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### REVIEW ARTICLE

## AN EMERGENCE OF ANTIBIOTIC RESISTANCE ENTEROCOCCUS INFECTION AND ITS MANAGEMENT; AN OVERVIEW.

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### Abstract

Enterococci have emerged as a leading cause of nosocomial infections and now rank among the most common nosocomial pathogens isolated from the bloodstream, surgical sites, and urinary tract infections. *E. faecalis* accounts for approximately 75% of all enterococcal infections, with *E. faecium* accounting for most of the rest. Enterococci have a remarkable ability to survive in an environment of heavy antibiotics. Indeed, it is resistance of organisms to multiple antimicrobial agents makes such feared opponents. Infections with vancomycin-resistant Enterococcus (VRE) have become a threat in nosocomial settings, with an increased incidence reported worldwide including India. The emergence of resistance to the most common anti enterococcal antibiotics has made the treatment of these infections a real challenge for clinicians. This review study providing the current and possible future therapeutic options for the management of infections caused by multidrug-resistant (MDR) *E. faecalis*.

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### Introduction:-

Enterococci are part of the normal intestinal flora of humans and animals but are also important pathogens responsible for serious infections. The genus Enterococcus includes more than 17 species, but only a few cause clinical infections in humans. With increasing antibiotic resistance, enterococci are recognized as feared nosocomial pathogens that can be challenging to treat [1].

Some of the common Enterococcus species includes *E. aquimarinus*, *E. asini*, *E. avium*, *E. caccae*, *E. camelliae*, *E. canintestini*, *E. canis*, *E. casseliflavus*, *E. cecorum*, *E. columbae*, *E. devriesei*, *E. dispar*, *E. durans*, *E. faecalis*, *E. faecium*, *E. flavescens*, *E. gallinarum*, *E. gilvus*, *E. haemoperoxidus*, *E. raffinosus*. Enterococcus species are hardy, facultative anaerobic organisms that can survive and grow in many environments. In the laboratory, enterococci are distinguished by their morphologic appearance on Gram stain and culture (gram-positive cocci that grow in chains) and their ability to (1) hydrolyze esculin in the presence of bile, (2) grow in 6.5% sodium chloride, (3) demonstrate pyrrolidonyl arylamidase and leucine aminopeptidase, and (4) react with group D antiserum. Before they were assigned their own genus, they were known as group D streptococci [2, 3].

Isolation of enterococci resistant to multiple antibiotics has become increasingly common in the hospital setting [4]. According to National Nosocomial Infections Surveillance (NNIS) data from January 2012 through December 2012,

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more than 28% of enterococcal isolates in ICUs of the more than 300 participating hospitals were vancomycin-resistant. Clonal spread is the dominant factor in the dissemination of multidrug-resistant enterococci in North America and Europe. Virulence and pathogenicity factors have been described using molecular techniques. Several genes isolated from resistant enterococci (agg, gelE, ace, cylLLS, esp, cpd, fsrB) encode virulence factors such as the production of gelatinase and hemolysin, adherence to caco-2 and hep-2 cells, and capacity for biofilm formation [5, 6].

### **Enterococcus faecalis**

*E. faecalis* are the most prevalent species cultured from humans, accounting for more than 90% of clinical isolates. Other enterococcal species known to cause human infection include *Enterococcus avium*, *Enterococcus gallinarum*, *Enterococcus casseliflavus*, *Enterococcus durans*, *Enterococcus raffinosus* and *Enterococcus mundtii*. *E. faecium* represents most vancomycin-resistant enterococci (VRE) [7]. *E. faecalis* is a ubiquitous bacterium that is capable of surviving in a broad range of natural environments, including the human host, either as a natural commensal or as an opportunistic pathogen involved in severe hospital acquired infections. Such opportunistic pathogens cause fatal infections is largely unknown but they should be equipped with sophisticated systems to perceive external signals and interact with eukaryote cells. Accordingly, being partially exposed at the cell exterior, some surface associated proteins are involved in several steps of the infection process. Among them are lipoproteins, representing about 25% of the surface associated proteins, which could play a major role in bacterial virulence processes. This present research focuses on the identification of 90 lipoprotein encoding genes in the genome of the *E. faecalis* clinical strain and their putative roles, and provides a transcriptional comparison of microarray data performed in environmental conditions including blood and urine. Taken together, these data suggest a potential involvement of lipoproteins in *E. faecalis* virulence, making them serious candidates for bacteriophage production [7].

### **Infection:**

Enterococci are a part of the normal human faecal flora. Sites less often colonized by enterococci include the oral cavity, genitourinary tract and skin especially in the perineal area. The main sites of colonization in the hospitalized patients are soft tissue wounds, ulcers and the gastrointestinal tract (GIT). Enterococci were traditionally regarded as low grade pathogens but have emerged causing increasingly nosocomial infections in the 1990s [8].

Enterococcal infections may be due to 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. solitarius*. Additional species such as *E. cecorum*, *E. columbae*, *E. saccharolyticus*, *E. dispar*, *E. sulfureus*, *E. seriolicidus* and *E. flavescens* have been proposed as added to this list [9].

Most clinical infections are due to either *E. faecalis* or *E. faecium*. Until recently *E. faecalis* had been the predominant enterococcal species, accounting for 80-90% of all clinical isolates, and *E. faecium* had accounted for 5 to 15%. Enterococci are the third most common pathogen isolated from bloodstream infections [10], the single most frequently reported type of pathogen in surgical-site infections in intensive care units [11], and the second most common nosocomial pathogen in the US [12].

Enterococci are responsible for three to four cases of nosocomial bloodstream infections per 10,000 hospital discharges [13]. These bacteria contribute significantly to patient mortality as well as to additional hospital stay [14]. The ability of enterococci to acquire, accumulate, and transfer genetic elements such as plasmids and transposons via conjugation is one of the major reasons for their increased importance as nosocomial pathogens [2]. Transfer of resistance determinants from enterococci to other more virulent Gram-positive bacteria, like staphylococci, has been observed in vitro [2]. The first isolation of a fully vancomycin-resistant *E. faecalis* strain in a patient previously colonized with VRE suggests the possibility of an in vivo exchange of resistance traits [15]. Enterococci can cause a variety of clinical syndromes including endocarditis; bacteremia, meningitis, intra abdominal wound, and urinary tract infections. There are well-defined patient populations (e.g. liver-transplant patients [16], neonates [17] patients with hematological malignancies [18] who would clearly benefit from improved treatment options for *Enterococcus* infections.

### **Urinary tract infections**

1. The most common type of infection caused by enterococci is usually nosocomial (associated with urinary tract catheterization or instrumentation).
2. Cystitis and pyelonephritis are common infections.

3. Occasionally, prostatitis and perinephric abscesses may develop.
4. Occasional infections may occur in young healthy women (< 5%).

### **Bacteremia**

1. Sources of Enterococcal bacteremia include the urinary tract, intra-abdominal foci, wounds, and intravascular catheters, especially catheters in femoral locations.
2. Community-acquired Enterococcus bacteremia is more commonly associated with endocarditis (up to 36% of cases) than nosocomial bacteremia (0.8%).
3. Nosocomial enterococcal bacteremia may arise from various sources. Polymicrobial bacteremia including enterococci and other bowel flora should increase the index of suspicion for an intra-abdominal source. Other sources may include surgical sites and burn wounds infections.
4. Blood cultures that grow enterococci may be positive because of contamination of the skin with these organisms. A blood culture positive for Enterococcus species in the absence of evidence of ongoing infection should raise this possibility.

### **Endocarditis**

1. Enterococci cause 5-15% of all endocarditis cases.
2. Enterococcal endocarditis usually occurs in older patients, particularly men.
3. The presentation of enterococcal endocarditis is typically subacute and infrequently associated with peripheral stigmata of endocarditis. Enterococcal endocarditis of native valves carries a relatively low short-term mortality rate.
4. Most cases of enterococcal endocarditis are left-sided. In two recent series of endocarditis caused by vancomycin-resistant enterococci (VRE), the aortic valve was involved more often than the mitral valve. (Stevens MP et al 2005).
5. *E. faecalis* causes most cases of endocarditis. Vancomycin-resistant *E. faecium* is more likely to cause endocarditis than other VRE species, especially cases acquired nosocomially. Risk factors for enterococcal endocarditis may include urinary tract infection or instrumentation [19].

### **Intra-abdominal and pelvic infections**

1. Such infections include biliary tract infection, intra-abdominal abscess, spontaneous bacterial peritonitis, endometritis and salpingitis.
2. Enterococci are usually part of mixed aerobic and anaerobic flora.
3. Antimicrobial regimens with minimal in vitro anti enterococcal activities are often effective in treating mixed infections; therefore, the pathogenicity of enterococci in this setting is questionable. Anti enterococcal bactericidal activity is recommended when blood culture results are positive for enterococci
4. In more seriously ill patients, enterococcal infections have been associated with higher risk of treatment failure and mortality. Consider administering antibiotics with anti enterococcal activity to immunocompromised patients at high risk for bacteremia, patients with peritonitis and valvular heart disease, patients with severe sepsis of abdominal origin who have recently received broad-spectrum antibiotics, and patients with persistent intra-abdominal fluid collections without clinical improvement [20].

### **Enterococcus antibiotic resistant pattern:**

The treatment of **Enterococcus** infections is limited by the intrinsic resistance among enterococci. In general, enterococci show intrinsic resistance to cephalosporins, lincosamides, and many synthetic  $\beta$ -lactams, such as the penicillinase-resistant penicillins [21]. **Enterococcus** species are also resistant to low levels of aminoglycosides, due to the decreased uptake of this antibiotic class. In this study, a majority of the **E. faecium** and **E. faecalis** isolates showed inherent resistance patterns which were consistent with previous studies [22]. For instance, both **E. faecium** and **E. faecalis** had intrinsic resistance to bacitracin, i.e., 90% of the isolates were inhibited at concentrations greater than 128 IU/ml.

Penicillin, vancomycin, aminoglycosides, chloramphenicol, ciprofloxacin, and quinupristin-dalfopristin all have been used in the treatment of enterococcal infections either in combination therapy, for optimal killing, or monotherapeutically [24]. Synergistic treatment includes the use of an aminoglycoside with the addition of a cell wall-active agent, such as vancomycin or penicillin. In the present study, there was an extremely low level of resistance to the aminoglycosides (**E. faecium**, 3%; **E. faecalis**, 0%), vancomycin (**E. faecium**, 0%; **E. faecalis**, 0%), and penicillin (**E. faecium**, 7%; **E. faecalis**, 0%). Chloramphenicol, also used synergistically in documented

cases, was shown to inhibit a majority of all isolates; 5% of *E. faecium* strains and only 3% of *E. faecalis* strains were resistant to chloramphenicol. According to Chow and Shlaes, enterococcal infections of less severity have been treated with a single antibiotic. Among the drugs in the NARMS 2001 panel, an example of such an antibiotic is ciprofloxacin, to which 27 (28%) of the *E. faecium* strains and 2 (5%) of the *E. faecalis* strains were found to be resistant. Quinupristin-dalfopristin can also be used for the treatment of *E. faecium* infections in humans, and in our study, 16 (16%) isolates were found to be resistant and 48 (49%) were intermediately resistant to these drugs. There is evidence that during the therapeutic use of quinupristin-dalfopristin for *E. faecium* bacteremia, super infection of *E. faecalis* can occur, posing concerns regarding such a high proportion of *E. faecium* resistance to these drugs [25]. Finally, and consistent with the literature [26], we found that all *E. faecalis* strains were susceptible to Nitrofurantoin, a drug frequently used for the treatment of *E. faecalis* urinary tract infections. When the data are taken together, there was a relatively low prevalence of resistance to most of the drugs used in clinical treatment of enterococcal infections in humans, especially for *E. faecalis*.

#### **Antibiotic resistance in *Enterococcus faecalis*:**

The emergence of resistance to the most common anti enterococcal antibiotics has made the treatment of these infections a real challenge for clinicians. We review the current and possible future therapeutic options for the management of infections caused by multidrug-resistant (MDR) *E. faecalis* [27].

#### **$\beta$ -lactams and synergism with aminoglycosides:**

Enterococci are often tolerant to the activity of penicillin and other  $\beta$ -lactams, this property differentiates enterococci from most streptococci which, in general, are also susceptible to much lower concentrations of  $\beta$ -lactams. Although rare, resistance to  $\beta$ -lactam antibiotics in *E. faecalis* is usually mediated by the production of a  $\beta$ -lactamase enzyme [2]. Non- $\beta$ -lactamase-mediated resistance to ampicillin and imipenem has also been reported in *E. faecalis* and appears to be associated with mutations of the *pbp4* gene [28]. Conversely, resistance to  $\beta$ -lactams in most clinical isolates of *E. faecium* is associated with mutations or overproduction of PBP5, with ampicillin MICs of >256 mg/L in some strains [2].

#### **Synergism with aminoglycosides:**

Gentamicin and streptomycin are the recommended aminoglycosides for synergistic therapy in combination with a cell wall agent and the use of other compounds of this family is not recommended because of the frequent presence of the aminoglycoside 6e-acetyltransferase (an intrinsic feature of *E. faecium*, precluding the use of tobramycin, kanamycin, netilmicin and sisomicin) and the *aph*-(3e)-IIIa gene that confers HLR to kanamycin and abolishes synergism with amikacin. Although enterococci are not susceptible to gentamicin and streptomycin at levels used for other organisms (considered to be a result of a decrease in the permeability of the cell wall), the addition of an agent that blocks peptidoglycan synthesis markedly increases the uptake of these antibiotics [2, 24]. Nonetheless, in recent years, the acquisitions of ribosomal mutations and/or aminoglycoside modifying enzymes that confer HLR to streptomycin or gentamicin continue to increase worldwide (although independent mechanisms, both can occur in the same strain).

#### **Acquired resistance to aminoglycoside:**

Some isolates of enterococci were highly resistant to streptomycin (minimal inhibitory concentration, >2000  $\mu$ g/mL, which eliminated any benefit of adding streptomycin to penicillin) but were not highly resistant to gentamicin led to the widespread use of gentamicin plus penicillin for enterococcal endocarditis [29]. Subsequently however, enterococci developed high level resistance to gentamicin that caused resistance to synergism between Gentamicin and penicillin (i.e., the of any benefit of adding Gentamicin to penicillin) [2].

#### **Glycopeptides and lipoglycopeptides:**

The isolation of vancomycin-resistant enterococci (VRE) has steadily increased worldwide subsequent to 1986. A recent study from the CDC indicates that, among 983 *E. faecium* isolates analyzed (2006–2007), 80% were resistant to vancomycin; conversely, just 6.9% of *E. faecalis* isolates were vancomycin-resistant ( $n = 1542$ ) [30]. In Europe, the emergence of VRE was initially correlated with the use of the glycopeptides, avoparcin, which was used as a growth promoter in animal husbandry. However, even after the ban of avoparcin, the European continent has continued to experience an important increase in the isolation of VRE (*E. faecium*) from hospitals, indicating that other factors are promoting the dissemination of VRE in Europe. The increased isolation of vancomycin-resistant *E. faecium* in hospitals worldwide has been attributed to the emergence of a specific genetic lineage designated clonal cluster 17 [31]. Vancomycin resistance continues to evolve in enterococci and newer phenotypes have been

described. Because of the increased presence of gene clusters conferring resistance to glycopeptides in *E. faecium*, vancomycin has become almost obsolete for the treatment of *E. faecium* infections (at least in the USA).

### Vancomycin

Prior to 1984, the glycopeptide class included few members beyond vancomycin, teicoplanin, ristocetin, and avoparcin. Vancomycin is a powerful, chlorine-containing antibiotic which often works when other antibiotics fail. It has been called the “antibiotic of last resort,” having saved the lives of many patients suffering from serious bacterial illnesses. Vancomycin hydrochloride is the hydrochloride of a mixture of related glycopeptides produced by the growth of certain strains of *Amycolatopsis orientalis* or by any other means.

### Discovery:

A last line of defense against the menace of was erected by the discovery of the antibiotic vancomycin by Eli Lilly in 1956. In the mid-1950s, scientists at Eli Lilly isolated vancomycin from a fermentation broth of the actinomycete *Streptomyces orientalis*, later renamed *Nocardia orientalis*, and finally reclassified as *Amycolatopsis orientalis*.

### Structure:

The chemical structure of vancomycin is given in figure and can be described as a seven-member peptide chain with two sugar moieties, vancosamine and glucose. Vancomycin has a large molecule: its chemical formula is  $C_{66}H_{75}Cl_2N_9O_{24}$ , with a molecular weight of 1486 Da. It is a white to almost white hygroscopic powder that is freely soluble in water and slightly soluble in alcohol [Figure 1].

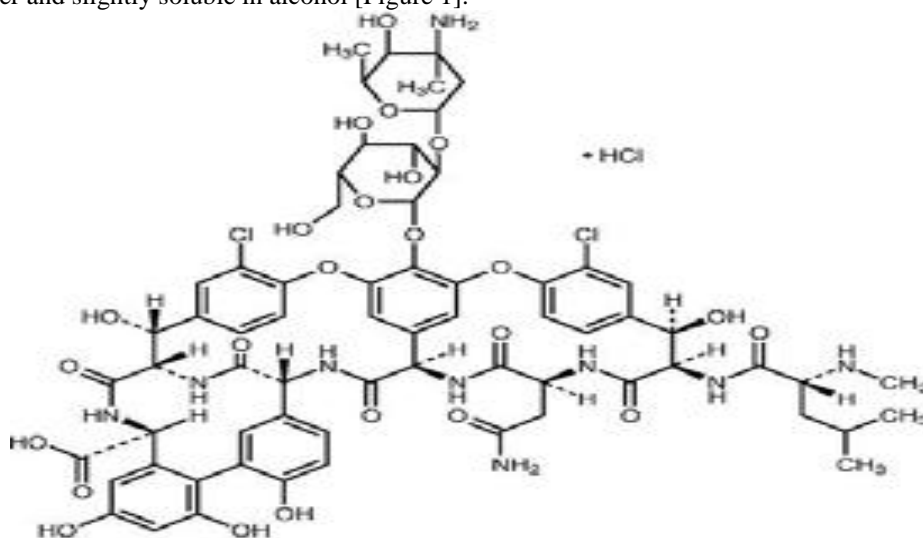


Fig 1:-Structure of Vancomycin

### Mode of action:

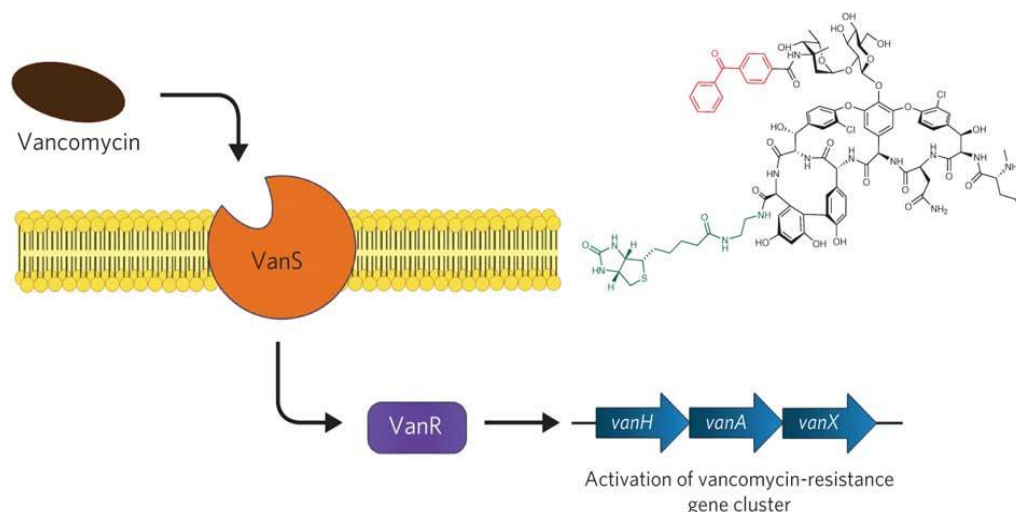
Vancomycin is a glycopeptide antibiotic that acts as an inhibitor of the biosynthesis of the major structural cell wall polymer peptidoglycan. It forms a complex with the D-alanyl-D-alanine peptidoglycan termini that are present in various phases of polymer synthesis and by this prevents the action of peptidoglycan polymerase and transpeptidase that would otherwise crosslink peptidoglycan intermediates by displacement of the terminal D-alanine. Thus, it displays bactericidal activity on bacteria in their growth phase.

### Vancomycin Resistance in Enterococci

#### Genes and Mechanism of Vancomycin resistance

Five types of vancomycin resistance have been reported in enterococci (VanA, VanB, VanC, VanD and vanE) [32, 33]. The mechanism of resistance (Figure 2) has been best characterized for the Van A cluster of seven genes found on the transposable (mobile) genetic element Tn1546. In the presence of an inducer like vancomycin, transcription of the gene necessary for resistance to vancomycin is activated as a result of the interactions of a sensory kinase and a response regulator. The transcribed genes are translated into enzymes, some of which make cell wall precursors ending in D-alanyl-D-lactate (D-Ala-D-Lac), to which vancomycin bind with very low affinity. Others prevent synthesis of or modify endogenous cell-wall precursors ending in D-alanyl-D-alanine (D-Ala-D-Ala), to which

vancomycin binds with high affinity [34]. All but one of the genes in the Van A clusters have homologues in Van B gene clusters that, in turn have a unique gene not found in the vanA clusters. Less is known about VanD types of resistance, but the gene for types A, B, D and E all appear to be acquired [35]. In contrast, the genes encoding the VanC type of resistance are endogenous, species-specific components of *E. gallinarum* (vanC-1) and *E. casseliflavus*/*E. flavescens* (vanC-2/vanC-3), respectively [36].



**Figure 2:-Mechanism of Vancomycin Resistance in Enterococcus species**

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### Conflict of Interest

The author declares no conflict of interest

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