'S instead of agar dilution.[38]Resistance associated with vanC is not usually detected by disc diffusion,but Van C strains grow on vancomycin screen agar. The need to differentiate VanA or VanB strains from VanC strains is quite evident for therapeutic, infection control and surveillance reasons. Because growth on vancomycin screen agar fails to help with this important distinction, species identification is necessary. Also resistance to other agents such as ampicillin and aminoglycosides is uncommon among VanC isolates.[56] Advanced methods of detection of VRE include automated culture and identification systems and chromogenic media. Vitek 2 and Phoenix systems have been shown to perform quite well in detecting vancomycin resistance in these organisms. Carroll et al. evaluated the capability of the BD Phoenix Automated Microbiology System to detect vancomycin resistant strains, all vancomycin-resistant strains were correctly identified by the system . Highly specific chromogenic substrates incorporated in CHROM ID VRE (BioMe'reiux) and the CHROMagar GRE (BD Diagnostics) are targeted by enzymes present specifically in *E. faecalis* or *E. faecium* and the degradation of these substrates leads to the species forming purple and blue-green colonies, respectively .In one study in 2008, the sensitivity and specificity of the CHROMID agar has been evaluated to be 96.9% and 99.4%, respectively.[56,57,58] Molecular methods used to detect the resistance genes have the advantage of being rapid. They can be used for surveillance of VRE using rectal swabs and stool samples and are less time consumed. Once standardized, these are also less expensive than the traditional culture screening methods. Since there are many genotypes of glycopeptides resistance, a multiplex PCR can prove helpful to detect which of the van genotypes is present in a particular isolate. A prospective study by Sue et al. found the overall performance of Multiplex PCR comparable to that of a chromogenic agar-based culture method for screening of VRE, and PCR could be an alternative or supportive method for effective control of nosocomial VRE infection.[59,60]

Macrolide, lincosamide, and streptogramin resistance

Macrolides, lincosamides, and streptogramin antibiotics inhibit protein synthesis by binding to the 50S subunit of the ribosome. The

different mechanisms of resistance to macrolides were target modification by precise mutation or by means of methylation of the 23S rRNA subunit so that it prevents binding of macrolides (i.e., genes ermA, ermB, ermC, ermTR), hydrolysis of the lactone ring of the antibiotic molecule, Efflux pumps, that remove antibiotic molecules from inside of the bacterial cell (i.e., genes mefA, mefE, msrA, msrC, mreA). The more frequent macrolide resistance determinants are erm genes coding for methyltransferase that acts on specific residues of the 23S rRNA subunit. This enzyme causes a N6-dimethylation of an adenine residue in the 23S rRNA subunit, inhibited erythromycin binding. The modification of the ribosomal target causes crossed resistance to macrolides, lincosamides and streptogramin B (MLS_B), or to macrolides and lincosamides, or to macrolides, ketolides and streptogramin A and B (MKS). Several erm genes have been described, erm (B) being the predominant one in *Enterococci* .[50] A second lincosamide resistance mechanism has been described in *E. faecium* ¸ mediated by a lincosamide nucleotidyl transferase encoded by linB that catalyzes 3-(5´-adenilation) of lincomicin and clindamicin. *Enterococcus* spp. may also contain export mechanisms for macrolide antibiotics.The genes responsible for this trait (mef) show a high mobility between diverse Gram-positive species .[50] The streptogramin B/A combination quinupristin-dalfopristin (Q/D) was the first drug approved by the US Food and Drug Administration (FDA) for the treatment of infections caused by vancomycin-resistant *E. faecium*. Both components bind to different sites of the 50S bacterial ribosome subunit and act synergistically by inhibition of the bacterial protein synthesis. The minimum inhibitory concentration of Q/D is quite effective on sensitive *E. faecium*, with MIC'S between 0.5 and 3 µg/ml, while *E. faecalis* is intrinsically resistant to this type of antibiotics There are three mechanisms by which acquired genetic elements cause streptogramin resistance: acetylation of the antibiotic, efflux of the antibiotic, and dimethylation of the 23S rRNA target site. Acquired resistance to Q/D in *E.faecium* can be mediated by Virginamycin acetyltransferase (vat) enzymes that acetylate streptogramin A (vat (B), vat (D), vat (E), or vat (G)). vatD and vatE have been isolated from plasmids alongside erm and vgbA genes (virginiamycin B lyase (Vgb)) that reduce susceptibility to streptogramin B—thus providing full resistance to quinupristin dalfopristin. Efflux of the antibiotic is by the ATP-binding cassette protein macrolide–streptogramin resistance protein (MsrC).The best understood mechanism of streptogramin resistance is dimethylation of the 23S rRNA.This resistance mechanism, which confers the MLS_A or MLS_B phenotype occurs through acquisition of either the ermA or ermB genes on broad host range plasmids such as pAMβ1. If these plasmids also contain vatE or vatD genes, then they confer resistance to quinupristin dalfopristin when acquired by a recipient cell.[61]

Linezolid resistance

Linezolid is the second of the two compounds that are approved by the FDA to treat *VRE* and is used worldwide. This oxazolidinone is bacte-riostatic and inhibits protein synthesis by interfering with the placement of the aminoacyl tRNA at the A site of the bacterial ribosome. Resistance to linezolid was still rare but has been documented in enterococcal outbreaks and even sporadically in patients who have never received the antibiotic. The most common mechanism of resistance found in enterococci involves G2576T mutations in genes encoding domain V of the 23S rRNA. This mutation interferes with the positioning of crucial nucleotides in the linezolid-binding site. As *E. faecium* and *E.faecalis* have six and four copies of the rRNA genes, respectively, the levels of resistance correlate with the a number of mutated alleles. Recombination between rRNA genes after the emergence of the G2576U mutation may enable the amplification of the level of linezolid resistance in enterococci under the selective pressure imposed by antibiotic treatment.[59]Rai et al.,have first reported linezolid resistant vancomycin resistance *Enterococcus faecium*(LRVRE) from India. However sequencing of the LRVRE isolates did not highlight any known or unknown mutations for linezolid resistance. Resistance may also be due to presence of a transferable plasmid-borne *cfr* gene encoding a methyl transferase enzyme responsible for methylation of A2503

in the 23SrRNA.Overall, linezolid resistance remains rare in *enterococci*.[62,63]

Daptomycin resistance

Daptomycin, a lipopeptide antibiotic, has potent in vitro bactericidal activity against *enterococci* (including ampicillin- and vancomycin-resistant *E. faecium*) and although it is not approved by the FDA it is often used by clinicians to treat *VRE* infections. As per CLSI, an MIC of ≤4 mg/L should be considered susceptible to this antibiotic. The mechanism of antimicrobial action for daptomycin is thought to involve calcium-dependent insertion into the cytoplasmic membrane followed by membrane depolarization, release of intracellular potassium ions, and rapid cell death.Daptomycin resistance have been observed in clinical isolates following daptomycin therapy as a result of mutations in chromosomal genes. The mechanism of daptomycin resistance in *enterococcus* remains unresolved; however reports have elucidated gene mutations associated with daptomycin resistance in enterococcus. Arias, et al.,explored the genetic basis of daptomycin resistance in *enterococci* by sequencing the genomes of a pair of *E. faecalis* strains isolated from the same patient before and after daptomycin therapy to identify polymorphisms contributing to resistance and found Unique sequence polymorphisms in cls, gdpD (both thought to be involved in phospholipid metabolism) and liaF .Changes in cls, liaF, liaS, or liaR were also identified in other daptomycin-resistant clinical isolates of enterococci, which suggests that such mutations play a key role in the development of daptomycin resistance in vivo. Comparative analysis of the resistant and susceptible isolates revealed that the resistant mutant has alterations in the ultrastructure of the cell membrane and cell wall, and that daptomycin is less able to depolarize the cell membrane of the resistant isolate than that of the susceptible isolate. LiaF is part of the three-component LiaFSR regulatory system, which is known to coordinate the response of the cell envelope to antibiotics and antimicrobial peptides in some Gram-positive bacteria, which suggests that perturbations in the activity of this signalling system may alter envelope properties in a such a way that daptomycin can no longer interact with, or insert into, the membrane efficiently.[64]

Tigecycline.

Tigecycline, a novel glycylcycline antibiotic, gained FDA approval in 2005 for complicated intra-abdominal infections, skin and soft tissue infections, and community-acquired pneumonia. It has been used off-label to successfully treat MRSA and GRE infections. Typical tigecycline MICs for enterococcus range from 0.125 mg/ml to 0.25 mg/ml, while MICs 0.5 µg/ml is considered resistant. Early surveillance studies of tigecycline showed no. cases of resistant enterococcus, although two case reports of E.faecalis strains with MICs of 2 µg/ml and 6 µg/ml, respectively, have been described. A more recent study from Taiwan reviewed antimicrobial resistance among 219 VRE isolates and found two isolates with a tigecycline MIC of 0.5 µg/ml, and one isolate with an MIC of 1 µg/ml, with a trend toward increasing tigecycline MIC over time. The mechanism of tigecycline resistance in *enterococcus* is unknown.[65]

Treatment of Vancomycin-Resistant Enterococcus*:*

Vancomycin resistant enterococci pose particular problems for treatment because the strains which harbor VanA and VanB resistance are also typically resistant to other classes of antibiotics. The drugs licensed by FDA for treatment of VRE infections are quinupristin/dalfopristin and linezolid. In uncomplicated cases, monotherapy based upon the antibiotic susceptibility profile is appropriate. Because of limited alternatives chloramphenicol, erythromycin, tetracycline and rifampin may be tested for VRE. In complicated cases such as endocarditis, the ideal therapy for VRE has not been determined. The use of quinupristin/dalfopristin, an injectable streptogramin has several limitations, included the need for central venous administration, metabolic interactions, an adverse-effect profile, a lack of bactericidal activity against *Vancomycin Resistant Enterococcus faecium* isolates, development of resistance and a lack of activity against *E. faecalis*. For these reasons Linezolid available for oral or intravenous administration has been used for VRE endocarditis in both *E. faecalis* and *E.*

faecium, both with and without additional agents. However the effectiveness of this agent for the treatment of VRE endocarditis remains controversial because of its bacteriostatic mode of action.[66.]Chong et al. in a small centre concluded that there was no significant difference between the efficacy of quinupristin-dalfopristin and linezolid. However, they found that prolonged bacteraemia and the development of resistance were more common in quinupristin-dalfopristin treated patients.[67] Monotherapy with daptomycin may be adequate in many VRE infections; however failure has been reported after prolonged treatment. Daptomycin is found to be inhibited by pulmonary surfactant so should not be used for pneumonias. Arias et al. reported subsequent response to the combination of daptomycin/gentamicin/ampicillin after failing daptomycin monotherapy, which may be explained by a synergistic effect of the triple therapy. Daptomycin synergy has been described in vitro with ampicillin, cephalosporins, imipenem, rifampin and gentamicin.[68,69]A meta-analysis by Chuang et al has shown that linezolid treatment for VRE bacteremia was associated with a lower mortality than daptomycin treatment.Gupta et al from India have reported all VRE isolates sensitive to Linezolid and Daptomycin highlighting the importance of susceptibility testing to Daptomycin of VRE isolates in patients on therapy with Daptomycin.[70,71] Tigecycline has an in vitro bacteriostatic activity against VRE, but its clinical use for serious enterococcal infections needs further evaluation.

CONCLUSION

Enterococci have demonstrated the potential to harbor and transfer resistance genes and as such have become an important clinical pathogen. Vancomycin still remains the mainstay of treatment for serious enterococcal infections, if the strain is found susceptible. However, with the emergence of resistance to vancomycin other antibiotics like linezolid, quinupristin-dalfopristin, tigecycline and daptomycin can also be considered. The data on local patterns of susceptibility of VRE to newer antimicrobial agents can help in guiding the treatment of these pathogens. Further studies were required to understand the mechanisms of resistance to Daptomycin and Tigecycline that will assist in prediction and prevention of epidemiologic spread. Future directions of research must also focus on development of new antimicrobial agents. Finally, efforts must continue to prevent development of antibiotic resistance and spread in the enterococci through infection control and antibiotic stewardship programs.

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