

## Review Article

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# Enterococcal infections & antimicrobial resistance

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Enterococci have traditionally regarded as low grade pathogens, have emerged as an increasingly important cause of nosocomial infections in the last decade. Although about a dozen enterococcus species have been identified, only two are responsible for the majority of human infections, *i.e.*, *Enterococcus faecalis* and *E. faecium*. The most common nosocomial infections produced by these organisms are urinary tract infections (associated with instrumentation and antimicrobial resistance), followed by intra-abdominal and pelvic infections. They also cause surgical wound infections, bacteraemia, endocarditis, neonatal sepsis and rarely meningitis. A major reason why these organisms survive in hospital environment is the intrinsic resistance to several commonly used antibiotics and, perhaps more importantly, their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons. The emergence of vancomycin-resistant enterococci (VRE) is a cause of concern, as once established, it is very difficult to control. Moreover, there can be transfer of resistant gene from enterococci to *Staphylococcus aureus* thereby posing a threat to the patient safety and also challenges for the treating physicians. This review highlights the shifting spectrum of enterococcal infections, along with their geographical distribution and growing nosocomial importance. Emergence of antimicrobial resistance, pathogenicity and virulence factors, current preventive, control and treatment modalities of severe enterococcal infections are also dealt with.

**Key words** *Enterococcus faecalis* - *Enterococcus faecium* - high-level aminoglycoside resistance (HLAR) - nosocomial infections - vancomycin resistant enterococci (VRE)

Enterococci were originally classified as enteric Gram-positive cocci and later included in the genus *Streptococcus*<sup>1</sup>. In the early 1930s, enterococci were classified as group D streptococci and were differentiated from the non-enterococcal group D streptococci by distinctive biochemical characteristics<sup>2</sup>. In the late 1930s, Sherman recommended that the term enterococcus be specifically used for the streptococci that grow at both 10 and 45°C, at pH 9.6, in the presence of 6.5

per cent NaCl, survive at 60°C for 30 min and hydrolyse esculin<sup>3</sup>. During the mid 1980s, studies involving fatty acid composition, nucleic acid hybridization and comparative oligonucleotide cataloguing of 16s RNA led to the acceptance that the enterococci were sufficiently different from other streptococci to merit their own genus<sup>4</sup>. Although about a dozen enterococcus species have been identified, only two are responsible for the majority of human infections.

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 as an increasingly important cause of nosocomial infections in the 1990s. These infections are recognized by 3 ts - tough, tenacious and oftentimes troublesome<sup>5</sup>.

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*<sup>6</sup>. Additional species such as *E. cecorum*, *E. columbae*, *E. saccharolyticus*, *E. dispar*, *E. sulfureus*, *E. seriolicida* and *E. flavescens* have been proposed as additions to this list<sup>6</sup>. 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 per cent of all clinical isolates, and *E. faecium* had accounted for 5 to 15 per cent<sup>7,8</sup>.

Prior to 1990s also enterococci have been recognized as an important cause of bacterial endocarditis for almost a century<sup>9</sup>. However, during the past decade, there has been a worldwide trend in increasing occurrence of enterococci (in the hospitals), a shift in the spectrum of enterococcal infections, and emergence of antimicrobial resistance among such isolates<sup>9</sup>. Enterococci were reported as the second most common cause of nosocomial infections in the US<sup>10</sup>. The most frequent infections caused by enterococci are urinary tract infections (UTIs)<sup>9</sup>. The second most frequent enterococcal infections generally have been intra - abdominal and intra - pelvic abscesses or post-surgery wound infections<sup>9</sup>. In these settings, enterococci are usually part of a mixed flora commonly found in the GIT (the exact role of enterococci in mixed infections is somewhat murky)<sup>6</sup>. The third most frequent infection caused by these organisms is blood stream infections (BSIs)<sup>10</sup>. Other infections caused with lower frequency are central nervous system (CNS) and neonatal infections. Enterococci rarely cause respiratory tract infections, osteomyelitis, or cellulitis<sup>11</sup>. Enterococci are currently ascendant nosocomial pathogens, having become the second most common organisms recovered from nosocomial UTI and wound infections and the third most common cause of

nosocomial bacteraemia in the United States<sup>10</sup>. Enterococci account for more than 9 per cent of BSIs in US and Canada (rates are lower in Latin America)<sup>9</sup>. The highest detected rate of enterococcal UTI was in Canada (16.8%), followed by the US (12.5%) and Europe (11.7%)<sup>9</sup>.

Further, enterococci with high level aminoglycoside resistance (HLAR),  $\beta$ -lactamase production and glycopeptide resistance including vancomycin resistant enterococci (VRE) have emerged posing a therapeutic challenge to physicians due to the ease of acquiring and transferring antimicrobial drug resistance<sup>11</sup>. In this review we discuss the spectrum of enterococcal infections along with their geographical distribution and growing nosocomial importance. Also emergence of antimicrobial resistance, pathogenicity and virulence factors will be discussed. Preventive, control and treatment modalities will also be dealt with.

### Shifting spectrum of enterococcal infections

Historically, the ratio of infections due to *E. faecalis* to those due to all other enterococcus species was approximately 10:1. In the recent years, there has been a progressive decline in this ratio<sup>12</sup>. While *E. faecalis* remains the predominant species in clinical infection, *E. faecium* isolates are increasing in proportion. The trend is particularly true for blood isolates where the ratio of *E. faecalis* to *E. faecium* has decreased from 3.7: 1 in 1996 to 1.9:1 in 1999<sup>5</sup>. This microbiologic shift is likely to be explained in part by the emergence of VRE and *E. faecium* being the dominant species identified among VRE. In a comparison of NNIS (National Nosocomial Infection Surveillance) pathogens from 1994 through 1998 and May 1999, there was a 47 per cent increase in VRE<sup>13</sup>. Emergence of "pan-resistant" *E. faecium* is a cause of concern; 31 per cent are resistant to the ampicillin, vancomycin, gentamicin, and streptomycin<sup>5</sup>.

In the early 1990s, there was a common tendency to clump together all enterococci, but the antibiotic resistance profiles of the two principal pathogenic species are distinct. Most notably, *E. faecalis* resistance is low against vancomycin and ampicillin while levels of resistance among *E. faecium* isolates are high (60 & 80% respectively) and rising<sup>5</sup>. The species difference is very unusual indeed, because the gene responsible for this resistance can be transferred easily in the laboratory between the two species carried on pheromone responsive plasmids or conjugative transposons. The proportion of isolates of motile enterococci (*E. gallinarum*, *E. casseliflavus*) remained

low, *i.e.* less than 2 per cent<sup>14</sup>. It is important to probably recognize the motile enterococci because they are intrinsically resistant to vancomycin (low level) and inappropriate treatment with vancomycin may contribute to morbidity and mortality. This low prevalence may be due, in part, to the inability of automated systems to recognize these species. Motility testing is required to distinguish these species from *E. faecium* because of the similarity in phenotypic characteristics (it is recognized retrospectively sometimes)<sup>14</sup>. Tests used to identify *E. gallinarum* and *E. casseliflavus* are motility test, yellow colony pigmentation and inability to ferment inulin<sup>6</sup>. The two species have been identified as a clinically significant cause of bacteraemia in immunocompromised patients, especially those who had received organ transplantation. And as the number of patients undergoing transplantation increases, the prevalence of disease due to motile enterococci may also rise<sup>14</sup>.

In a prospective study, *E. faecium* (42.90%) and *E. faecalis* (40.00%) constituted the predominant isolates. *E. faecium* was the commonest blood culture isolate while *E. faecalis* predominated pus and urine samples. Other species isolated were *E. mundtii*, *E. dispar*, *E. durans*, *E. avium*, *E. raffinosus* and *E. gallinarum*<sup>15</sup>. In another study from New Delhi<sup>16</sup>, *E. faecium* (66%) was the most common isolate followed by *E. faecalis* (20%) in blood samples. However, *E. faecalis* (55%) followed by *E. casseliflavus* (24%) and *E. faecium* (12%) were reported from Chandigarh<sup>17</sup> from urinary isolates.

### Geographic distribution & spread of VRE within hospitals & community

VRE were first detected in Europe (United Kingdom & France) in 1986 and soon after a Van B *E. faecalis* clinical isolate was reported in the United States<sup>18</sup>. These have now been reported from Australia, Belgium, Canada, Denmark, Germany, Italy, Malaysia, Netherland, Spain and Sweden<sup>19</sup>. But the incidence of human VRE infections in European countries is relatively low (1-3%) compared with the high and rising rate in the US<sup>20</sup>. The rates have steadily increased from 0.3 to 7.9 percent (CDC)<sup>20</sup>. The increase is mainly due to 34-fold rise (0.4 -13.6%) of VRE infections in the intensive care unit (ICU) patients, although an increase has been noted in non ICU patients also<sup>20</sup>.

These geographic differences might be due to the use of the glycopeptide (avoparcin) as growth promoter in animal feeds (licensed since 1975) in some European countries. It has been fed to broiler chickens, swine,

and cattle. Avoparcin causes cross-resistance to vancomycin and teicoplanin among bacteria<sup>21</sup>. Here animal -to -human food chain appears to be a significant factor in antibiotic resistance, based on periodic examination of sewage and on comparisons of manure of animals fed with or without antibiotic growth promoters (these organisms readily colonize the intestinal tract of animals for which avoparcin was used as a feed supplement)<sup>22</sup>. However, this does not explain the greater frequency of VRE in the US hospitals, because in the US, avoparcin is not a licensed feed additive for animals, and culture surveys of a limited number of chickens in several cities have failed to detect VRE<sup>23</sup>. In fact, there is little evidence to suggest that transmission of VRE occurs in healthy adults in the US community. Attention is focused only in hospitals<sup>5</sup>.

The fact that vancomycin use in the US hospitals has increased dramatically in the past 10 to 15 years is the more likely explanation. The injudicious use of antimicrobial agents and the rising colonization pressure are the largest contributors to selection of vancomycin resistance<sup>24</sup>. At present, hospitalized patients with gastrointestinal carriage of VRE appear to be the major reservoir of the organism in the US (endogenous infection)<sup>25</sup>. This might explain the importance of VRE as a cause of catheter-related sepsis. It may increase the risk of cross-infection or blood culture contamination, which may explain the spontaneous resolution of bacteraemia in VRE<sup>25</sup>.

There is also evidence for direct exogenous acquisition of infection<sup>26</sup>. Oropharyngeal colonization may provide a source for cross-colonization, particularly if hospital staff consider manipulations (such as tracheostomy or endotracheal tube care) to be clean and do not subsequently wash their hands<sup>26</sup>. Additionally, skin colonization associated with diarrhoea or faecal incontinence has been reported<sup>27</sup>.

Environmental surfaces and medical equipment items in the patient's room frequently become contaminated with VRE and may also serve as a reservoir especially in the rooms of patients who have diarrhoea<sup>28</sup>. Examples of items that may be contaminated include patient gowns and linen, beds, bedside rails, overbed tables, floors, door knobs, wash basins, glucose meters, blood pressure cuffs, electronic thermometers, ECG monitors, ECG wires, *etc.*<sup>28-30</sup>. VRE may remain viable on such surfaces for days or weeks and may act as a source from which health care workers (HCWs) may contaminate their hands or clothing.

Once colonized, the patients remain so for weeks or months (even up to 1 yr)<sup>27</sup>. Thus, as colonized patients leave the hospital environment, the possibility that transmission might occur in the community cannot be discounted. In fact, two cases of apparent community-acquired VRE urinary tract infection in New York City have been reported<sup>31</sup>. Another aspect to be kept in mind is that hospitals should develop means of prompt identification of such patients at the time of re-admission so that they can be placed in isolation right away<sup>28,30</sup>.

### Emergence of antimicrobial resistance

An important feature in the emergence of the enterococci as a cause of nosocomial infection is their increasing resistance to a wide range of antibiotics. They demonstrate both intrinsic and acquired resistance<sup>32</sup>.

**Intrinsic resistance:** Enterococci exhibits intrinsic resistance to penicillinase-susceptible penicillin (low level), penicillinase-resistant penicillin, cephalosporin, nalidixic acid aminoglycoside and clindamycin<sup>1</sup>. Enterococci are resistant to most beta-lactam antibiotics because of low affinity penicillin binding proteins (PBP), which enable them to synthesize cell wall components even in the presence of modest concentration of most beta-lactam antibiotics<sup>1</sup>. Due to the overproduction of low affinity PBP-5 (a protein that can take over the function of all PBPs), higher level of resistance is observed in *E. faecium* (MIC 16-64 µg/ml) in comparison to *E. faecalis* which can be inhibited by concentration of penicillin achievable in plasma (MIC 1-8 µg/ml)<sup>8</sup>. Enterococci exhibit a low to moderate level resistance to aminoglycosides (MIC 62 to 500 µg/ml) related to the slow uptake or permeability of these agents. However, aminoglycoside uptake can be enhanced when enterococci are exposed to a beta-lactam<sup>33</sup> (which increases the intracellular uptake). They also exhibit high level aminoglycoside resistance (MIC >2000 µg/ml) which is either ribosomally mediated or due to the production of inactivating enzymes.

The three types of resistance of most significance in the enterococci are high-level resistance to the aminoglycosides, ampicillin resistance caused by beta lactamase production, and glycopeptide resistance<sup>34</sup>.

**Acquired resistance:** Acquired resistance in enterococci can occur either via mutations in existing DNA or through the acquisition of new DNA. High-level resistance (MIC  $\geq$  2000 µg/ml) is usually due to the transferable plasmid-mediated production of aminoglycoside-inactivating enzymes. Since enterococcal resistance to gentamicin and streptomycin occur by different mechanisms, it is important to test susceptibilities to both agents. High-level gentamicin resistance is associated with a bifunctional enzyme possessing acetylase (6') and phosphotransferase (2') activities (Table I)<sup>33</sup>, conferring resistance to all aminoglycosides except streptomycin. High-level streptomycin resistance may be ribosomally mediated or due to the production of streptomycin adenylyltransferase; these strains remain susceptible to gentamicin. Penicillin-aminoglycoside synergy does not occur in high-level aminoglycoside resistant enterococci (streptomycin MIC  $\geq$  2000 µg/ml; gentamicin MIC  $\geq$  500 µg/ml)<sup>34</sup>.

Although high-level aminoglycoside resistant (HLAR) enterococci are usually defined as enterococci the MIC of >2000 µg/ml, some investigators propose using gentamicin at a concentration of 500, or 1000 µg/ml<sup>33</sup>. Whether 500, 1000 or 2000 µg of gentamicin per ml is the most appropriate concentration to use for testing is undecided. Use of any one of these concentrations will probably accurately detect high-level gentamicin resistance.

Studies on HLAR have been done almost exclusively on *E. faecalis*. The incidence of HLAR is increasing (approximately 50% of isolates show this resistance). In two studies conducted in Delhi, 81 per cent of *E. faecium* and 72 per cent of *E. faecalis* isolates exhibited HLAR in one study<sup>15</sup>, while only 66 per cent of HLAR isolates were detected in another<sup>16</sup>.

**Table I.** Activity of aminoglycoside-modifying enzymes in *E. faecalis* showing HLAR phenotype

Enzyme	Activity on aminoglycoside			
	Gentamicin	Streptomycin	Tobramycin-Netilmicin	Amikacin-Kanamycin
Streptomycin adenylyltransferase	Absent	Present	Absent	Absent
3' Phosphotransferase	Absent	Absent	Absent	Present
2' Phosphotransferase & 6' acetyltransferase (acetylase)	Present	Absent	Present	Present

Since gentamicin is the most widely used aminoglycoside (in combination with a cell wall active agent) for treatment of serious enterococcal infections, an HLAR screen for gentamicin is usually sufficient. However, if an isolate demonstrates HLAR to gentamicin, screening for high-level streptomycin resistance is needed so that streptomycin could be used therapeutically<sup>33</sup>. There may be rare isolates that lack HLAR to gentamicin and streptomycin but show HLAR to amikacin and kanamycin. These would not be detected by the above methods but would require kanamycin (120 µg disc or 2000 µg/ml) as amikacin is a poor predictor for the enzyme responsible. But these are rarely used, so the clinical significance of such resistance is minimal<sup>33</sup>.

Most *E. faecium* isolates produce an enzyme (6' acetyltransferase - acetylase) that makes them inherently resistant to amikacin, kanamycin, netilmicin and tobramycin<sup>33</sup>. This resistance may not be expressed as HLAR, but synergy will not occur with these agents. Therefore, gentamicin predicts only gentamicin resistance, and streptomycin predicts only streptomycin resistance in *E. faecium*<sup>33</sup>.

Beta-lactamase producing enterococci are infrequently isolated. The enzyme hydrolyses penicillin, ampicillin, and piperacillin. Beta-lactamase production is sometimes encoded on a transferable plasmid which also carries high-level gentamicin resistance (unlike most staphylococci, where beta-lactamase production is inducible, in enterococci it is constitutive, low-level and inoculum dependant). The nitrocefin (chromogenic cephalosporin) is the only reliable method for the detection of beta-lactamase production in *E. faecalis*. It is easy to perform and detects most known beta-lactamases<sup>33</sup>.

*Vancomycin resistance*: From the perspective of *E. faecium* antimicrobial resistance, there is an association between ampicillin and vancomycin resistance. Ampicillin resistant *E. faecium* isolates are most often detected before vancomycin resistance is detected. Together, the genetic linkage in *E. faecium* between ampicillin, PBP- 5, and vancomycin<sup>34</sup> and clinical studies that have shown prior beta-lactam use as a leading predisposing factor suggest that antimicrobial agents such as cephalosporins contribute to the emergence of vancomycin resistant *E. faecium*<sup>35</sup>. The linkage between a beta-lactam resistant PBP and vancomycin resistance does not appear to have occurred yet in *E. faecalis*, which may account for the sporadic detection of vancomycin resistant *E. faecalis*.

**Table II.** Phenotypes of glycopeptide-resistant enterococci

Phenotype	Characteristic		
	Vancomycin MIC (µg/ml)	Teicoplanin MIC (µg/ml)	Mode of acquisition of resistance
VanA	64->1,000	16-512	Acquired
VanB	4-1,024	≤0.5	Acquired
VanC	2-32	d>0.5	Intrinsic
VanD	128	4	Acquired
VanE	16	0.5	Acquired

Phenotypic classification- There are five recognized phenotypes of vancomycin resistance, VanA, VanB, VanC, VanD, and VanE<sup>36-38</sup>. Two of these (VanA and VanB) are mediated by newly acquired gene clusters not previously found in enterococci. VanA and VanB resistance phenotypes were described primarily in *E. faecalis* and *E. faecium*. VanA-resistant strains possess inducible, high-level resistance to vancomycin (MICs ≥ 64 µg/ml) and teicoplanin (MICs ≥ 16 µg/ml)<sup>36</sup> (Table II). The details of vancomycin resistance have been best documented with the *vanA* gene cluster found on the transposon, or “jumping” genetic element, Tn1546<sup>36</sup>.

VanB isolates were initially believed to be inducibly resistant to more modest levels of vancomycin (MICs 32 to 64 µg/ml) but are susceptible to teicoplanin. It is now known that levels of vancomycin resistance among VanB isolates may range from 4 to ≥ 1,000 µg/ml whereas susceptibility to teicoplanin is retained. VanB resistance determinants also reside on large mobile elements that can be transferred from one strain of enterococcus to another<sup>39,40</sup>. VanC resistance phenotype was described in *E. casseliflavus* and *E. gallinarum*, which demonstrate intrinsic, low-level resistance to vancomycin (MICs 2 to 32 µg/ml) and are susceptible to teicoplanin<sup>41</sup>.

Certain limitations of this classification method have become evident over time *e.g.*, the genetic determinants of the VanA phenotype have now appeared in *E. gallinarum* and other enterococcal species<sup>41</sup>. Additionally, mutants derived from VanB strains may exhibit resistance to teicoplanin and thus be phenotypically indistinguishable from VanA strains<sup>42</sup>. Nevertheless, this phenotypic classification scheme is still useful, because it usually corresponds well with the genotypic classification and utilizes information that can be derived simply and inexpensively in a laboratory<sup>43</sup>.

Genotypic classification - Under normal conditions of peptidoglycan synthesis in enterococci, two

molecules of D-alanine are joined by a ligase enzyme to form D-Ala-D-Ala, which is then added to UDP-*N*-acetylmuramyl-tripeptide to form the UDP-*N*-acetylmuramyl-pentapeptide. This pentapeptide, when incorporated into the nascent peptidoglycan (transglycosylation), permits the formation of cross-bridges (transpeptidation) and contributes to the strength of the peptidoglycan layer<sup>43</sup>. Vancomycin binds with high affinity to the D-Ala-D-Ala termini of the pentapeptide precursor units, blocking their addition to the growing peptidoglycan chain and preventing subsequent cross-linking<sup>44</sup>.

*VanA glycopeptide resistance:* The *vanA* gene and the other genes involved in the regulation and expression of vancomycin resistance (*vanR*, *vanS*, *vanH*, *vanX*, and *vanZ*) are located on a 10,581-bp transposon (Tn 1546) of *E. faecium*, which often resides on a plasmid<sup>45</sup>. Expression of these genes results in the synthesis of abnormal peptidoglycan precursors terminating in D-Ala-D-lactate instead of D-Ala-D-Ala. Vancomycin binds to D-Ala-D-Lac with markedly lower affinity than it does to the normal dipeptide product<sup>46</sup>.

The proteins involved in the synthesis of D-Ala-D-Lac are as follows (i) VanA protein is a ligase of altered substrate specificity which produces D-Ala-D-Lac in preference to D-Ala-D-Ala<sup>47</sup>, (ii) VanH protein is a D-hydroxy acid dehydrogenase which creates a pool of D-lactate for use in the above reaction<sup>48</sup>, (iii) VanX protein is a D,D-dipeptidase lacking activity against D-Ala-D-Lac. This enzyme reduces pools of D-Ala-D-Ala produced by the native enterococcal ligase, thereby minimizing the competing synthesis of normal pentapeptide<sup>44</sup>. VanR and VanS proteins constitute a two component regulatory system that regulates the transcription of the *vanHAX* gene cluster. VanS apparently functions as a sensor to detect the presence of vancomycin or, more likely, some early effect of vancomycin on cell wall synthesis. VanS then signals VanR, the response regulator, which results in activation, or turning on, of the synthesis of some other proteins (VanH, VanA, and VanX) involved in resistance. In VanA phenotype strains, either vancomycin or teicoplanin can induce the transcription, but the precise signals are still unknown<sup>49</sup>. *VanY* and *VanZ* may contribute to but are not essential for resistance.

*VanB glycopeptide resistance:* VanB glycopeptide resistance in enterococci is mediated by an abnormal ligase (VanB) that is structurally related to VanA ligase. VanB protein also favours the production of the

pentadepsipeptide terminating in D-Ala-D-Lac<sup>50</sup>. Genes analogous to their class A resistance counterparts are designated *vanH<sub>B</sub>*, *vanX<sub>B</sub>*, *vanY<sub>B</sub>*, *vanR<sub>B</sub>*, and *vanS<sub>B</sub>*<sup>49</sup>. However, there is no gene counterpart of *vanZ* in these organisms. The regulatory system in class B strains appears insensitive to induction by teicoplanin<sup>51</sup>. Teicoplanin induces the synthesis of 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<sup>52</sup>.

VanA- and VanB-type resistances differ in other ways too. VanA is more widely distributed and is by far the predominant type of resistance reported in Europe. While VanB strains are fairly common in the United States, with some hospitals reporting VanB exclusively, VanA still predominates<sup>53</sup>. The *vanA* ligase gene is found not only in a wider range of enterococcal species but in non-enterococcal species as well, while *vanB* is confined primarily in *E. faecalis* and *E. faecium*. The difference in the dissemination of these resistant traits may be related to the observation that the *vanA* gene cluster is often located on a transposon which, in turn, can be a part of a conjugative (transferable) plasmid<sup>45</sup>. The *vanB* cluster is often located on the host chromosome and initially was thought not to be transferable to other bacteria. However, it can also occur on plasmids, and, even when it is chromosomal, this gene cluster has been transferable as part of large mobile elements, perhaps related to large conjugative transposones<sup>39</sup>.

Conjugal transfer of VanA-type vancomycin resistance genes from enterococci to other Gram-positive bacteria has been accomplished *in vitro*. The Gram-positive organisms include group A and viridans group streptococci, *Listeria monocytogenes* and *Staphylococcus aureus*<sup>54</sup>. This gives rise to concern that such transfer in humans under natural conditions might be feasible.

*VanC glycopeptide resistance:* Low-level resistance to vancomycin is typical of *E. gallinarum*, *E. casseliflavus*, and *E. flavescens*. VanC ligase of *E. gallinarum* favours the production of a pentapeptide terminating in D-Ala-D-Ser. Substitution of D-Ser for D-Ala is presumed to weaken the binding of vancomycin to the novel pentapeptide<sup>23</sup>.

*VanD glycopeptide resistance:* *VanD*, a novel vancomycin resistance gene was first described in a New York Hospital in 1991<sup>38</sup>. It appears to be located on the chromosome and is not transferable to other enterococci. The deduced amino acid sequence of *VanD* showed 67 per cent identity to those of *VanA* and *VanB*<sup>23</sup>.

*VanE glycopeptide resistance:* The *VanE* vancomycin resistance gene has been described in *E. faecalis* isolates which show low level of resistance to vancomycin and susceptibility to teicoplanin. This phenotype has similarities to the intrinsic *VanC* type of resistance<sup>23</sup>.

*Vancomycin-dependent enterococci:* An interesting phenomenon that has developed in some strains of *VanA*- and *VanB*-type VRE is that of vancomycin dependence<sup>19,55</sup>. These enterococci are not just resistant to vancomycin but require it for growth. A likely explanation for this phenomenon 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 is 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.

### Status of VRE in India

In India, at All India Institute of Medical Sciences, New Delhi, five isolates of *E. faecalis* were found to be resistant to vancomycin by the disk diffusion and agar screen methods. On PCR, four had *VanA* phenotype and one had *VanB* phenotype<sup>56</sup>. In another study from Lady Hardinge Medical College, New Delhi<sup>16</sup>, Chandigarh<sup>17</sup> and Mumbai<sup>57</sup> indicate 8, 5.5 and 23 per cent VRE respectively and all being of *Van B* phenotype.

### Pathogenicity and virulence

Surprisingly little is known about the factors that contribute to the ability of enterococci to cause infections in man<sup>5</sup>. Studies have documented high mortality rates (42 to 68%) in patients with enterococcal bacteraemia<sup>18,58</sup>.

Although not a classic virulence factor, the resistance of enterococci to multiple antimicrobial agents allows them to survive and proliferate. Key to understanding enterococcal infection at the molecular and cellular level is that nosocomial enterococcal disease is predominantly a two-stage process. There is initial, usually asymptomatic colonization of gastrointestinal tract by enterococcal strains possessing various traits, such as antibiotic resistances, cytolytic toxin genes, or possibly aggregation substance or the protease gelatinase upon hospital admission<sup>59</sup>. Subsequently this population is expanded, often facilitated by antibiotic elimination of competitors. For a select number of patients, there is subsequent tissue invasion, directly or indirectly, from the expanded gastrointestinal tract reservoir. Given the two stage model, virulence factors may act by functioning at either or both levels. This explains how antibiotic resistance helps.

Traits that may enhance the virulence include the usual suspects cytolysin, aggregation substance, adhesions, extracellular superoxide (ESO), extracellular surface protein (ESP), haemolysin and gelatinase<sup>5</sup>. Mechanisms by which they enhance virulence are not completely understood but they act on any of the two stages. Most cytolytic strains also produce aggregating substance, so in all likelihood they act synergistically. Reactive oxygen species associated with superoxide generation are destructive in mammal tissues.

Another variable trait that appears to be associated with enterococcal virulence is extracellular surface protein. The *esp* gene encodes a large bacterial surface protein with an interesting structure<sup>60</sup>. The central core consists of tandem repeating units, with a slightly divergent C- terminal cell wall anchor domain and an apparently globular N- terminal domain. It is hypothesized that the central repeat region acts as a retractable arm and may actually facilitate immune evasion<sup>60</sup>. As such, very little is known about host immune responses to enterococci. We have no information about evasive tactics of *E. faecium* and clearly need more research in this direction.

As sophisticated support currently available to prolong life of patients with severely debilitating underlying conditions, a fraction of enterococcal disease is attributable to ordinary commensal strains. Therefore, collections of infection-derived isolates should contain a spectrum of types of strains, from pure commensals to those harboring the most synergistic combinations of various virulence traits.

### Risk factors

Earlier studies in US revealed that most patients with VRE were in ICUs<sup>30</sup>. However, VRE is now being seen with increasing frequency among patients with chronic renal failure, cancer, organ transplant recipients and patients who experience prolonged hospitalization. They tend to occur in more debilitated or seriously ill hospitalized patients<sup>28,30,61,62</sup>.

Risk factors include longer duration of hospitalization, longer duration of stay in ICU, gastrointestinal colonization with VRE (it may precede infection in many patients but all colonized patients do not get infected), previous antimicrobial therapy (especially with multiple antibiotics), severity of illness, exposure to contaminated medical equipment, proximity to a previously known VRE patient, exposure to a nurse who has assigned on the same shift to another known patient, haematological malignancy/bone marrow transplantation, parenteral/oral vancomycin and receipt of third-generation cephalosporins and drugs with activity against anaerobes<sup>28,30,63</sup>. Vancomycin most probably predisposes patients to colonization and infection with VRE by inhibiting the growth of the normal Gram-positive bowel flora and by providing selective advantage for VRE that may be present in small numbers in the individuals bowel. Oral vancomycin use may also be a risk factor for VRE colonization<sup>29</sup>, and this has led to recommendations discouraging the use of this agent for the primary treatment of antibiotic-associated diarrhoea<sup>64</sup>.

The specific association between cephalosporin exposure and resistant infection may be due to the exclusively nosocomial nature of this infection. Moxalactam, a third generation cephalosporin used in 1980s with significant activity against anaerobes poses a risk factor for colonization and acquisition of VRE. The same holds true for metronidazole and clindamycin<sup>23</sup>.

The emergence of VRE has alarmed the global infectious diseases community for several reasons. Because of the limited therapeutic options for treating serious infections caused by enterococci, it has emerged as one of the leading clinical challenges for physicians. The limited successes over the past decade of prevention and control strategies for containing vancomycin resistance (as well as methicillin resistance in *Staphylococcus* sp.) highlight the difficulty of limiting the problem once it is established.

### Prevention and control

The traditional view that enterococcal infections were endogenous in origin came from studies based on antibiotic resistance pattern and biochemical profiles. Evidence of hospital-acquired or inter-hospital spread of enterococcal infection came during the 1980s with molecular methods. It has been demonstrated that enterococci, including VRE, can be spread by direct patient-to-patient contact or indirectly via transient carriage on the hands of personnel<sup>28</sup>, contaminated environmental surfaces<sup>28,30</sup> or patient care equipment<sup>62</sup>.

In response to the dramatic increase in vancomycin resistance in enterococci, the CDC Hospital Infection Control Practices Advisory Committee (HICPAC) has made the following recommendations<sup>64</sup>:

(i) Prudent use of vancomycin: Encouraging the appropriate use of oral and parenteral vancomycin is an important component of HICPAC recommendations. Other measures include formulary policies discouraging the use of third-generation cephalosporins and agents most likely to cause *C. difficile* colitis.

(ii) Education of hospital staff: Continuous education programmes for health care workers should include information about the epidemiology of VRE and the potential impact of this pathogen on the cost and outcome of patient care.

(iii) Effective use of the microbiology laboratory: Early detection of patients colonized or infected with VRE is an essential component of any hospital programme designed to prevent nosocomial transmission of VRE. There are at present eight available susceptibility test methods (agar dilution, disk diffusion, E-test, agar screen plate, Vitek GPS-TA and GPS-101, and MicroScan overnight and rapid panels). Enterococci may also be tested for vancomycin resistance by using PCR assays designed to detect the genes responsible for glycopeptide resistance in these organisms. Surveillance cultures for VRE are time consuming and expensive for the laboratory to perform.

(iv) Implementation of infection control measures: Including the use of gloves and gowns and isolation or cohorting of patients, as appropriate to specific conditions.

### Treatment

Treatment is difficult due to resistance and because of the fact that it is necessary to use specialized techniques to demonstrate their true susceptibility in

clinical laboratory. The laboratory must look for HLAR/time-kill curves. Likewise, standard testing will fail to demonstrate penicillin/ampicillin resistance in many beta-lactamase producing strains.

However, uncomplicated enterococcal infections are usually treated with single-drug therapy such as ampicillin, penicillin or vancomycin. Serious enterococcal infections (*e.g.*, bacteraemia and endocarditis) require treatment with a bactericidal combination of antibiotics that should include a penicillin (ampicillin or penicillin G) to which the isolate is susceptible and an aminoglycoside (gentamicin or streptomycin) to which it does not exhibit high-level resistance<sup>65</sup>. Vancomycin can also be used in combination with an aminoglycoside, and is recommended as a drug of choice in patients with serious penicillin allergy or in treatment of ampicillin and penicillin-resistant strains of bacteria<sup>65</sup>. As of today, enterococci are becoming increasingly resistant to traditional antibiotic therapy. In addition to HLAR and ampicillin resistance, rapid spread of vancomycin resistance has resulted in limited therapeutic options<sup>1</sup>.

If the infecting VRE is highly resistant to ampicillin, there are a few treatment options and one should ask the laboratory to test various other antimicrobials including tetracyclines, erythromycin, chloramphenicol, high levels of aminoglycosides, rifampin, fluoroquinolones, novobiocin, and, for urinary tract infections, nitrofurantoin<sup>66</sup>. When enterococci have high-level resistance to both gentamicin and streptomycin, no regimen currently available is likely to produce a reliable bactericidal effect.

A number of new approaches to the treatment of VRE infections including beta lactam - beta lactam, beta lactam - glycopeptide, and beta lactam fluoroquinolone combinations have been explored in experimental animal models<sup>67</sup>. Each approach has its limitations and the treatment is at best, experimental. Dalfopristin-quinupristin (RP 59500), a streptogramin antibiotic, is the first antibiotic approved for the treatment of patients with serious or life-threatening infections associated with vancomycin-resistant *E. faecium* bacteraemia<sup>68</sup>. Although daptomycin (LY146032), an acidic lipopeptide, gave promising results *in vitro*<sup>68</sup>, it has been withdrawn because of the associated toxicity in humans. Similarly, ramoplanin, a lipoglycopeptide, which is more active *in vitro*, seems unsuitable for systemic use because of its toxicity<sup>68</sup>. Another agent active against VRE is a semisynthetic glycopeptide designated LY333328, which demonstrates bactericidal as well as

bacteristatic activity against enterococci<sup>69</sup>. Although its mechanism of action is still unknown, it is thought to be similar to that of vancomycin. However, lack of data on pharmacokinetics in humans and its toxicity creates uncertainties about its use in humans. Amongst all the other agents, it is the oxazolidinones, a new class of synthetic antibiotics, which have a good anti-enterococcal activity<sup>70</sup>.

### Conclusion

To conclude enterococci are Gram-positive cocci presenting as harmless commensals to multifaceted deadly pathogens. The most frequent infections caused by them are urinary tract infections followed by wound infections and blood stream infections. Most clinical infections are due to either *E. faecalis* or *E. faecium*. Although in bacteraemia cases the predominance of *E. faecium* has been observed, *E. faecalis* is the most common isolate clinically. The three types of resistance of most significance in the enterococci are high-level resistance to the aminoglycosides, ampicillin resistance caused by beta lactamase production, and glycopeptide resistance including vancomycin resistance. Conjugal transfer of VanA-type vancomycin resistance genes from enterococci to other Gram-positive bacteria has been accomplished *in vitro*. The occurrence of VRE is a persisting clinical problem in all geographic areas and continues to be exacerbated by clonal dissemination within the health care facility leading to limited therapeutic options. The steady pandemic spread of VRE along with acquisition of resistance to newer antimicrobials warrants continued surveillance of these versatile pathogens.

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